Cell cycle regulatory proteins in renal disease: role in hypertrophy, proliferation, and apoptosis

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Shankland, Stuart J., and Gunter Wolf. Cell cycle regulatory proteins in renal disease: role in hypertrophy, proliferation, and apoptosis. Am J Physiol Renal Physiol 278: F515–F529, 2000.—The response to glomerular and tubulointerstitial cell injury in most forms of renal disease includes changes in cell number (proliferation and apoptosis) and cell size (hypertrophy). These events typically precede and may be responsible for the accumulation of extracellular matrix proteins that leads to a decrease in renal function. There is increasing evidence showing that positive (cyclins and cyclin-dependent kinases) and negative (cyclin-dependent kinase inhibitors) cell cycle regulatory proteins have a critical role in regulating these fundamental cellular responses to immune and nonimmune forms of injury. Data now show that altering specific cell cycle proteins affects renal cell proliferation and improves renal function. Equally exciting is the expanding body of literature showing novel biological roles for cell cycle proteins in the regulation of cell hypertrophy and apoptosis. With increasing understanding of the role for cell cycle regulatory proteins in renal disease comes the hope for potential therapeutic interventions.

cyclin; cell cycle; kidney; glomerulus; mesangial cell

DURING DEVELOPMENT, KIDNEY growth is initially due to an increase in glomerular and tubular epithelial cell number because of proliferation. Thereafter, there is a physiological increase in cell size. Thus kidney size depends on cell number and on the individual cell size and mass (10). Cell number reflects the balance of new cell generation by proliferation and loss by apoptosis (99). There is very little cell turnover in the mature adult kidney under normal physiological circumstances (81). However, cell number and cell size may change after many forms of pathological renal injury. For example, glomerulonephritis can be associated with an increase in glomerular cell number due to proliferation (47), whereas increased apoptosis after ureteral obstruction and acute tubular necrosis are initially associated with a decrease in tubular epithelial cell number (59). Moreover, renal cell proliferation and apoptosis are often closely linked within a given renal cell population, and the balance of these growth and death processes ultimately determines renal cell number. Certain forms of renal injury such as diabetic nephropathy and a reduction in nephron number are associated with an increase individual cell size by hypertrophy rather than an increase in cell number (42, 90).

Thus, depending on the underlying form of renal injury, proliferation, hypertrophy, and apoptosis may contribute to the development of renal scarring in glomerular and tubulointerstitial diseases. Although such diverse processes appear to have nothing in common at the first glance, this review will show that the ultimate fate of a cell is regulated at the level of the cell cycle by a complex interaction of specific cell cycle regulatory proteins.

THE CELL CYCLE

It has been known for more than 100 years that cell proliferation is ultimately a nuclear event and is divided into different phases in what is now called the cell cycle (72, 74) (Fig. 1). Nondividing (quiescent) cells are in G0 phase and enter the cell cycle at G1, followed by the S phase, where DNA replication occurs. Cells then progress through G2, and enter mitosis (M phase).
which is further subdivided into prophase, metaphase, anaphase, and telophase, which is followed by cytokinesis (cell division). Transition from one cell cycle phase to another is a coordinated, sequential, and synchronized process that occurs with precise timing in a well-defined order to ensure that the cellular machinery is ready for DNA replication, that DNA replication occurs only once in each cycle, that DNA replication is completed before mitosis starts, and that chromosomes are replicated into identical sets in daughter cells (31).

After passing a "restriction point" in late G1, cells are no longer responsive to extracellular signals and complete the cell cycle under the control of specific cell cycle regulatory proteins. The average cell cycle time is 12 h for G1, 6 h for S, G2 lasts 6 h, and mitosis is completed in 30 min (70).

Renal injury can result in proliferation, hypertrophy, or apoptosis, which we believe are linked at the level of the cell cycle (101). Proliferation requires normal progression through the cell cycle; hypertrophy occurs when cells engage the cell cycle but cannot progress beyond late G1 (G1/S arrest); apoptosis is associated with exit from the cell cycle, which typically occurs in G1. Thus these processes may share common pathways and may explain why certain cell populations undergo proliferation and apoptosis, whereas proliferation and hypertrophy are independent events.

Cell Cycle Regulatory Proteins

The cell cycle is ultimately controlled by cell cycle regulatory proteins, which localize predominantly in the nucleus. Transition between each phase of the cell cycle is positively regulated by the kinase activity of a distinct holoenzyme, which is composed of two subunits: cyclins and their partner, cyclin-dependent kinases (CDK) (65, 112, 114) (Table 1). Cyclins have very short half-lives (<30–60 min), and levels fluctuate throughout the cell cycle (84). In contrast, CDK proteins are constitutively expressed, and levels typically remain unchanged throughout the cell cycle (57). However, CDK are posttranslationally activated on binding by a partner cyclin (118). CDK inhibitors negatively regulate cell cycle progression by inhibiting cyclin-CDK complexes, resulting in cell cycle arrest (115).

Entry of Quiescent Cells Into the Cell Cycle

Entry of quiescent cells (G0) into early G1 requires D-type cyclins (D1, D2, D3) (6), which are expressed in a cell-type-specific manner (48). D-cyclins are transcriptionally regulated, and levels are increased by specific mitogens such as growth factors (6). D-cyclin levels decrease on mitogen withdrawal (114), and growth inhibitors such as interferon (127) and transforming growth factor-β (TGF-β) (28) suppress D-type cyclin transcription.

D-type cyclins associate with and activate CDK4 and 6 in G1 (112, 113). A critical substrate for cyclin D-CDK is the 110-kDa protein product of the retinoblastoma gene (pRb) (8), which regulates G1/S transition (96, 133) (Fig. 2). pRb is hypophosphorylated during G0 and early G1 and is growth restrictive by sequestering the transcription factor E2F (133). Cyclin D phosphorylation by 10.220.33.2 on April 8, 2017 http://ajprenal.physiology.org/ Downloaded from...
lates some of the sites on pRb, but only to the point in which pRb is still hypophosphorylated, active, and growth restrictive. Cyclin E phosphorylates the remaining sites, so that pRb is now hyperphosphorylated, inactive, and growth permissive and releases E2F, which binds to the promoter regions of several target genes essential for further cell cycle progression, including immediate early genes, thymidine kinase, and dihydrofolate reductase.

G1/S Transition

The transition from late G1 into the S phase determines the cell’s growth characteristics. For example, G1 arrest results in antiproliferation or hypertrophy (90), G1 exit is associated with apoptosis (64), and G1/S transition results in DNA synthesis and proliferation (112). Cyclin E levels increase in late G1, where it associates with and activates CDK2, thereby playing a pivotal role in G1/S transition (77, 78). Cyclin E induction is less dependent on exogenous growth factors and is regulated by intrinsic factors of the cell cycle such as E2F. Cyclin E-CDK2 also phosphorylates pRb (see above), and a positive cyclin E-synthesis feedback loop exists through the phosphorylation of pRb, leading to release of E2F (Fig. 2). Recently, a novel cyclin E2 was cloned, which associates with CDK2 (32).

Cyclin A levels peak in late G1, are maximal during the S phase, and persist through G2. Cyclin A activates CDK2 (15), which is essential for DNA synthesis (29). Forced overexpression of cyclin A induces DNA synthesis, and reducing cyclin A levels prevents cell proliferation (29, 82).

Entry Into Mitosis

Although mitosis is the “final” phase of the cell cycle, it was the first phase to be carefully delineated, and from these studies has arisen much of our present understanding of cell cycle proteins (76, 89). The first cyclin identified was cyclin B, which is required for mitosis (16). Cyclin B levels fluctuate due to synthesis and degradation, whereas its partner, cdc2 (formerly called CDK1), does not (Fig. 3). Cyclin B-cdc2 activity, similar to CDK2, depends on its phosphorylation status (17).

Monomeric cdc2 is unphosphorylated and inactive. cdc2 undergoes a conformational change on binding to cyclin B, which results in the phosphorylation on threonine 14 (Thr 14), tyrosine 15 (Tyr 15), and Thr 161 amino acid residues on cdc2 (89). Phosphorylation of Thr 14 and Tyr 15 by the kinases Wee1 and Myt1 are growth inhibitory, which dominates over Thr 161 phosphorylation, which is growth activating. Consequently, the triple phosphorylated cyclin cdc2-cdc2 heterodimer is inactive. Dephosphorylation of Thr 14 and Tyr 15 by the dual-specific phosphatase cdc25 is essential for entry into mitosis. Active cdc2-cyclin B phosphorylates substrates (H1 histone, lamins, nucleolin) required for chromosome condensation, nuclear envelope breakdown, and formation of the mitotic spindle (30). On completion of mitosis, cyclin B is degraded via the ubiquitin-proteasome pathway, leading to the dissociation and inactivation of the complex (69), and cdc2 is finally dephosphorylated by kinase-associated phosphatase.

CDK Inhibitors: Negative Regulators of the Cell Cycle

Cyclin-CDK complexes are negatively regulated by cell cycle proteins called CDK inhibitors (20, 87, 115). CDK inhibitors are relatively small molecules that bind to specific cyclin-CDK complexes and in so doing inhibit their activity. There are two families of CDK inhibitors, which are based on the target cyclin-CDK they inhibit, and on shared homologous sequences. Individual CDK inhibitors are named according to their molecular weight. The INK4 family only inhibit cyclin D-CDK complexes and share an ankyrin repeat. The Cip/Kip family are more promiscuous and inhibit CDK 2, 4, and 6, and share a CDK2-binding domain (39) (Table 1).
The molecular mechanisms whereby CDK inhibitors inhibit cyclin-CDK complexes are still incompletely understood.

CIP/KIP Family of CDK Inhibitors

p21. The CDK inhibitor p21\textsuperscript{Cip1,WAF1,SDI1} (p21), a 21-kDa protein, is the founding member of the Cip/Kip family. p21 is transcriptionally regulated in a p53-dependent (18) and a p53-independent manner (19). However, p21 expression can also be modulated through posttranslational mechanisms (3). In addition to binding CDKs, p21 can also associate with PCNA, a processivity factor of DNA polymerase α, through the COOH-terminal domain, which may be sufficient for G\textsubscript{1} arrest (132). A somewhat surprising observation was that p21 expression increases during proliferation and that p21 remains bound to CDK complexes in proliferating cells (152). p21 may therefore act as a scaffold to facilitate the assembly of cyclins and CDKs required for DNA synthesis. Recent studies have demonstrated that a single p21 molecule is sufficient for CDK inhibition (38). Because p21 knockout mice do not have developmental deficiencies or tumors, it has been suggested that the role of p21 is check-point control of G\textsubscript{1}/S-phase transition rather than withdrawal from the cell cycle and differentiation (7). More recently, studies have shown that p21 inhibits G2/M phase of the cell cycle and thus may also regulate mitosis, which further distinguishes it from other members of the Cip/Kip family of CDK inhibitors.

p27. The 27-kDa protein p27\textsuperscript{Kip1} (p27) is widely expressed in nonproliferating (quiescent) renal (11, 107) and nonrenal cells (75). In contrast to p21, p27 expression is posttranscriptionally regulated by changes in protein translation and degradation through the ubiquitin proteolytic pathway (73, 130) and is also posttranslationally modified by phosphorylation (111). Thus, in contrast to p21, p27 is not regulated by p53. p27 regulates growth arrest in response to TGF-β, rapamycin, cAMP, and contact inhibition (75). The interaction of p27 with cyclin-CDK complexes is more complicated than previously thought because p27 can be both an inhibitor and a substrate for cyclin E-CDK2. Cyclin E-CDK2 may be inhibited in G\textsubscript{0} by p27. However, after growth factor-mediated activation of cyclin D-CDK4, p27 preferentially binds to these complexes and redistributes p27 to cyclin D-CDK4 results in activation of cyclin E-CDK2 with subsequent phosphorylation of p27, which enhances p27 degradation (111). The final result may be activation of more cyclin E-CDK2, which facilitates G\textsubscript{1} progression. Low levels of p27 have been shown in a variety of tumors, and p27 levels are critical in renal cell differentiation, apoptosis, proliferation, and hypertrophy (see below) (102).

p57. The CDK inhibitor p57\textsuperscript{Kip2} (p57) is expressed in many differentiated cells and in many adult tissues (153). p57 binds CDK2, 3, and 4, and overexpression leads to G\textsubscript{1} arrest (154). Further studies suggest a close cooperation of p27 and p57 to control proliferation and differentiation in multiple tissues during development (154). However, the precise function of p57 in cell cycle regulation, and in particular in the kidney, remains to be elucidated.

INK4 Family of CDK Inhibitors

The INK4 family (p15, p16, p18, p19, p20) consist of four or more ankyrin repeats and inhibit CDK4 and CDK6 complexes in G\textsubscript{1} (116). In contrast to the Cip/Kip family, INK4 members are tumor suppressor genes (12), where p15 and p16 are deleted and mutated in a variety of tumors, and the selected disruption of p19 in mice predisposes to tumor development (150). TGF-β-mediated induction of p15 blocks activation of cyclin D-CDK4 complexes by displacement of p27 from these complexes to downstream binding and inhibition of cyclin E-CDK2 heterodimers (95). The expression of INK4 CDK inhibitors may be required to maintain quiescent cells in G\textsubscript{0} (36).

CELL CYCLE REGULATION OF RENAL CELL HYPERTROPHY

What Is Hypertrophy?

An organ can increase in size at the cellular level due to an increase in cell number (increased proliferation or decreased apoptosis) or an increase in individual cell size (hypertrophy). Cell hypertrophy is defined as cell enlargement due to an increase in protein and RNA content without DNA replication (21, 22) and can be due to cell cycle-dependent or -independent mechanisms (60, 90). Normal entry into G\textsubscript{1} phase of the cell cycle is associated with increased protein and RNA synthesis, which occurs in “anticipation” of DNA synthesis in the S phase. The present paradigm suggests that hypertrophy is an active process requiring entry into the cell cycle, without progression through the S phase (134, 136) (Fig. 4). Hence hypertrophy can also be defined as G\textsubscript{0}/G\textsubscript{1}-phase arrest (91), which explains why hypertrophy and proliferation are exclusive at a single cell level. Thus certain growth factors, hormones, extracellular matrix, mechanical forces, and hyperglycemia that induce hypertrophy facilitate entry into the cell cycle. In contrast, tubular cell hypertrophy can be cell cycle independent due to the inhibition of protein degradation (25).

Glomerular Cell Hypertrophy

Glomerular cell hypertrophy occurs during many forms of chronic renal disease and may anteced the development of glomerulosclerosis (27, 42, 151). Glomerular diseases associated with glomerular hypertrophy include diabetic nephropathy (155), relapsing minimal change nephropathy (129), focal segmental glomerular sclerosis (24, 68), and a reduction of nephron mass due to disease, surgery, or congenital aplasia (120). The consequence of glomerular hypertrophy depends on the underlying disease and is considered compensatory after a decrease in renal mass such as uninephrectomy and is not therefore associated with glomerulosclerosis. In contrast, glomerular hypertrophy in diabetic nephropathy antecedes and probably underlies the development of glomerulosclerosis (93).
Diabetic nephropathy. A characteristic finding in early diabetic nephropathy is glomerular hypertrophy, which predominantly involves the mesangial cell but can also involve the glomerular endothelial cell (51, 144). This contrasts to most forms of mesangial cell injury that are associated with proliferation (47). Glomeruli from diabetic rats and cultured mesangial cells grown in high-glucose concentrations are associated with an increase in expression of immediate early-response genes (52, 108). That immediate early genes are expressed in the early G1 phase of the cell cycle (137) suggests that high glucose induces quiescent glomerular cells to actively enter the cell cycle.

Although short-term exposure of mesangial cells to high glucose levels induces a limited proliferation in vitro (13) and in vivo (149), prolonged exposure to high glucose has two effects on mesangial cell growth. First, glucose inhibits mesangial cell proliferation (142) and, second, glucose induces hypertrophy (110). Cell cycle analysis reveals glucose arrests cells in the G1 phase, an effect mediated in part by the autocrine synthesis and activation of TGF-β (142). Indeed, glomerular TGF-β expression increases in vivo during the phase of hypertrophy (109, 148), and application of neutralizing anti-TGF-β antibodies attenuates hypertrophy in diabetic mice (110).

A body of literature is emerging on the role of specific cell cycle proteins in diabetic glomerular hypertrophy (Tables 2 and 3). First, glucose-induced mesangial cell hypertrophy in vitro is not associated with an increase in the protein expression for cyclin E or CDK2 (56). We have also been unable to show a significant increase in glomerular expression for G1- and S-phase cyclins in experimental diabetic nephropathy (56), and glomerular expression of the retinoblastoma protein gene product remains in an underphosphorylated (growth-restrictive) state (104), consistent with G1/S arrest. In contrast, Huang and Preisig (44) showed that cyclin D kinase is activated during tubular epithelial hypertrophy in experimental diabetes. Taken together, these studies suggest that hyperglycemia does not typically increase the mesangial cell expression of cyclins and CDKs required for DNA synthesis.

Table 2. Expression and activity of cell cycle proteins in renal cells in vitro

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cyclin-CDK Complex</th>
<th>CDK Inhibitor</th>
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</thead>
<tbody>
<tr>
<td>PDGF, bFGF, ET-1</td>
<td>CDK4 activity (143)</td>
<td>p21 (124)</td>
</tr>
<tr>
<td>TGF-β</td>
<td>CDK2 activity (100)</td>
<td>p21, p27</td>
</tr>
<tr>
<td>SPARC</td>
<td>CDK4 activity (100)</td>
<td>p21, p27</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>CDK2 activity (56)</td>
<td>p21 (143)</td>
</tr>
<tr>
<td>Glucose</td>
<td>CDK2 activity (56)</td>
<td>p21 (143)</td>
</tr>
</tbody>
</table>

PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; ET-1, endothelin 1; TGF, transforming growth factor; SPARC, secreted protein acidic and rich in cysteine; MC, mesangial cell; PCT, proximal tubular epithelial cell; ↑, increased; ↓, decreased; ↔, no change. Ref. nos. are in parentheses.
Table 3. Cell cycle regulatory proteins in experimental renal disease

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>Cell Type</th>
<th>Cyclins</th>
<th>CDK</th>
<th>CDK Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulus Thy1</td>
<td>MC</td>
<td>D1 (146)</td>
<td>CDK4 (146)</td>
<td>p15 (146)</td>
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<tr>
<td></td>
<td></td>
<td>A (108)</td>
<td>CDK2 (108)</td>
<td>p21 (108)</td>
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<td></td>
<td></td>
<td></td>
<td>p27 (108)</td>
</tr>
<tr>
<td>Remnant GEN model</td>
<td>MC</td>
<td>E (107)</td>
<td>CDK2 (107)</td>
<td></td>
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<tr>
<td></td>
<td>GEN</td>
<td>A</td>
<td>CDK2</td>
<td></td>
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<tr>
<td></td>
<td>VEC</td>
<td>A</td>
<td>CDK2</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>A (106)</td>
<td>CDK2 (106)</td>
<td>p21 (106)</td>
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<td>p27 (106)</td>
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<td></td>
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<td></td>
<td></td>
<td>p15, p57</td>
</tr>
<tr>
<td>Tubulointerstitium</td>
<td></td>
<td>D1 (147)</td>
<td>p21 (63)</td>
<td></td>
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<tr>
<td>Ischemia</td>
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<tr>
<td>Ureteral obstruction</td>
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<tr>
<td>Cisplatinum</td>
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<tr>
<td>Glomerulus</td>
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<tr>
<td>STZ-diabetes db/db rat</td>
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<td>Uninephrectomy</td>
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GEN, glomerular endothelial cell; VEC, podocyte; PHN, passive Heymann nephritis; STZ, streptozotocin; |, mild increase; |||, marked decrease.

These studies have lead to the hypothesis that glucose-induced glomerular cell hypertrophy is regulated by CDK inhibitors at the level of the cell cycle (Fig. 4). Incubation of mouse mesangial cells in high-glucose medium stimulates p27 protein expression, independent of mRNA abundance (141). High-glucose-stimulated expression of p27 involves the activation of protein kinase C (PKC) and is partly dependent on the induction of TGF-β. The increase in p27 preferentially associates with CDK2 (141), which differs from ANG II-mediated hypertrophy, where p27 associates with cyclin D-CDK4 (143). Lowering p27 levels with specific antisense oligonucleotides, but not control missense, inhibited high-glucose-stimulated hypertrophy and facilitated cell cycle progression (141). A similar role for p27 has been shown in vivo. The glomerular levels for p27 are enhanced in db/db mice (model of type II diabetes mellitus), and the increase was restricted to mesangial cells (140). Furthermore, mesangial cells cultured from db/db mice grown in medium containing normal glucose concentrations displayed no difference in p27 expression compared with mesangial cells obtained from non-diabetic db/+ animals (140). However, raising glucose concentrations to 275–450 mg/dl induced cell cycle arrest and increased the expression of p27 (140). These studies indicate that high glucose was the primary stimulus for p27 induction.

To further characterize the role of p27 in high-glucose-induced hypertrophy, we studied cultured mesangial cells derived from p27-knockout (p27−/−) mice and control p27 wild-type (p27+/+) animals. Our preliminary studies showed that high glucose concentrations (450 mg/dl) stimulated hypertrophy in p27+/+ mesangial cells compared with control p27+/+ cells grown in normal glucose concentrations (100 mg/dl) (G.Wolf and S.J. Shankland, unpublished observations). In contrast, in p27−/− mesangial cells, high glucose concentrations caused cell cycle progression with DNA synthesis, but not cell hypertrophy. However, when p27 levels were reconstituted in p27−/− mesangial cells with an inducible expression vector, high glucose concentrations arrested cells at G1 and induced cell hypertrophy in a similar manner to p27+/+ cells. Taken together, these studies show a critical role for p27 in the development of diabetic glomerular hypertrophy.

In addition to regulating p27, high glucose also increases p21 expression in cultured mesangial cells (56). Moreover, there is an increase in glomerular p21 immunostaining in experimental diabetes (streptozotocin model) during the phase of early glomerular hypertrophy (56). Recent studies have shown that streptozotocin-induced diabetes is associated with glomerular hypertrophy in p21+/+ mice but that the same increase in hyperglycemia was not associated with glomerular hypertrophy in diabetic p21−/− mice (4).

We propose the following pathway of cell cycle-dependent regulation of glomerular cell hypertrophy in diabetes (Fig. 4). High ambient glucose in vivo or in vitro stimulates G1 entry. After one or two completions through the cell cycle leading to a limited proliferation, the concomitant induction of TGF-β, most likely mediated by PKC activation, stimulates an increase in the CDK inhibitors p21 and p27. CDK inhibitors preferentially bind to and inactivate cyclin E-CDK2 complexes, resulting in underphosphorylation of pRb. The final result is G1-phase arrest with increased protein content and cellular enlargement, without DNA synthesis (hypertrophy).

Reduced nephron mass. A reduction in nephron number induces compensatory glomerular hypertrophy in the remaining nephrons (120). Uninephrectomy does not influence the protein expression of cyclins D1 and D2, nor of CDK2 and 4, when total renal lysates are studied at day 7 (85). However, when specific renal compartments were examined, studies showed that cyclin E-CDK2, but not cyclin D1-CDK4, was active during compensatory hypertrophy induced by surgical uninephrectomy (61). However, severe renal ablation such as a 5/6 nephrectomy is also accompanied by an early glomerular cell proliferative response, which is associated with an increase in cyclin E expression and the phosphorylation of pRb (105).

A picture is thus emerging from cell culture experiments demonstrating how cell cycle proteins regulate hypertrophy. However, studies are necessary to characterize the role of these heterogeneous components in renal hypertrophy in vivo.

Tubular Epithelial Cell Hypertrophy

The capability of the adult kidney to grow to replace “lost” tissue was first recognized by Aristotle (384–322 BC), who showed that animals born with a single kidney have a larger organ compared with animals with two kidneys (145). Further studies have shown an
increase in renal size, predominantly due to proximal tubular epithelial cell hypertrophy, in the remaining kidney after a decrease in nephron number due to disease or surgical resection (93). The initial tubular epithelial cell hypertrophy is considered “compensatory” and “adaptive” hypertrophy (134, 135, 145). However over time, tubular cell hypertrophy becomes “mal-adaptive” (42, 50) and is associated with the subsequent infiltration of macrophages/monocytes, T cells, and fibroblasts into the tubulointerstitial space, which results in tubular atrophy and tubulointerstitial fibrosis, which are the ultimate common end points of many renal diseases with diverse etiologies. The biochemical aspects, growth factors, and potential signal transduction pathways of renal hypertrophy have been reviewed elsewhere (21, 22, 93).

ANG II-induced tubular cell hypertrophy. To identify potential molecular mechanisms underlying tubular epithelial cell hypertrophy, we induced proximal tubular cell hypertrophy in vitro with ANG II (139; Table 2). In this model, ANG II stimulated the expression of immediate early genes, which is consistent with a cell G0/G1 phenotype. However, ANG II-induced G1 arrest was associated with increased protein synthesis and cellular enlargement but not progression through the cell cycle and DNA synthesis, findings consistent with hypertrophy (139, 143).

ANG II treatment of proximal tubular cells increased the protein levels for the CDK inhibitor p27, without altering the mRNA abundance (143). More recently, we showed that ANG II-induced p27 expression was mediated by superoxide anions generated/generated by membrane-bound NAD(P)H oxidase (35). Moreover, p27 preferentially associated with cyclin D-CDK4 complexes, which inhibited the kinase activity (143). Lowering p27 levels in proximal tubular cells with p27 antisense oligonucleotides abolished ANG II-mediated G1-phase arrest and hypertrophy and facilitated entry into the S phase, thereby converting a hypertrophic phenotype to a proliferative one (143). Recent preliminary data suggest that ANG II also induces p21 expression via a JAK2-STAT1 pathway to mediate proximal cell hypertrophy. Terada et al. (123) showed that ANG II-induced proximal tubular cell hypertrophy.

TGF-β-induced tubular cell hypertrophy. The role of the cytokine TGF-β in inducing hypertrophy is also of particular interest to nephrologists (9). For example, we showed that ANG II-induced proximal tubular cell hypertrophy depends on the concomitant induction of endogenous TGF-β (138). In the model of TGF-β converting mitogen-induced tubular epithelial cell proliferation to hypertrophy, Franch et al. (26) showed that TGF-β prevents entry into the S phase by maintaining pRb in an underphosphorylated state. TGF-β had no effect on cyclin D-CDK4 activity, but rather TGF-β prevented cyclin E kinase activity (26).

CELL CYCLE CONTROL OF RENAL CELL PROLIFERATION

In contrast to other organs such as the gastrointestinal tract or liver, there is very little cell turnover in the normal adult kidney (81). Labeling murine glomerular cells with [3H]thymidine, a marker of DNA synthesis, showed that only 1–2% of cells of glomerular endothelial and, to a much lesser extent, mesangial cells, proliferate, whereas podocytes do not (81). The calculated mean lifespan of glomerular cells is ~5–100 days (81). About one tubular epithelial cell per human nephron sloughs into the urine daily under normal physiological conditions (92), which explains why there is very little proliferation in the normal human kidney.

In contrast to normal physiological conditions, glomerular and tubular epithelial cell proliferation and cellularity increase in pathological situations such as renal injury. Addis (1881–1949) and the pathologist Olivier (2) suggested that proliferation of intrinsic glomerular cells contributes to the overall cellularity under certain pathological conditions (2). Indeed, proliferation of intrinsic glomerular cells such as mesangial cells is the characteristic response to many forms of immune (IgA nephropathy, lupus, membranoproliferative glomerulonephritis), metabolic (diabetes), and hemodynamic (remnant kidney)-mediated glomerular injury (for review, see Ref. 47). Moreover, glomerular cell proliferation is also closely linked to extracellular matrix protein accumulation and the subsequent decline in renal function. The regulation of glomerular cell proliferation by growth factors and intracellular signaling pathways has been reviewed elsewhere (1, 119), and blocking mesangial cell proliferation at these levels decreases glomerular matrix protein accumulation.

Mesangial Cell Proliferation: Cyclins and CDKs

Mesangial cell proliferation induced by a variety of known mitogens in vitro is associated with changes in specific cell cycle proteins (Table 2). For example, platelet-derived growth factor, endothelin-1, and basic fibroblast growth factor (bFGF) are associated with an increase in D-type cyclins (43, 46, 100) in early G1, and cyclin E in late G1 (100, 105), and cyclin A in the S phase (107). Lowering cyclin D1 levels with antisense reduces DNA synthesis. Mitogens also increase the activity of CDK4 (100) and CDK2 in mesangial cells in vitro (107), and antimitogens such as TGF-β1 (100, 107) and secreted protein acidic and rich in cysteine (SAGE) (personal communication) reduce mesangial cell proliferation by decreasing CDK4 and CDK2 activity, thereby preventing phosphorylation of pRB, which causes G1/S arrest. Finally, Riley et al. (97) showed that the phosphorylation status of pRB determines the mesangial cell’s proliferative response to mitogens.

The expression of positive cell cycle proteins has also been shown in experimental glomerular diseases in the past few years (Table 3). Cyclins D, E, and A are increased in glomerular diseases characterized by mesangial cell proliferation such as experimental mesangial proliferative glomerulonephritis (Thy1 nephritis) (106, 121, 146). CDK2 protein levels and activity are also increased in Thy1 (106). Furthermore, inhibiting CDK2 activity with specific purine analogs, without
altering CDK2 protein levels, markedly decreases mesangial cell proliferation (88). Moreover, inhibiting CDK2 activity also decreases the accumulation of matrix proteins and improves renal function compared with control subjects (88). More recently, we have also shown that inhibiting CDK2 activity reduces glomerular cell proliferation in another model of immune-mediated glomerular injury. These studies provide potential targets for future therapeutic interventions in glomerular diseases associated with mesangial cell proliferation.

Mesangial Cell Proliferation: CDK Inhibitors

CDK inhibitors are critical determinants for the onset and magnitude of renal cell proliferation. Although there are two families of CDK inhibitors (115), most renal studies reported have focused on the Cip/Kip family (p21, p27, p57). The CDK inhibitor p21 is normally only present in low abundance in quiescent glomerular cells (106). However, p21 levels increase during mitogen-induced mesangial cell proliferation. Studies in nonrenal cell have suggested p21 provides a “framework” for the assembly of cyclins, CDKs, and PCNA required for DNA synthesis. We have shown that the antimitogenic TGF-β1 increases p21 levels in mesangial cells in vitro, and Terada et al. (125) showed that forced overexpression of p16 and p21 reduces mitogen-induced mesangial cell proliferation in vitro. Interestingly, there is a de novo expression of p21 in Thy1 glomerulonephritis that coincides with increased TGF-β expression and the resolution of mesangial cell proliferation in this model (106). From a therapeutic standpoint, it should be noted that glucocorticoids increase p21 expression in vivo. However, lowering the levels of p21 by itself, in the absence of mitogenic signals, is not sufficient to induce mesangial cell proliferation.

In contrast to p21, p27 is constitutively expressed in normal quiescent mesangial cells in vitro (107). The onset of mesangial cell proliferation in vitro induced by specific mitogenic growth factors is associated with a decrease in p27 protein levels (109). Furthermore, p27 dissociates from cyclin A-CDK2 complexes on mitogen stimulation, which coincides with increased A-CDK2 activity. The onset and magnitude of mitogen-induced mesangial cell proliferation in vitro are further augmented when p27 levels are lowered with antisense (107). However, lowering the levels of p27 by itself, in the absence of mitogenic signals, is not sufficient to induce mesangial cell proliferation.

p27 is also constitutively expressed in normal glomerular cells (106), and mesangial cell proliferation in vivo also correlates closely with p27 levels. The peak of mesangial cell proliferation in experimental mesangial proliferative glomerulonephritis coincides with almost undetectable p27 levels (106). To determine the role of p27 in glomerular cell proliferation in inflammatory renal disease, immune-mediated glomerulonephritis was induced in p27−/− and p27+/+ mice. The lack of p27 resulted in a marked increase in the onset and magnitude of glomerular cell proliferation in nephritic p27−/− mice compared with control animals (80). This was associated with an increase in glomerular matrix proteins in nephritic p27−/− mice compared with control mice. Tubular cell proliferation was also markedly increased after non-immune-mediated injury in p27−/− mice compared with control animals (80). Taken together, these results show that the level of the CDK inhibitor p27 is a critical determinant in the renal response to immune and nonimmune forms of injury. Terada et al. (122) showed that lovastatin inhibited mesangial cell proliferation by increasing the levels of p27 in vitro and in vivo (personal communication). We have recently shown that preventing p27 degradation by blocking the ubiquitin pathway inhibits mitogen-induced mesangial cell proliferation in vitro.

Lack of Podocyte Proliferation: Role of Cell Cycle Proteins

In contrast to mesangial and glomerular endothelial cells, adult visceral glomerular epithelial cells (VEC, also called podocytes) do not readily proliferate in response to the same forms of injury (53). However, during glomerulogenesis VEC proliferate during the S-shaped phase, which ceases during the comma phase. The cessation of VEC proliferation coincides with the de novo expression of the CDK inhibitors p27 (11) and p57 in VEC (71). Nagata et al. (71) showed that p21 is also transiently expressed by VEC during glomerulogenesis. These studies show that the transition from a proliferating immature VEC phenotype to a mature nonproliferating and quiescent phenotype during glomerulogenesis coincides with, and may be due to, the de novo expression of specific CDK inhibitors (Fig. 5). Interestingly, p57−/− mice are born with fused foot processes (153), suggesting that this CDK inhibitor may be more critical for VEC development than other members of this family of CDK inhibitors, as p21−/− and p27−/− mice have a normal glomerular development.

In contrast to mesangial cells, mature VEC undergo little, if any, proliferation in vivo in response to immune (membranous nephropathy, minimal change), metabolic (diabetes), hemodynamic (reduced nephron mass) and other (focal segmental glomerulosclerosis) forms of injury (53). Kriz (53). Kriz et al. (54), Rennek (94), Pavenstadt (86), and others have proposed that the inability of VEC to proliferate and replace VEC lost by detachment after injury results in a denuded glomerular basement membrane, which underlies the development of glomerulosclerosis in these diseases. However, in certain forms of VEC injury such as collapsing glomerulopathy, VEC proliferation is prominent and may be associated with a rapid decline in renal function that characterizes this form of focal glomerulosclerosis (14).

Why do VEC not proliferate in most glomerular diseases, yet do proliferate in others? In experimental membranous nephropathy, complement-mediated VEC injury is associated with a slight increase in expression for cyclin A and its partner, CDK2 (103). Thus VEC have the nuclear “machinery” capable of undergoing DNA synthesis. However, the answer to the relative lack of VEC proliferation probably lies with the CDK inhibitors. Complement-mediated injury to VEC was
associated with a marked increase in expression for the CDK inhibitors p21 and p27 in VEC in experimental membranous nephropathy (103). Moreover, both these inhibitors were bound to and inhibited cyclin A-CDK2 activity (103).

Kriz et al. (55) and Floege et al. (23) showed that the mitogen bFGF increases DNA synthesis in VEC in rats with membranous nephropathy. To determine whether this was due to changes in the levels of specific CDK inhibitors, we administered bFGF to rats with passive Heymann nephritis. Our results showed that, indeed, the increase in VEC DNA synthesis was associated with a decrease in levels of p21, but not p27 (103). To further determine whether levels of the CDK inhibitor p21 determine the capacity of mature VEC to reengage the cell cycle and proliferate in vivo, experimental glomerulonephritis was induced in p21−/− and p21+/+ mice with an antiglomerular antibody. Nephritic p21−/− had a marked increase in VEC DNA synthesis compared with control mice, and this was associated with an increase in the number of multilayered cells in Bowman’s space (49). This was also associated with increased glomerular tuft collapse, and a decline in renal function, somewhat analogous to that seen in collapsing glomerulonephritis. Interestingly, VEC proliferation was associated with the loss of VEC-specific markers (such as WT-1, GLEPP-1), suggesting that if VEC dedifferentiated after injury, they can reenter the cell cycle and proliferate (49). Thus it is tempting to speculate that the marked VEC proliferation in nephritic p21−/− mice occurs because p21 inhibits both G1/S and M phases of the cell cycle.

As stated earlier, p57 is constitutively expressed in quiescent mature human VEC (71), and we have shown that p57-knockout mice are born with fused glomeruli.
foot processes (153). We have recently shown that p57 levels decrease in VEC that have reengaged the cell cycle after antibody-induced injury in experimental glomerulonephritis. Further studies are ongoing to test the hypothesis that p57 is required to maintain a terminally differentiated and quiescent VEC phenotype.

In summary, these studies show that specific cell cycle proteins have two important roles in VEC biology (Fig. 5). First, the "switch" to a quiescent VEC phenotype during glomerulogenesis coincides with de novo expression of specific CDK inhibitors (p21, p27, p57), and maintaining a quiescent and terminally differentiated VEC phenotype may be due to p27 and p57. Second, the inability of mature terminally differentiated VEC to proliferate after injury is due to an upregulation of CDK inhibitors p21 and p27 and the maintenance of p57 expression, and lowering these levels is associated with VEC proliferation.

CELL CYCLE AND TUBULINTERSTITIAL PROLIFERATION

In contrast to tubular epithelial cell hypertrophy in chronic renal disease, tubular epithelial cell proliferation is the major growth response after acute tubular injury such as ischemia or obstruction (128). After acute tubular ischemia, surviving tubular cells migrate along the basement membrane, proliferate, and differentiate to highly specialized cells of the appropriate nephron segment, a process that can be accelerated by the administration of exogenous growth factors (98). These studies show that cell proliferation is essential in the restoration and repair of renal function after acute injury (34, 41).

Cyclins and CDKs in Tubulointerstitial Cell Proliferation

Cyclins D and A, and CDK2 and 4, increase in proliferating tubular epithelial cells after ischemic injury (147). The role of CDK inhibitors has been shown in tubulointerstitial cell injury. Megyesi et al. (63) showed that cisplatinum-induced injury caused a p53-independent increase in p21 expression in tubular epithelial cells and that p21 increased in acute ischemic tubular injury. The same group showed a marked increase in tubular cell proliferation in p21−/− mice after cisplatinum-induced injury compared with control p21+/+ mice (62). Morissey et al. (66, 67) showed that p21 increased after ureteral obstruction. However, when ureteral obstruction was performed in p21−/− mice, a somewhat surprising finding was that proliferation was markedly increased in the myofibroblast cell population, rather than tubular cells in obstructed p21−/− mice compared with obstructed p21+/+ mice (45). Finally, tubular cell DNA synthesis is significantly increased in hyperglycemic p21−/− mice compared with hyperglycemia p21+/+ mice (4). These results suggest that the role of p21 may depend on the type of tubulointerstitial injury and on the cell type injured.

Although tubulointerstitial cell proliferation and apoptosis characterize unilateral ureteral obstruction, p27−/− mice showed that cisplatinum-induced injury caused a p53-independent increase in p21 expression in tubular epithelial cells and that p21 increased in acute ischemic tubular injury. Megyesi et al. (63) showed that cisplatinum-induced injury caused a p53-independent increase in p21 expression in tubular epithelial cells and that p21 increased in acute ischemic tubular injury. The same group showed a marked increase in tubular cell proliferation in p21−/− mice after cisplatinum-induced injury compared with control p21+/+ mice (62). Morissey et al. (66, 67) showed that p21 increased after ureteral obstruction. However, when ureteral obstruction was performed in p21−/− mice, a somewhat surprising finding was that proliferation was markedly increased in the myofibroblast cell population, rather than tubular cells in obstructed p21−/− mice compared with obstructed p21+/+ mice (45). Finally, tubular cell DNA synthesis is significantly increased in hyperglycemic p21−/− mice compared with hyperglycemia p21+/+ mice (4). These results suggest that the role of p21 may depend on the type of tubulointerstitial injury and on the cell type injured.

Although tubulointerstitial cell proliferation and apoptosis characterize unilateral ureteral obstruction, the contralateral kidney undergoes compensatory hypertrophy. Morissey et al. (66) reported that p21 mRNA levels do not increase in the contralateral kidney at days 1–8 postobstruction. Tubular epithelial cell proliferation of the obstructed kidney is considerably increased in p27−/− mice compared with p27+/+ mice (80). We have extended these studies to the contralateral kidney, and although we detected a high protein expression of p21 and p27 in the obstructed kidney by Western blotting, there was no increase in the contralateral kidney during the early phase of compensatory hypertrophy (J. Gerth and G. Wolf, unpublished observations). This somewhat surprising finding may indicate that compensatory hypertrophy in the contralateral kidney after unilateral ureteral obstruction is independent of these specific CDK inhibitors.

CELL CYCLE PROTEINS AND RENAL CELL APOPTOSIS

As stated earlier, total organ cell number reflects the balance of proliferation and apoptosis (programmed cell death) and many forms of glomerular and tubular cell injury are associated with increased proliferation and apoptosis (59, 131). However, the consequence of glomerular cell apoptosis depends on the type of glomerular injury and the glomerular cell type injured. For example, apoptosis may be beneficial during the resolution phase of mesangial proliferative glomerulonephritis to normalize the increase in mesangial cell number (5). In contrast, a decrease in mesangial cell number due to excess apoptosis may underlie the development of glomerulosclerosis after a decrease in mesangial cell number (37). Likewise, endothelial cell apoptosis results in a denuded capillary lumen in thrombotic microangiopathy (R. Johnson, unpublished observations) and in other forms of endothelial injury (117) and may predispose to the development of progressive glomerulosclerosis. Podocyte loss in diabetes (83) and membranous nephropathy (103) may also underlie the development of glomerulosclerosis. Finally, proliferation and apoptosis have also been reported in proximal and distal tubular epithelial cells in many types of acute and chronic tubulointerstitial diseases. Apoptosis, in excess of proliferation, decreases cell number, leading to interstitial fibrosis (59, 126).

These studies show a possible link between certain growth (proliferation) and death (apoptosis) pathways in renal disease. How are these processes linked at the level of the cell cycle? In contrast to transition and progression through the cell cycle that occurs with proliferation, in vitro studies have shown that cells typically exit the cell cycle in late G1 phase during apoptosis. However, apoptotic cells can exit during any phase of the cell cycle (64).

CDK Inhibitors in Renal Cell Apoptosis

The first clue to a possible role for cell cycle proteins in glomerular cell apoptosis came from the observation that the peak in mesangial cell apoptosis in experimental (Thy1) glomerulonephritis coincided with the maximum decrease in p27 levels (106). Moreover, apoptosis
was markedly increased in nephritic p27−/− mice compared with control p27+/+ mice (80). Furthermore, tubulointerstitial cell apoptosis was also increased in p27−/− after unilateral ureteral obstruction compared with control mice (80). In contrast, apoptosis is not a marked feature of glomerular diseases associated with increased p27 levels, such as membranous nephropathy and diabetic nephropathy (56).

To determine whether the CDK inhibitor p27 indeed protects mesangial cells from apoptosis, we studied mesangial cells in vitro derived from p27−/− and p27+/+ mice. Apoptosis induced by growth factor deprivation or cyclohexamide was markedly increased in p27−/− mesangial cells compared with p27+/+ cells (40). Moreover, reconstituting p27 levels in p27−/− cells rescued cells from apoptosis. An increase in growth factor-deprived induced apoptosis was also observed in rat mesangial cells when p27 levels were lowered with p27 antisense compared with control rats (40). Recent studies in nonrenal cells showed that the COOH termini of p21 and p27 are truncated by caspases in apoptotic cells (58). Taken together, in addition to its role in proliferation and hypertrophy (105), our studies showed that a novel function for the CDK inhibitor p27 is to protect renal cells from apoptosis (Fig. 6).

CDKs in Renal Cell Apoptosis

The role for cyclin dependent kinase 2 (CDK2) in DNA synthesis has been well established. However, we and others have recently shown that CDK2 also has a critical role in apoptosis. In growth factor-deprived p27−/− mesangial cells undergoing apoptosis, CDK2 activity was markedly increased without any increase in CDK2 protein levels (40). Moreover, CDK2 activity increased in the absence of DNA synthesis. Furthermore, inhibiting CDK2 activity pharmacologically or with a dominant negative mutant decreased apoptosis in growth factor-deprived mesangial cells (40). We have also shown that tubular epithelial cell apoptosis in vivo after ureteral obstruction is associated with increased CDK2 activity (80). More recently, studies have also shown that CDK2 activity is increased in apoptotic nonrenal cells (33, 58).

As discussed earlier, normal cell cycle progression (and DNA synthesis) requires the sequential activation of CDK2 by cyclin E in late G1 phase, and cyclin A in the S phase (112). To determine whether the CDK2-dependent mesangial cell apoptosis was due to the activation of CDK 2 by a specific cyclin, total cell protein was immunoprecipitated with antibodies to cyclins E and A, the partners for CDK2. Our results showed that apoptosis was due to increased cyclin A-CDK2 activity, without a preceding increase in cyclin E-CDK2 activity. These studies suggest that an unscheduled and uncoordinated increase in cyclin A-CDK2 activity (without a preceding cyclin E-CDK2 activation) may lead to a catastrophic G1/S phase, resulting in apoptosis (40).

These studies show that growth (proliferation) and death (apoptosis) pathways share common cell cycle pathways (Fig. 6) and may provide an explanation why glomerular cell proliferation and apoptosis are often closely linked in many forms of glomerular disease.

CONCLUSIONS

The progressive decline in renal function in glomerular and tubulointerstitial disease is ultimately due to the increase in extracellular matrix proteins, which leads to scarring. However, these late changes are preceded by, and are closely linked to, changes in cell number (balance of proliferation and apoptosis) and/or cell size (hypertrophy). It has only recently been recognized that these earlier cellular events are closely linked to the cell cycle in pathological states such as occur in many forms of renal disease. Recognition that the growth (proliferation) and death (apoptosis) responses to injury share common cell cycle pathways may explain why these processes are often closely linked in renal disease. Furthermore, our understanding of the cell cycle also explains why proliferation and hypertrophy are exclusive within the same cell.

There are a number of compelling reasons to study cell cycle regulatory proteins in renal disease. First, although cell proliferation, apoptosis, and hypertrophy are regulated by growth factors, signaling pathways, and extracellular matrix and immediate response genes, the ultimate fate of the cells’ response to injury may be governed by cell cycle regulatory proteins within the nucleus. Second, each renal cell type has a different constitutive expression pattern of cell cycle proteins. Third, the role of each cell cycle protein is probably cell type specific and also depends on the form of injury, and hence its role in renal disease is not always predictable. Finally, many new and exciting therapeutic strategies are being developed to target specific cell cycle proteins and further research into these applications in renal disease may provide some hope to our patients.

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REFERENCES


