Cell Cycle and Glomerular Disease: A Minireview

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Key Words

Cell cycle · Kidney · Glomerulus · Proliferation · Podocyte · Mesangial cell · Progressive glomerulosclerosis · Hypertrophy · Apoptosis

Abstract

Globally, glomerular diseases are a leading cause of chronic and end-stage renal disease. In the mature glomerulus, under normal conditions, glomerular cells have a low turnover rate. However, in disease, a variety of pathophysiological stimuli can lead to disturbances in glomerular cell biology, including toxins, immune-mediated stresses, metabolic derangements, drugs, infections, hemodynamic changes, growth factors, and cytokines. Not only does the form of injury govern the histologic and clinical manifestations of disease, but also the nature of the response to injury. This response to injury is largely cell-type specific, and the glomerulus represents a rare microcosm of the larger organism in which one can study the cellular responses of three very distinct cell types: mesangial cells, visceral epithelial cells or podocytes, and endothelial cells. These cells can undergo several cell fates in response to injury, including proliferation, de-differentiation, hypertrophy, senescence, apoptosis, or necrosis. The regulation of these responses occurs at the level of the cell cycle, coordi-

nated by positive regulators, cyclins and cyclin-dependent kinases, and negative regulators, cyclin-dependent kinase inhibitors. There is now a large body of literature confirming the importance of cell cycle regulatory proteins in the glomerular cellular response to injury. The recent advances in cell cycle biology in diseases of the mesangial cell and the podocyte are the focus of this minireview.

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Introduction

Glomerular diseases are a leading cause of chronic and end-stage kidney failure worldwide. Diseases of the glomerulus have been divided clinically into two primary groups based on the presenting clinico-pathologic syndrome: nephritic versus nephrotic. An alternate classification, preferred by the authors, characterizes glomerular diseases according to the principal cell type injured. Glomerular diseases are considered consequences of injury primarily to the three cell types existing within the glomerular tuft: mesangial cells, podocytes (visceral epithelial cells), and endothelial cells. In addition to cell type, the form of injury determines the response to injury, ultimately governing the histologic and clinical manifestations of disease.

Table 1. Cell cycle regulatory proteins in glomerular disease

Experimental model	Cell type	Cyclin	CDK	CDK Inhibitor
<i>Proliferation</i>				
In vitro exposure to mitogens (PDGF, BFGF)	MC	↑D1, ↔E, ↑A	↑CDK4, ↑CDK2	↑p21, ↓p27, ↔p57
Thy1, early phase	MC	↑D1, ↑↑↑A	↑CDK4, ↑↑↑CDK2	↑p15, ↑p21, ↓↓p27
Remnant kidney	MC	↑E	↑CDK2	
Anti-glomerular antibody	Podocyte	↑A	↑CDK2, ↓CDK5	
PHN	Podocyte	↑A	↑CDK2	↑↑↑p21, ↑↑↑p27 ↔p15, ↔p57
Collapsing glomerulopathy	Podocyte	↑A, ↓D1	↓CDK5	↓p27, ↓p57, ↓p21
<i>Hypertrophy</i>				
In vitro exposure to high glucose	MC	↑D1	↑CDK4, ↔CDK2	↑p21, ↑p27
In vitro exposure to TGF-β	MC			↑p21, ↑p27
In vitro exposure to CTGF	MC			↑p15, ↑p21, ↑p27
STZ-diabetes, BBdp rat (type 1)	MC	↔E, ↔A	↔CDK2	↑↑↑p21, ↑p27
db/db rat, Zucker rat (type 2)	MC		↔CDK2	↑↑↑p27
Mechanical stretch	Podocyte	↓D1, ↓A, ↓B1	↓Cdc2	↑p21, ↑p27, ↑p57
ZDF-fa/fa rat (type 2)	Podocyte			↑p27, ↑p21, ↔p57
<i>Apoptosis</i>				
PHN	Podocyte	↑A	↑CDK2	↑p21, ↓p57
Anti-glomerular antibody	Podocyte	↑A	↑CDK2	↑p21, ↑p27, ↓p57
Mechanical stretch	Podocyte	↑D1, ↑A, ↑B1	↑Cdc2	↑p21, ↑p27, ↑p57
Thy1, resolution phase	MC	↑E, ↑A	↑CDK2	↓p27

PDGF = Platelet-derived growth factor; BFGF = basic fibroblast growth factor; MC = mesangial cell; PHN = passive Heymann nephritis; STZ = streptozotocin; TGF-β = transforming growth factor β; CTGF = connective tissue growth factor; Thy1 = Thy1 mesangial proliferative glomerulonephritis.

↑ = Mild increase; ↑↑↑ = marked increase; ↔ = no change; ↓ = mild decrease; ↓↓↓ = marked decrease.

In response to injury, glomerular cells may undergo several cell fates, including proliferation, de-differentiation, hypertrophy, senescence, apoptosis, or necrosis. However, common to these outcomes is their ultimate regulation at the level of the cell cycle by specific cell cycle regulatory proteins (table 1). Although first described and identified for their roles as coordinators of cell cycle progression and proliferation, recent research has characterized a more extensive involvement of cell cycle regulatory proteins in other fundamental biologic processes, including development, differentiation, hypertrophy, and apoptosis.

Glomerular cell cycle control is particularly fascinating given the divergent responses of the resident cell types to injury. Due to space limitation, the authors are unable to provide a detailed background on cell cycle regulatory proteins, and the reader is directed to suggested references [1, 2] and to figure 1. Briefly, specific cell cycle regulatory proteins govern each phase of the cell cycle. Progression, from the quiescent phase (G₀) through DNA

synthesis (S phase) and mitosis (M phase), requires the activation of cyclin-dependent kinases (CDK) by specific cyclins. Two families of CDK-inhibitors bind to and inhibit cyclin-CDK complexes, thereby preventing cell cycle progression. Recent advances in cell cycle biology in diseases of the mesangial cell and the podocyte will be reviewed.

Mesangial Cell's Response to Injury

The mesangial cell's roles in the glomerular capillary include (i) structural support of the capillary tuft, (ii) glomerular hemodynamic modulation, and (iii) phagocytosis of macromolecules and immune complexes. An array of glomerular diseases is distinguished by mesangial cell injury, including IgA nephropathy, lupus nephritis, deposition diseases such as amyloidosis, membranoproliferative glomerulonephritis, and diabetic nephropathy. Typical mesangial cell fates that are regulated by cell cycle

Fig. 1. The cell cycle. With mitogenic stimuli, quiescent cells in G₀ re-enter the cell cycle at early G₁, followed by S phase, during which DNA synthesis occurs. During G₂ phase, cell growth continues and proteins are synthesized in preparation for entry into the M phase, where mitosis occurs. The transition from late G₁ into the S phase determines the cell's growth characteristics. G₁ arrest results in hypertrophy; G₁ exit is associated with apoptosis; and G₁/S transition results in DNA synthesis and proliferation. Cyclin-dependent kinases (CDKs) and cyclins act as positive regulators. CDKs, a family of serine-threonine kinases, are constitutively expressed throughout the cell cycle. Each CDK complex contains one of many acti-

vating subunits, cyclins, whose levels fluctuate throughout the cell cycle. The cyclin-CDK complexes are regulated by multiple phosphorylation and dephosphorylation events and by several subunits named CDK-inhibitors. Two classes of CDK-inhibitors have been identified: the INK4 family and the Cip/Kip family. Individual CDK-inhibitors are named according to their molecular weights. For simplicity, the Cip/Kip family is represented as strictly inhibitory. However, this family of CDK-inhibitors actually plays dual roles as both activators and inhibitors at different stages of the cell cycle [1, 2].

proteins include proliferation, apoptosis, and hypertrophy. For a summary of the mesangial cell's response to injury, see figure 2.

Mesangial Cell Proliferation

Mesangial cell proliferation underlies, and likely causes, an accumulation in extracellular proteins and subsequent glomerular scarring [3]. Thus, understanding the mechanisms governing proliferation is critical. Mesangial proliferative glomerulonephritis (Thy1 model), induced in rats by an antibody directed against the mesangial cell Thy1 antigen, offers a useful model to examine the regulation and consequences of mesangial cell proliferation

[4]. In this model, the initial complement-dependent mesangiolysis is followed by a phase of marked mesangial proliferation, a response correlated with increases in cyclin D1, cyclin A, CDK4, and CDK2 [5]. The level and activity of CDK2, normally present in low abundance in quiescent mesangial cells, are also augmented in other experimental models characterized by mesangial proliferation, including the non-immune-mediated remnant kidney model, and in cultured mesangial cells exposed to mitogens [5].

To define better the biological significance of increased CDKs, the ATP binding site competitor roscovitine, a potent inhibitor of Cdc2, CDK2, and CDK5, was used to

Fig. 2. Mesangial cell's response to injury. In response to injury, the mesangial cell can undergo several cell fates, including apoptosis, proliferation, and hypertrophy.

inhibit CDK activity in experimental mesangial proliferative glomerulonephritis. Roscovitine, when administered either immediately after disease induction (i.e., prior to the onset of mesangial cell proliferation) or once mesangial proliferation was already established, significantly reduced mesangial cell proliferation [6]. Moreover, reduction in proliferation was associated with reduced glomerular extracellular matrix proteins (collagen IV, laminin, fibronectin), and improved renal function. Subsequent studies have shown that roscovitine also ameliorated immune-mediated glomerulonephritis induced by the administration of an anti-glomerular antibody in mice. These findings suggest that amplification or overexpression of cell cycle kinases may play a critical role in inflammatory glomerulonephritis. These cell cycle kinases may be potential therapeutic targets in diseases characterized by glomerular cell proliferation. However, given the promiscuous targeting of kinases by roscovitine, including potentially non-cell cycle kinases and kinases in unaffected and normal cells, significant toxicity may result. To reduce potential toxicities, there are new generations of CDK-inhibiting drugs in development, specifically for treatment of malignancies, which may be applicable in future therapy for glomerular proliferative diseases.

A role for specific CDK-inhibitors has also been shown in mesangial cells [5, 7]. In quiescent mesangial cells, p27^{Kip1} is expressed constitutively, whereas p21^{Cip1} and p57^{Kip2} are barely detected. Immune-mediated injury in experimental mesangial proliferative glomerulonephritis leads to a striking decrease in p27^{Kip1} levels. Yet, during the resolution phase of disease, there is de novo synthesis of p21^{Cip1}, concomitant with a decrease in proliferation. To explore further the role of p27^{Kip1}, experimental glomerulonephritis was induced in p27^{Kip1}−/− mice. The onset of glomerular cell proliferation occurred earlier, and the magnitude of proliferation was greater, in nephritic p27^{Kip1}−/− mice compared with nephritic p27^{Kip1}+/+ mice. Increased proliferation was coupled with extracellular matrix expansion and deterioration in renal function [8]. Taken together, these studies show that p27^{Kip1} regulates the onset and magnitude of mesangial cell proliferation and, therefore, matrix accumulation and subsequent decline in renal function.

Mesangial Cell Apoptosis

Studies have shown that apoptosis is an essential mechanism in normalizing mesangial cell number in the reparative phase of injury. Nevertheless, the cellular pathways linking these opposing responses, proliferation and

apoptosis, remain poorly defined. Still, cell cycle proteins are predicted to have a crucial role in both processes, as many cells undergoing apoptosis have re-entered the cell cycle before exiting by apoptosis.

The resolution phase of mesangial proliferative glomerulonephritis is typified by mesangial cell apoptosis, a process that peaks when the levels of p27^{Kip1} are at their lowest [5]. Furthermore, in both glomerulonephritis and ureteral obstruction, there is an increase in glomerular cell proliferation following injury, which is associated with a marked increase in glomerular cell apoptosis in p27^{Kip1}–/– mice when compared with wild-type controls [8]. Moreover, apoptosis was also increased in p27^{Kip1}–/– mesangial cells in culture after exposure to apoptotic triggers, with the reconstitution of p27^{Kip1} levels by transfection rescuing the cells from apoptosis [9]. In addition, in rat mesangial cells, apoptosis was increased after treatment with anti-p27^{Kip1} antisense oligonucleotides [9]. Taken together, these results confirm the importance of p27^{Kip1} in both the regulation of proliferation and the protection from apoptosis. Thus, the CDK-inhibitor p27^{Kip1} has a critical role in governing overall mesangial cell number.

Mesangial Cell Hypertrophy

A hallmark of diabetic nephropathy is mesangial cell hypertrophy. On entry into G₁, cells normally undergo a physiologic increase in protein synthesis before S phase DNA synthesis. In hypertrophic cells, an increase in protein content is not matched by a concurrent increase in DNA. Consequently, one mechanism underlying cellular hypertrophy is cell cycle arrest at the G₁/S checkpoint, leading to increased protein to DNA ratio (the biochemical definition of hypertrophy). Studies have shown that glomerular hypertrophy predicts, and likely causes, increased extracellular matrix proteins.

In vitro, upon exposure to high glucose, mesangial cells enter the cell cycle and exhibit a biphasic growth response [10]. The initial proliferative phase is followed by G₁ arrest, with progressive hypertrophy. Hyperglycemia increases the expression of cyclin D1 and the activation of CDK4, evidence of cell cycle entry [11]. Recent studies have shown a role for CDK-inhibitors in mesangial cell hypertrophy. Hyperglycemia increases the levels of both p21^{Cip1} and p27^{Kip1} in cultured mesangial cells, and antisense oligonucleotides to p21^{Cip1} or p27^{Kip1} reduce the hypertrophic effects of hyperglycemia. Fan and Weiss [12] recently showed that p21^{Cip1} antisense oligonucleotide leads to a dose-dependent reduction of hyperglycemia-induced hypertrophy in human mesangial cells. Fur-

thermore, in p21^{Cip1}–/– and p27^{Kip1}–/– mesangial cells in culture, hypertrophy is not induced by hyperglycemia. Providing further support for the role of p27^{Kip1} in hyperglycemia-induced hypertrophy, the reconstitution of p27^{Kip1} by transfection in p27^{Kip1}–/– mesangial cells restores the hypertrophic phenotype [13, 14]. More recently, studies showed that both p21^{Cip1} and p27^{Kip1} are required for maximal mesangial cell hypertrophy induced by transforming growth factor (TGF)-β [12]. Taken together, following an initial entry into the cell cycle upon exposure to high glucose, the subsequent increase in CDK-inhibitors leads to hypertrophy by arresting the cell cycle before S phase, thereby preventing the DNA replication that typically follows the increase in protein content.

These findings have been corroborated in experimental models of type 1 (streptozotocin-induced mouse model) [15] and type 2 (*db/db* mouse) diabetic nephropathy. Diabetic p21^{Cip1}–/– mice are protected from glomerular hypertrophy and development of progressive renal failure [16]. Diabetic p27^{Kip1}–/– mice exhibit only mild mesangial expansion without glomerular hypertrophy, despite an increase in glomerular TGF-β [17]. Taken together, the cell culture and experimental models show that p21^{Cip1} and p27^{Kip1} play a critical role in mediating diabetes-associated glomerular hypertrophy and the consequential increase in matrix proteins, associated with the deterioration in renal function.

The Podocyte's Response to Injury

The podocyte is a highly specialized and terminally differentiated epithelial cell overlying the outer aspect of the glomerular basement membrane, whose complex cytoarchitecture determines its elaborate function as a critical component of the glomerular filtration barrier. In this capacity, podocytes function to prevent urinary protein leakage, maintain the integrity of the underlying glomerular capillary loops, oppose intracapillary hydrostatic pressure, and synthesize the glomerular basement membrane [18]. Thus, the mechanisms governing podocyte differentiation, and therefore proliferation, are essential to the normal function of this cell. Podocyte response to injury includes effacement, detachment, and glomerulosclerosis. Figure 3 summarizes the podocyte's response to injury at the level of the cell cycle.

Podocyte Proliferation and Differentiation

Due to its highly differentiated state, analogous to the neuron, the podocyte typically does not proliferate in

Fig. 3. Podocyte's response to injury. In response to injury, the normally quiescent and highly differentiated podocyte can undergo several cell fates, depending on the type of injury. De-differentiation, due to a decrease in CDK-inhibitors, permits cell cycle re-entry, and proliferation occurs when cyclins and CDKs are activated. In contrast, most forms of injury lead to a decrease in podocyte number (podocytopenia) because of apoptosis, hypertrophy, lack of proliferation, or presence of DNA damage. Levels of cyclins, CDKs, CDK-inhibitors, or other checkpoint proteins may determine which of these fates podocytes undergo.

vivo. The tight regulatory control of cell cycle quiescence in podocytes, sustained by a strong and specific upregulation of CDK-inhibitors, may guarantee their highly specialized structure and function and is a prerequisite for terminal differentiation. In sharp contrast to the proliferative capacity of neighboring mesangial and endothelial cells in response to injury, as podocytes detach, apoptose, and necrose following injury, there is a reduction in podocyte number, associated with a relative lack of proliferation. The consequential podocyte loss results in areas of denuded basement membrane, ultimately leading to proteinuria, the development of scarring, known as focal glomerulosclerosis, and progressive deterioration in kidney function.

During glomerulogenesis, immature podocyte precursor cells proliferate. In the S-shaped body stage of glomerulogenesis, presumptive podocytes express proliferative tissue markers, including proliferating cell nuclear antigen (PCNA) and Ki-67, together with cyclins A and B1. In both mouse and human glomerulogenesis, immunostaining for p27^{Kip1} and p57^{Kip2} is absent at this early stage. With transition to the capillary loop state, a fundamental phenotypic switch occurs with cessation of mitotic activity, reorganization of the cytoskeleton, expression of podocyte maturity markers (including synaptopo-

din and glomerular epithelial protein-1), and development of foot-process interdigitation. In parallel to these changes, there is a decrease in levels of cell cycle promoters (cyclins and CDKs), and a reciprocal upregulation of p27^{Kip1} and p57^{Kip2}. These events coincide with the cessation of proliferation, as podocytes become terminally differentiated and quiescent – necessary requirements for normal function [7, 19]. However, null mice have shown that p21^{Cip1}, p27^{Kip1}, and p57^{Kip2} alone are not essential for glomerular development.

In the mature glomerulus, podocytes do not readily proliferate under normal conditions or in response to a wide variety of injuries. The podocyte's inability to proliferate in many diseases is most likely the consequence of robust expression, or even upregulation, of the CDK-inhibitors p21^{Cip1}, p27^{Kip1}, and p57^{Kip2} with disease progression. However, in idiopathic collapsing glomerulopathy and HIV-associated nephropathy, dysregulation of the differentiated phenotype and proliferation can occur [18]. In collapsing glomerulopathy, Barisoni et al. [20] showed that podocyte number was increased in affected tissue and that, accompanying this proliferation, there was associated de-differentiation (loss of maturity markers, including synaptopodin) and phenotypic dysregulation (loss of Wilm's tumor protein). In proliferating podocytes,

cytes overlying areas of collapse, p57^{Kip2} and p27^{Kip1} are reduced and cyclin A increased. This form of non-reparative proliferation is characterized by the podocyte's escape from cell cycle blockade, leading to a disruption of glomerular architecture followed by rapidly declining renal function.

Further support of a critical role for p21^{Cip1} and p27^{Kip1} in limiting the proliferative response of podocytes in many disease processes comes from studies in p21^{Cip1}−/− and p27^{Kip1}−/− mice. With the administration of an anti-glomerular antibody, podocyte de-differentiation and proliferation increased in p21^{Cip1}−/− and p27^{Kip1}−/− mice compared with wild-type mice [21]. Podocyte proliferation was accompanied by extracellular matrix accumulation with associated decline in renal function. Inhibiting podocyte proliferation with the CDK-inhibitor roscovitine leads to reduced glomerulosclerosis and improved renal function. Taken together, proliferation is detrimental to the normal biological function of podocytes, and the decision to proliferate is likely controlled by a reduction in CDK inhibition.

Podocyte DNA Damage and Lack of Proliferation Abnormalities in DNA Synthesis

The passive Heymann nephritis (PHN) model, induced by the administration of an antibody reactive against the rat podocyte Fx1A antigen, has many similarities to human membranous nephropathy. In both diseases, complement activation, with the assembly of the terminal complement components to form C5b-9, the membrane attack complex, is the principal mediator of glomerular injury. Nucleated cells are relatively resistant to C5b-9 attack and may actually become activated by it, leading to release of inflammatory mediators, calcium, oxidants, growth factors, matrix components, and proteases. C5b-9 is also central to mesangial cell injury in mesangial proliferative glomerulonephritis. However, in mesangial cells, C5b-9 attack leads to DNA synthesis and a marked proliferative response [22]. In contrast, although podocytes also re-engage the cell cycle following C5b-9 injury, as demonstrated by an increase in cyclins and CDKs required for proliferation (cyclin A and CDK2 protein levels increase), they undergo limited DNA synthesis, and podocyte number typically remains constant, and even decreases with time. These findings are consistent with a defect in DNA synthesis (S phase of cell cycle). Indeed, the levels of p21^{Cip1} and p27^{Kip1} increase specifically in podocytes after induction of PHN, supporting the notion that increased CDK-inhibitors prevent podocyte cell cycle progression, and therefore, DNA synthesis.

Abnormalities in Mitosis

An important question is whether abnormalities in podocyte mitosis can account for additional or alternate mechanisms for post-injury limitation of proliferation. Recent studies in PHN rats showed that podocytes do increase their levels of cyclin B-Cdc2, the cell cycle proteins required for mitosis. However, mitosis is infrequent. This suggests an abnormality in the G₂/M checkpoint, which precedes mitosis. Indeed, sublytic C5b-9 injury causes DNA damage in podocytes in vitro and in vivo, accompanied by a delay or inhibition in entering mitosis, i.e., G₂/M phase arrest. In contrast, upon exposure to sublytic C5b-9, DNA damage is not seen in mesangial cells. In podocytes, DNA damage is associated with a marked increase in cell cycle checkpoint proteins checkpoint kinase 1 (Chk-1), checkpoint kinase 2 (Chk-2), tumor suppressor p53, and p21^{Cip1}, which converge on and inhibit the G₂/M phase checkpoint, thus limiting mitosis [23]. These observations support the hypothesis that p21^{Cip1}, a downstream effector of p53 in the DNA damage checkpoint pathway, plays a critical role in limiting proliferation.

When p21^{Cip1}−/− mouse podocytes were exposed to sublytic C5b-9, at time points beyond 6 h, there were increased levels of DNA damage compared to p21^{Cip1}+/+ podocytes. This greater degree of DNA damage in p21^{Cip1}−/− podocytes was not accompanied by an increase of DNA damage checkpoint proteins (including Chk-1, Chk-2, p53, and growth arrest and DNA damage inducible gene (GADD45α)) as was seen in the wild-type podocytes [unpubl. data] (for an overview of G₂/M cell cycle checkpoints, see figure 4) [24]. Taken together, p21^{Cip1} likely plays a critical role in the podocyte's cell cycle arrest in response to DNA damage and in the regulation of DNA damage response effectors.

DNA damage may also account for the inability of podocytes to proliferate in response to injury in other disease models. In the puromycin aminonucleoside nephropathy animal model of minimal-change disease progressing to focal segmental glomerulosclerosis, DNA damage has been documented and data supports that this is mediated by reactive oxygen species. Puromycin aminonucleoside also induced DNA damage in cultured mouse podocytes, accompanied by an upregulation of p21^{Cip1}, GADD45α, p53, Chk-1, Chk-2, and the repair enzymes DNA polymerase-β and AP-endonuclease [unpubl. data]. Therefore, in both immune- and non-immune-mediated podocyte injury, in vitro and in vivo, the podocyte's lack of proliferation may be due to injury-induced DNA damage with upregulation of cell cycle checkpoint proteins, including p21^{Cip1}, leading to G₂/M phase arrest.

Fig. 4. Cell cycle checkpoints and the limitation of podocyte mitosis. Cell cycle checkpoint proteins, enforcing the orderly execution of the cell cycle, are response elements that operate to maintain the fidelity of DNA replication, repair, and, ultimately, cell division. The proteins that act as sensors of damaged DNA, not yet fully characterized, serve to activate signaling pathways that lead not only to cell cycle arrest, but also to activation of DNA repair or other cell fate pathways. The immediate targets of the sensor proteins are two related protein kinases, designated ATM (mutated in ataxia telangiectasia) and ATR (ATM and Rad3-related), which are activated in response to DNA damage. Two checkpoint kinases, Chk-1 and Chk-2, link ATM and ATR to downstream effectors, including p53 and the Cdc25 phosphatase family. Phosphorylation of Cdc25 (leading to inactivation and nuclear export) and p53, with subsequent transcriptional induction of downstream targets p21^{Cip1} and GADD45 α , leads to a block of Cdc2-cyclin B and G₂/M cell cycle arrest. These events prevent podocyte mitosis, and therefore limit proliferation [24].

Recently, Milovanceva-Popovska et al. [25] examined the role of roscovitine in PHN. Treatment with roscovitine caused a statistically significant reduction in glomerular mitotic figures, bromodeoxyuridine-positive cells, and PCNA-positive cells. In addition, cyclin D1 decreased in the roscovitine group. This further supports that even in podocyte-injury models that have conventionally been considered non-proliferative, in response to injury, the cells may re-engage the cell cycle but are unable to successfully undergo mitosis/cytokinesis. It is currently unclear whether blocking the pro-proliferative cell cycle regulatory proteins in a non-proliferative disease model will prove beneficial in reducing progressive renal deterioration.

Podocyte Hypertrophy

Recent studies have shown that podocytes also undergo hypertrophy following injury, which may be the podocyte's attempt to cover denuded areas of glomerular basement membrane following the loss of neighboring cells [18]. A final common pathway to progressive glomerular scarring is increased intraglomerular capillary pressure, which results in increased mechanical stretch on resident glomerular cells. Applying mechanical stretch to cultured mesangial cells activates a variety of pro-proliferative sig-

naling pathways, resulting in proliferation [26]. In contrast, mechanical stretch decreases podocyte proliferation [27]. Recent studies in cultured podocytes show that mechanical stretch decreases cyclins D1, A, and B1, and Cdc2, and increases CDK-inhibitors p21^{Cip1} and p27^{Kip1} [27]. Interestingly, exposing cultured mouse podocytes to cyclic mechanical stretch also induces a hypertrophic phenotype in podocytes [28]. The mechanisms may be due to p21^{Cip1}. In contrast to the growth arrest and hypertrophy induced in wild-type cells exposed to stretch, p21^{Cip1}–/– podocytes continued to proliferate and did not hypertrophy. Thus, mechanical stretch inhibits podocyte proliferation and prompts the cell to adopt a hypertrophic phenotype, both events regulated by the CDK-inhibitor p21^{Cip1} [28].

Diabetic nephropathy is characterized by glomerular hypertrophy. Upon study of Zucker diabetic fatty rats (ZDF-*fa/fa*), a model of type 2 diabetes, Hoshi et al. [29] found that tissue from diabetic rats, by morphometric analysis, had significantly increased glomerular volume and sclerosis. This was associated with increased expression of p27^{Kip1}, predominantly localized to podocytes, and p21^{Cip1}, but unchanged levels of p57^{Kip2}. An in vitro correlate confirmed that podocytes exposed to hyperglycemia exhibited cell hypertrophy, with accompanying in-

creased levels of p27^{Kip1}, but unchanged levels of p21^{Cip1} and p57^{Kip2}. This study demonstrated that CDK-inhibitors may underlie the podocyte's tendency to undergo hypertrophy in states of hyperglycemia. Thus, in many injury models, although podocyte hypertrophy is initially compensatory in response to podocyte loss, this response is likely maladaptive and contributes to the development of glomerulosclerosis [29].

Podocyte Apoptosis

As the reduction in podocyte number is closely associated with the development of glomerulosclerosis, the biological significance of apoptosis in podocyte depletion has recently been examined. In wild-type podocytes, upon exposure to TGF- β_1 , a cytokine important in glomerular disease induction and progression, Takehiko Wada et al. found that p21^{Cip1} levels were increased, which was associated with an increase in podocyte apoptosis. To examine further the role of p21^{Cip1} in podocyte apoptosis, p21^{Cip1}−/− mouse podocytes were exposed to TGF- β_1 . Interestingly, in p21^{Cip1}−/− podocytes, no significant apoptosis was seen when compared to p21^{Cip1}+/+ cells. This finding was associated with an increase in anti-apoptotic Bcl-2 in p21^{Cip1}−/− podocytes, a finding not observed in p21^{Cip1}+/+. Restoration of p21^{Cip1} by an adenoviral transfection prevented the TGF- β_1 -induced increase in Bcl-2 expression, resulting in increased apoptosis. These findings suggest that the upregulation of anti-apoptotic Bcl-2 is suppressed in the presence of the CDK-inhibitor p21^{Cip1} under disease conditions, supporting a critical role for p21^{Cip1} in triggering apoptosis in response to injury [pers. commun.].

Novel Cell Cycle Proteins and Podocytes: CDK5

Recently, CDK5, a non-classical and novel CDK that increases in certain post-mitotic cells, with highest levels observed in the brain, has been shown to increase in podocytes during differentiation in culture and in developing fetal kidneys.

CDK5 declines in experimental diseases associated with podocyte de-differentiation and proliferation, including anti-glomerular antibody disease and HIV-associated nephropathy, implicating a role for CDK5 in maintaining the mature podocyte phenotype. Indeed, in vitro, inhibiting CDK5 activity, pharmacologically and with small interfering RNA, significantly altered podocyte shape, with a dramatic reorganization of the actin cytoskeleton. CDK5 inhibition had no effect on proliferation. Accordingly, a paradigm is developing that CDK5 is involved in maintaining the podocyte's complex morphology, critical for its highly specialized function [30].

Conclusion

A brief overview of the extensive body of literature supporting a critical role for cell cycle regulatory proteins in diseases of the glomerulus has been provided (table 1). The late changes of increased extracellular matrix and progressive glomerulosclerosis are preceded by, and closely linked to, the various fates that a cell can follow in response to injury including proliferation, apoptosis, hypertrophy, and senescence, all of which are regulated at the level of the cell cycle. The expectation is that attempts to understand the role of the cell cycle in the pathophysiology of glomerular diseases ultimately will lead to the development of novel therapeutic approaches to preserve renal function.

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An unabridged list of references is available from the authors upon request.