New players in the pathogenesis of focal segmental glomerulosclerosis

Christoph Schell¹,² and Tobias B. Huber¹,³

¹Renal Division, University Hospital Freiburg, Freiburg, Germany, ²Spemann Graduate School of Biology and Medicine, Freiburg, Germany and ³BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-University Freiburg, Freiburg, Germany

Correspondence and offprint requests to: Tobias B. Huber, E-mail: tobias.huber@uniklinik-freiburg.de

Abstract
Focal segmental glomerulosclerosis (FSGS) is the most common primary glomerular disorder causing end-stage renal disease. Since the first description of this clinicopathological entity in the early 1930s, various studies have identified numerous underlying pathogenetic mechanisms. Nevertheless, FSGS is still a complex, only partially understood and its classification sometimes confusing disease. A unifying pathophysiological concept has not been identified and might not even exist. However, research efforts of past decades identified FSGS as a podocytopathy with several podocyte molecules being key players in the development and the course of FSGS. Podocytes are crucially involved in the formation of the glomerular barrier and any assault on their delicate physiological balance and architecture can result in the development of proteinuria. The following review article will introduce most recent examples identifying novel players in the complex pathogenesis of FSGS.

Keywords: Arhgap24; CD2AP; FSGS; podocyte; suPAR

Introduction

The term focal segmental glomerulosclerosis (FSGS) stands for a complex clinicopathological entity, which integrates various clinical presentations as well as different underlying pathophysiological aetiologies [1–5]. However, FSGS is uniformly characterized by the occurrence of proteinuria and the involvement of the podocyte, a specialized epithelial cell, which is essential for the formation and the integrity of the glomerular barrier. For decades, FSGS was mainly investigated in terms of histological description and these efforts culminated in the classification in five morphological subtypes [6]. From an aetiological and functional standpoint, FSGS can be divided into primary and secondary FSGS. The difference between these two groups has major therapeutic implications, as primary FSGS is usually treated empirically with immunomodulatory agents. This treatment concept is mainly based on the theory that a dysregulated immune system contributes to the pathogenesis of FSGS. However, recent studies also identified off-target effects of established immunomodulatory drugs, such as rituximab and cyclosporine A, that can directly affect and modulate podocyte function [7–9].

While primary FSGS subsumes all idiopathic cases of FSGS, the group of secondary FSGS presents a kaleidoscopic array of different diseases, all resulting in different degrees of nephron loss and podocyte damage. The greatest contribution in understanding how the podocyte is particularly affected was done by the identification of causative genes in hereditary forms of nephrotic syndrome. Here we will only briefly review some of the most important genes...
Fig. 1. Schematic overview depicting so far identified players in genetic forms of FSGS. Several different cellular podocyte compartments and functions can be affected by genetic mutations. (i) Transcriptional regulation modulated by podocyte transcription factors like LMX1B and WT-1. (ii) Mutations in INF2 and α-ACTININ-4 lead to an unbalanced actin assembly. (iii) Mutations affecting slit diaphragm (SD) molecules or SD associated molecules like nephrin, Cd2ap, podocin and Trpc6 are involved in the development of hereditary nephrotic syndrome. (iv) Adhesion molecules like integrins and laminin-β2 play important roles in the pathogenesis of FSGS.

(see therefore also Figure 1); for more details we refer to several published reviews on this topic (e.g. [4]). All identified causative genes in hereditary FSGS locate to the podocyte, where a broad range of cellular functions and compartments can be affected. It was in 1998 that Karl Tryggvason et al. identified, by positional cloning, the slit diaphragm core component nephrin (NPHS1) as a novel gene, which was found to be mutated in congenital nephrotic syndrome of the Finnish type [10]. Since then more and more mutations in the Nphs1 gene were reported and also linked with late/adult-onset of FSGS [11, 12]. Podocin (NPHS2) was the next identified major player in hereditary FSGS [13]. Podocin physically interacts with nephrin, localizes to the slit diaphragm and is enriched in lipid rafts (cellular compartments of high signalling activity) [14–17]. The genotype phenotype correlation emphasized the importance of the slit diaphragm as a signalling platform for the podocyte [13, 18, 19]. Another molecule identified to be linked with FSGS was CD2-associated protein [20] (CD2AP). CD2AP is an 80 kDA protein and functions as an adaptor/linker molecule and is critical for stabilizing contacts between T-cells and antigen-presenting cells [21]. By generating constitutive knockout mice, Shaw et al. identified the fundamental role of C2d2ap for the integrity of the podocyte [20, 22] and in the following years mutations in the CD2AP gene could be associated to the occurrence of FSGS [23]. The identification of mutations in CD2AP highlighted once more the essential role of signalling events taking place at the slit diaphragm, since CD2AP also links podocin and nephrin to the phosphoinositide 3-OH kinase [15, 24]. Two further identified genes pinpoint to the exclusive role of the podocyte cytoskeleton in the pathogenesis of FSGS, namely mutations found in alpha-actinin-4 and inverted formin 2. While alpha-actinin-4 serves as an actin cross-linking protein [25], inverted formin 2 is promoting actin nucleation [26]. Reported gain-of-function mutations in the alpha-actinin-4 gene lead to an increased binding to F-actin fibres and finally results in unbalanced assembly properties [27]. Mutations in INF2 on the other hand affects actin nucleation processes and loss of function might be reflected by disorganized actin bundles in podocyte foot processes [26, 28]. Furthermore, the ion channel TRPC6, as a podocyte Ca2+ influx pathway and upstream regulator of podocyte cytoskeleton, has been identified to cause both genetic [29–31] and acquired [32] FSGS. Interestingly, not only monogenic mutations, but also heterozygous oligogenic mutations of key podocyte molecules can result in FSGS in mice [33] pointing towards the importance of genetic modifiers of glomerular diseases. Indeed, gene association studies led to
a breakthrough in identifying genomic modifiers explaining the excessive risk for chronic kidney disease in non-dia-
betic African Americans [34]. One of these studies re-
vealed that coding sequence variants within the APOL1
gene are responsible for a significant part of observed
FSGS risk in African Americans [35].

In summary, the genes mentioned so far play pivotal
roles in podocyte physiology and have shed light on fund-
amental cellular properties, namely the slit diaphragm
and the regulation of the cytoskeleton. The aim of this
review is to summarize innovative concepts based on
recently published examples of new players in the patho-
genesis of secondary FSGS.

New identified players in the pathogenesis of FSGS

Role of Arhgap24 in the regulation of cytoskeletal prop-
eries in podocytes. The association of podocyte foot
process effacement with proteinuria might represent a
switch in podocyte actin networks [36] that are paralleled
by changes in podocyte foot process motility [37]. It is
assumed that podocytes under physiological conditions
might exhibit a rather stationary phenotype, which is
mainly controlled by the activity of Rho-A with low
activity of Cdc42/Rac1 [38]. Under stress and in patho-
physiological situations, this phenotype might be shifted
to a more migratory behaviour of podocytes, characterized
by an increase in Cdc42/Rac1 activity [38–40]. Small
GTPase in-/activating proteins (so-called GAPs and
GEFs) play a decisive role in this kind of ying and yang
balance between RhoA and Cdc42/Rac1 [41]. By modu-
ulating the GTPase activity of Rho GTPase molecules, the
cytoskeletal functions will be fine tuned and regulated
[41]. Using a combined approach of podocyte cell culture
and genetic studies, Shaw et al. could identify Arhgap24,
which is a reported Rac1-inactivating GAP, as a highly
podocyte-specific protein. Membrane ruffling, which can
be interpreted as a surrogate marker for a migratory podo-
cyte phenotype, is negatively regulated by Arhgap24. In-
terestingly, the expression of the Arhgap24 transcript
significantly increases with podocyte differentiation,
suggesting that podocytes shift to a more stationary pheno-
type, when they become highly differentiated. In con-
trast, the loss of function mutations of the Arhgap24 gene
can be found in FSGS patients indicating the critical
importance of Arhgap24 for human glomerular disease.
The authors identified an Arhgap24 sequence variation
(Q158R), which was only present in the patient cohort.
In one pedigree, the mutation could be detected in at least
two FSGS-affected family members. The sequence
variant is affecting the essential GAP domain of
Arhgap24, and when transferred to cell systems, this
mutation causes similar phenotypes as knockdown of
Arhgap24, indicating that the mutation is indeed affecting
Arhgap24 activity.

In summary, this innovative study demonstrates the
delicate involvement of Rho-GTPase modulation by GAPs
in podocyte homeostasis (see Figure 2). Compared with
the usual suspects (e.g. Nphs2), the frequency of the re-
ported sequence variation in the Arhgap24 gene appears
rather minuscule, but nevertheless fundamentally supports
the concept of Rho-GTPase regulated podocyte pheno-
types and related patho-/physiological conditions [42].

Myo1e a novel human FSGS gene regulating podocyte
motility. Another important study by the PodoNet Con-
sortium identified two mutations in the MYO1E gene,
which are linked to a childhood-onset and glucocorticoid-
resistant form of FSGS [43]. In an approach, using
whole-genome linkage analysis, they could discover and
sequence these mutations in 52 unrelated patients with
FSGS. In further experiments, this group could not only
demonstrate that MYO1E is highly enriched in human po-
docytes, but furthermore discovered that mutations
(A159P and Y695X) were linked with decreased motility
behaviour of human podocytes and mislocalized expres-
sion of the MYO1E protein in vitro [43]. This
important study again underlines how sensitive podocytes
react to an unbalanced cytoskeletal homeostasis leading to
the development of FSGS.

CD2AP is linked to the modulation of proteolytic pro-
grammes via cathepsin L. Recent work could demon-
strate that the induction of a cytosolic variant of the
protease cathepsin L is a key event in the development of
proteinuric diseases [9, 37, 44]. While the spectrum of
functions for cathepsin L is very broad [45], quite specific
implications for podocyte homeostasis were determined:
for instance, it was shown that cathepsin L proteolytically
degrades dynamin [44] and synaptopodin [9] causing dys-
regulated podocyte actin networks, foot process efface-
ment and glomerular disease. In a current report, Reiser
et al. used the well-established C2d2ap knockout mouse as
a model system [20, 46] for FSGS to study the regulatory
programmes of cathepsin L action.

So far, it has already been shown that TGF-beta
levels are massively elevated in C2d2ap knockout mice
[47–49]. Further studies revealed that TGF-beta drives
the nuclear relocation of dendrin resulting in enhanced
TGF-beta-mediated apoptosis of podocytes [50]. By
combining transgenic animal models and podocyte cell
culture techniques, Reiser et al. could now demonstrate
that CD2AP directly regulates the TGF-β1-dependent
translocation of dendrin from the slit diaphragm (SD) to
the nucleus. Interestingly, nuclear dendrin was identified
as a transcription factor to promote expression of cyto-
solic cathepsin L. As a consequence of elevated TGFβ
levels and consecutively increased expression of cathep-
sin L, the podocyte cytoskeleton is affected by proteo-
lytical degradation of synaptopodin and dynamin (for
schematic illustration, see Figure 3). However, cathepsin
L does not only proteolyse dynamin and synaptopodin,
but also CD2AP—thereby promoting a positive feed-
back loop with a sustained action of cathepsin L and an
increased apoptotic susceptibility of podocytes to TGF-
β1 [51]. As CD2AP is an integral part of the slit dia-
aphragm, these findings underline once more the unique
signalling properties of this specialized cell–cell junc-
tion. In summary, under physiological conditions, the
SD requires intact CD2AP, which appears to act as a
kind of gatekeeper as it anchors transcription factors (e. g.
dendrin) to the plasma membrane. Any assault upon
CD2AP (e.g. mutations or enzymatic destruction) might affect its stabilizing function and result in the translocation of dendrin to the nucleus subsequently sensitizing podocytes towards injury (see Figure 3).

Altogether, Reiser et al. provide a novel concept linking the slit diaphragm to the regulation of podocyte proteolytic programmes [51]. Future studies will have to examine the role of this regulatory link for its role in human glomerular diseases.

mTOR signalling balances podocyte homeostasis and disease. Mammalian target of rapamycin (mTOR) represents an evolutionarily conserved protein kinase regulating an array of essential cellular processes, including translation, transcription and autophagy. mTOR forms two distinct functional complexes, termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), whereby mTORC1 is sensitive to rapamycin and consists of multiple components including Raptor. Recent genetic studies
Role of mTOR for podocyte maintenance and disease. Different extracellular stimuli result in altered mTOR activity in the podocyte. While mTOR is responsible for podocyte maintenance under physiological situations, excessive activation of this pathway, in conditions like diabetes, lead to maladaptive responses finally resulting in glomerulosclerosis.

could now dissect the role of the mTOR complexes in podocyte maintenance and disease [52, 53]. mTORC1 centrally regulates the podocyte growth, hypertrophy and de-differentiation whereby the temporospatial context of mTOR activation appears to define the outcome of mTOR action in podocytes: on the one hand, mTOR is required for podocyte development and podocyte maintenance. On the other hand, in glomerular diseases such as diabetic nephropathy mTOR is persistently hyper-activated and ultimately causes podocyte loss, glomerulosclerosis and disease progression [52, 53] (Figure 4). These studies thereby might explain the conflicting results that have been obtained with mTORC1 inhibition in the past by preventing glomerular diseases in many animal models, while also causing significant proteinuria in some patients [54]. In summary, these studies indicate that mTOR is a central regulator of podocyte function and further emphasize that a detailed understanding of context-dependent mTOR function is necessary for directed manipulation of mTOR activity to possibly prevent glomerular diseases.

**suPAR as a circulating FSGS factor.** A study with potentially outstanding clinical implications is the identification of soluble urokinase receptor (suPAR) as a factor involved in the pathogenesis of FSGS [55]. The basis for this work was the clinical observation that in close to 30% of all transplanted FSGS cases, the disease reoccurs in the transplanted graft [56]. As plasmapheresis or immunoadsorption attenuates the course of the disease in many cases, a soluble and circulating factor has been assumed for a long time. Based on their previous work, where they identified the urokinase receptor as an important player in podocyte homeostasis [57], Reiser et al. hypothesized that the soluble form of uPAR might be an FSGS factor candidate regulating podocyte foot process motility. One striking feature of this study was the confirmation that suPAR is not only elevated in serum of two-thirds of FSGS patients, but also correlated with the risk of FSGS recurrence after transplantation. Mechanistically, the authors could show that increased levels of suPAR (or serum of FSGS patients) resulted in beta-3 integrin activation in human podocytes, which was interpreted by the authors as a first step in the pathogenesis of FSGS. Future studies will have to determine whether therapeutic approaches targeting suPAR will change the clinical course of FSGS before and after transplantation.

**Role for parietal cells in the pathogenesis of FSGS.** Besides the loss of podocytes, progressive sclerosis represents a hallmark of FSGS. Moeller et al. could now demonstrate by elegant genetic tracing studies that parietal epithelial cells (PECs) participate in the formation of sclerotic lesions [58]. Interestingly, the loss of podocytes triggers a focal activation of PECs, which subsequently form cellular adhesions with the capillary tuft. At the site of these adhesions, PECs can migrate onto the capillary tuft, where they contribute to fibrotic lesions. These findings might lead to novel therapeutic applications aiming to prevent the profibrotic dysregulation of PECs.

**Conclusion and future perspectives**

In the field of glomerular research, focal segmental glomerulosclerosis still appears as a chameleon in terms of its complex and kaleidoscopic pathophysiological mechanisms [1]. Nevertheless, insights from hereditary forms of FSGS have contributed to the currently accepted concept that podocytes are the pacemaker of this disease and diverse aspects of podocyte dysfunction can contribute to the development of FSGS. In this review, we have presented very recently published examples of significant clinical relevance identifying novel molecular mechanisms in the pathogenesis of FSGS. These studies highlight the importance of the fine tuning of the podocyte cytoskeleton, the impact of metabolic signalling pathways and the critical role of systemic soluble factors for podocyte homeostasis. In addition, we have learned that the podocentric view on FSGS over the last decade has to be extended on PECs. Some of these findings might potentially change our future diagnostic and treatment strategies of FSGS. However, there is still a lot to learn in terms of understanding which distinct signals or pathways are integrated by podocytes and PECs. Concurrent development of new high throughput sequencing techniques will further enable us to identify novel FSGS genes and focus on specific targets responsible for the development of FSGS. Combining traditional hypothesis-driven research as well as unbiased screening approaches will further deepen our understanding of the podocyte and will hopefully translate into novel therapeutic approaches within this decade.

**Acknowledgements.** We apologize to the colleagues whose work has not been cited because of length restrictions. We thank all members of our laboratory for their support and helpful discussions.

**Funding.** This work is supported by grants to T.B.H. (DFG, BMBF GerontoSys II - NephAge (031 5896A) and Else Kröner Foundation), as well as by the Excellence Initiative of the German Federal and State Governments (EXC 294).

**Conflict of interest statement.** None declared.
References

Programmed necrosis in acute kidney injury

Andreas Linkermann¹, Federica De Zen¹, Joel Weinberg², Ulrich Kunzendorf¹ and Stefan Krautwald¹

¹Division of Nephrology and Hypertension, Christian-Albrechts-University, Kiel, Germany and ²Division of Nephrology, Department of Internal Medicine, Veterans Affairs Ann Arbor Healthcare System, University of Michigan, Ann Arbor, MI, USA

Correspondence and offprint requests to: Ulrich Kunzendorf; E-mail: kunzendorf@nephro.uni-kiel.de

Abstract
Programmed cell death (PCD) had been widely used synonymously to caspase-mediated apoptosis until caspase-independent cell death was described. Identification of necrosis as a regulated process in ischaemic conditions has recently changed our understanding of PCD. At least three pathways of programmed necrosis (PN) have been identified. First, receptor-interacting protein kinase 3 (RIP3)-dependent necroptosis causes organ failure following stroke, myocardial infarction and renal ischaemia/reperfusion injury. Necroptosis can be mediated either by a large intracellular caspase-8-containing signalling complex called the ripoptosome or by the RIP1–RIP3-containing necroptosome and is controlled by a caspase-8/FLICE inhibitory protein heterodimer in the latter case. Second, mitochondrial permeability transition mediates apoptotic or necrotic stimuli and depends on the mitochondrial protein cyclophilin D. The third PN pathway involves the poly(ADP-ribose) polymerase-calpain axis that contributes to acute kidney injury (AKI). Preclinical interference with the PN pathways therefore raises expectations for the future treatment of ischaemic conditions. In this brief review, we aim to summarize the clinically relevant PCD pathways and to transfer the basic science data to settings of AKI. We conclude that pathologists were quite right to refer to ischaemic kidney injury as ‘acute tubular necrosis’.

Keywords: AKI; necroptosis; programmed cell death; RIP1; RIP3

Introduction

From apoptosis to programmed necrosis
Caspase-dependent apoptosis is referred to as ‘extrinsic’ if it is triggered by death receptors that are members of the tumour necrosis factor receptor (TNFR) superfamily, such as Fas, TNFR1, TRAILR, FN14 and others [1, 2]. These pathways critically involve caspase-8 in mice and both caspase-8 and caspase-10 in humans. ‘Intrinsic’ apoptosis is independent of death receptors and requires the release of cytochrome c from the mitochondrial intermembrane space. Extrinsic and intrinsic apoptosis signalling pathways are interconnected.

The commonly used term ‘programmed cell death’ (PCD) had been used synonymously to apoptosis until caspase-independent cell death (CICD) was discovered [3]. Within CICD, programmed necrosis (PN) is currently being intensively investigated and our understanding of PCD in general is increasing at an extraordinary pace. The fact that a necrotic cellular phenotype is the result of a genetically determined programme is widely accepted in the basic science community, but the translation into clinical nephrology has yet to be fully performed. From the clinician’s point of view, PN opens the door for future therapeutic interference with these pathways, once they are understood and safe inhibitors are generated.

Classical apoptosis
The apoptotic phenotype is characterized by cellular shrinkage, nuclear condensation and membrane blebbing. The extrinsic and intrinsic signalling pathways are known to mediate this characteristic morphology via the activation of caspases (Figure 1).

The extrinsic or death receptor-mediated pathway results in trimerization of death receptors and intracellular formation of the DISC (see Table 1 for common abbreviations) which allows association of adapter molecules, such as TRADD and FADD, which subsequently recruit pro-caspase-8 and FLIP isoforms through interactions of...