

## Full Review

# Cell cycle control in the kidney

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### ABSTRACT

Proper control of the cell cycle is mandatory during homeostasis and disease. The balance of p53 and MDM2 integrates numerous signalling pathways to regulate the cell cycle, which is executed by multiple proteins including the cyclins, cyclin kinases and cyclin kinase inhibitors. Mutations or environmental factors that affect cell cycle control can lead to inappropriate hyperplasia or cancer as well as to cell loss and tissue atrophy. Normal kidney function is maintained largely by post-mitotic quiescent cells in the G0 phase with a low turnover. Early cell cycle activation during kidney injury contributes to cell death via mitotic catastrophe, i.e. death via mitosis, e.g. of cell with significant DNA damage. At later stages, cell cycle entry supports tissue regeneration and functional reconstitution via cell hypertrophy and/or cell proliferation. It is of note that so-called proliferation markers such as Ki67, PCNA or BrdU identify only cell cycle entry without telling whether this results in cell hypertrophy, cell division or mitotic catastrophe. With this in mind, some established concepts on kidney injury and regeneration are to be re-evaluated. Here, we discuss the components and functional roles of p53/MDM2-mediated cell cycle regulation in kidney homeostasis and disease.

**Keywords:** acute kidney injury, chronic kidney disease, crescent, renal fibrosis, renal function

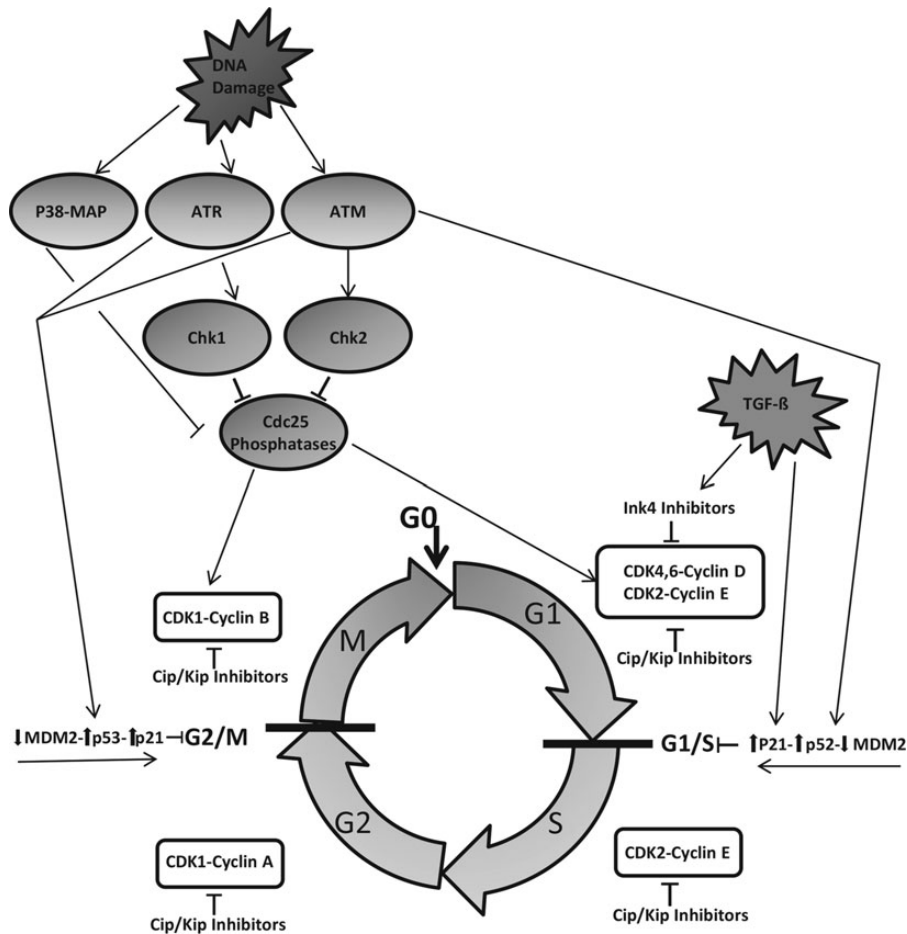
### INTRODUCTION

The life span of cells can vary from a few hours to several decades, hence, cell renewal from symmetric or asymmetric division of respective progenitor cells varies accordingly. During homeostasis cell turnover in the kidney is low, which implies cell cycle arrest in the G0 phase for most of the cells.

Upon injury, many cells activate the cell cycle because hypertrophy and cell division are two important compensatory mechanisms to prevent organ dysfunction. But which factors determine hypertrophy versus cell division upon cell cycle activation and assure that responses are always appropriate? Does inappropriate cell cycle control contribute to disease and disease manifestations, e.g. like known for uncontrolled hyperplasia of tumour cells in cancer? In this review, we discuss the regulatory role of the MDM2-p53 pathway in cell cycle regulation during homeostasis, injury and repair of the kidney as well as current and future drug options for cell cycle modulation in kidney disease.

### THE CELL CYCLE AND ITS CHECKPOINTS

The cell cycle is a unidirectional pathway that, if completed, can lead to cell division (Figure 1). The cell cycle involves four tightly controlled phases, i.e. G0 phase, G1 phase, S phase, G2 phase and M (mitosis) phase. G0 is an exit phase of quiescent cells that fulfil their physiological functions with the tissue. For example, the low cell turnover of the adult kidney implies that most renal cells remain in G0 during homeostasis. When cells activate the cell cycle into G1 they start gene transcription and protein translation for the production of new cell organelles. In the S phase, DNA synthesis duplicates the chromatin as a prerequisite for the division into two diploid progeny cells. The G2 phase is characterized by an increase in cell size, more protein synthesis and preparation for cell division. During the M phase the chromosomes and the mitotic spindle form to segregate the chromatin, which involves large parts of the actin cytoskeleton, hence, the cell rounds up. In podocytes, this would result in loss of adherence with glomerular basement membrane and with adjacent podocytes, events that are all incompatible with maintaining podocyte function which would be followed by podocyte detachment [1]. Similarly, the



**FIGURE 1:** Cell cycle regulation. The figure illustrates the pathways and molecules that regulate the cell cycle upon cytokine insults or DNA damage, including the MDM2-p53-p21 signalling cascade. Inhibition of CDK2-cyclin E is essential for G1/S arrest and blocking CDK1-cyclin B is necessary for G2/M arrest. ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; Chk, checkpoint kinase; Cdc25, cell division cycle 25; CDK, cyclin-dependent kinases; Rb, retinoblastoma protein; MDM2, mouse double minute 2; cip/kip, CDK interacting protein/Kinase inhibitory protein; INK4, inhibitor of kinase 4.

acquisition of functional specialization in highly differentiated cell types such as neurons or cardiomyocytes is coupled with the permanent exit from the cell cycle. Finally, completing mitosis also requires cytokinesis, i.e. the separation of the cytoplasm, a process that does not happen in podocytes and therefore can lead to multinucleated (polyploidy) podocytes followed by mitotic catastrophe (=death by mitosis) [2]. Mitotic catastrophe is not considered a 'pure' cell death executioner pathway, but a mechanism that is initiated by perturbations of the mitotic apparatus during the M phase of the cell cycle is paralleled by mitotic arrest and ultimately triggers cell death or senescence. Mitotic catastrophe is driven by a complex and poorly understood signalling cascade but, from a functional perspective, it is an oncosuppressive mechanism that precedes (and is distinct from) apoptosis, necrosis or senescence [3–5].

### The cell cycle checkpoints

To ensure the fidelity of the cell division, the cell possesses control mechanisms called checkpoints. These checkpoints verify whether the processes at each phase of the cell cycle have been accurately completed before progression into the

next phase. The first checkpoint is the G1/S checkpoint, which checks for the presence of DNA damage, stalls cell cycle for DNA repair and allows only the cells with intact DNA to proceed into the next phase of the cell cycle, the mechanism extremely important in preventing carcinogenesis. If the DNA damage escapes the G1/S checkpoint or occurs during the S phase, the cell cycle can be halted by intra-S phase checkpoint. The G2/M checkpoint will determine whether or not the cell proceeds to complete mitosis. The final checkpoint, metaphase or spindle checkpoint ensures proper chromosome alignment prior to cell division. Checkpoint signalling is activated in response to incomplete DNA replication due to stalled replication forks, and damaged DNA induced by both internal and external sources such as UV light, ionizing radiation, reactive oxygen species or DNA damaging chemotherapeutic agents [6]. The cell cycle pathway is intrinsically linked to cell survival and cell death. Failure to meet the quality check requirements in cell cycle checkpoints will result in cell death, e.g. during tissue injury. This process contributes to organ failure and also prevents growth of potentially cancerous cells with significant DNA damage. Therefore, any use of cell death inhibitors must be with caution as they might promote cancer.

## CYCLE REGULATION BY CYCLINS, CYCLIN-DEPENDENT KINASES AND THEIR INHIBITORS

The majority of the cell cycle pathway is regulated by three classes of proteins: cyclic proteins, called cyclins, cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CKIs) (Figure 1). Cyclins and CDKs have very low catalytic activity on their own; however, they become active, when cyclins and CDKs form complexes that enable CDKs to phosphorylate and activate specific cell cycle intermediates. When cells enter the cell cycle out of quiescence (G<sub>0</sub>), cyclin D will bind to CDK4/6 and this complex will phosphorylate and inactivate the retinoblastoma (Rb) gene. In G<sub>0</sub> state, Rb is bound to DNA and blocks the transcription of specific genes; however, when Rb is released, genes necessary for the cell cycle become accessible [7]. Two distinct families of CDK inhibitors, the 'CDK interacting protein/Kinase inhibitory protein' (cip/kip) family and the inhibitor of kinase 4 (INK4) family, have been identified, which prevent the progression of the cell cycle. The INK4 family specifically inhibits cdk4 and cdk6, which are active in G<sub>1</sub> phase while the cip/kip family proteins block cyclin-CDK complexes of both G<sub>1</sub>- and S-phase.

### Cell cycle signalling in response to DNA damage

In response to DNA damage, eukaryotic cells activate two major canonical kinase signalling networks ataxia telangiectasia mutated/checkpoint kinase2 (ATM/Chk2) and/or Rad3-related protein/checkpoint kinase1 (ATR/Chk1) to arrest the cell cycle and initiate DNA repair. The ATM/Chk2 module is activated after DNA double-strand breaks and the ATR/Chk1 pathway responds primarily to DNA single-strand breaks. These kinases control the G<sub>1</sub>/S, intra-S and G<sub>2</sub>/M checkpoints through activating their downstream effector kinases Chk2 and Chk1, respectively [6]. The locally increased ATM activity is important for efficient phosphorylation of ATM substrates including the downstream effector kinase Chk2 and the prominent tumour suppressor protein p53. In normal cells, p53-dependent signalling results in G<sub>1</sub>/S arrest, mainly mediated by transcriptional up-regulation of p21, which in turn inhibits the Cdk2/cyclin E complex, blocking thus dissociation of Rb protein and transcription factor E2F and hence the cell cycle progression [8]. In addition, p21 can also break the cell cycle progression in the G<sub>2</sub>/M checkpoint after  $\gamma$ -irradiation [9] or transforming growth factor beta (TGF- $\beta$ ) stimulation in renal epithelial cells [10]. If the DNA damage is extensive, however, then p53-dependent pathways target the damaged cell for cell death [11]. TGF $\beta$  signalling can, however, arrest the cell cycle also in G<sub>1</sub>/S phase by preventing the phosphorylation of Rb protein through p16ink4a31-mediated blocking of Cdk4/6 interaction with cyclin D1.

Both the ATM/Chk2 and the ATR/Chk1 pathway converge to inactivate members of the Cdc25 family of dual-specificity phosphatases, which are positive regulators of cell cycle progression. Of the three known Cdc25 family members Cdc25A seems to be a critical Chk1 substrate for the intra-S phase

checkpoint. A novel cell cycle checkpoint kinase pathway that integrates global stress responses with DNA damage is p38MAPK/MK2. The p38MAPK/MK2 pathway responds to various stress-cellular stimuli, including cytokines, hyperosmolarity and UV irradiation [12] and halts the cell cycle progression in G<sub>2</sub>/M phase via inactivation of Cdc25.

## CELL CYCLE IN HOMEOSTASIS, INJURY AND REGENERATION

### Homeostasis

**Podocytes.** Under normal conditions, epithelial turnover in the kidney is slow [13]. In healthy mice and humans, mature podocytes are quiescent, express high levels of CDK inhibitors and are differentiated, suggesting that these cells lack the ability to renew during adult life. Podocytes express cyclin A, B1 and D1 and CDK inhibitors, such as p21, p27 and p57. Early in development, Ki-67, a marker of proliferation, is highly expressed in potential podocytes while cyclin D1 and CKIs are markedly down-regulated. At the capillary loop stage, cell cycle proteins and CDKs are markedly altered; in contrast, CKIs and cyclin D1 are intensely increased and Ki-67, cyclin A and cyclin B1 are not detectable [14, 15]. These changes are associated with podocyte exit from the cell cycle and their differentiation into mature podocytes expressing podocyte marker proteins, such as WT-1 or podocalyxin [14, 15]. The constitutive and intense production of CKIs is indeed necessary for the maintenance and function of the differentiated quiescent podocytes [14].

**Tubular epithelial cells.** Renal tubule cells divide at a very low rate, as evaluated by proliferative cell nuclear antigen (PCNA) and Ki-67 immunoreactivity [13]. Of note is that both of these markers are expressed in all phases of the cell cycle, so their detection does not necessarily mean proliferation *per se*, but it marks also hypertrophic cells, arrested in G<sub>1</sub> or S phase. This cell production balances the loss of tubular epithelial cells into the urine which under physiological conditions is minimal, estimated to one tubular epithelial cell per human nephron daily [16]. Nevertheless, this turnover rate must be under tight control as even a small imbalance between cell loss and cell division would soon lead to nephron loss or marked increases in nephron and kidney size over time. In unstressed kidney, tubular epithelial cells are maintained in G<sub>0</sub>-G<sub>1</sub> phase [17], upon injury, the tubular cells re-enter the cell cycle, producing high levels of Cyclin D and A and CDK2 and CDK4 [18].

**Mesangial cells.** Mesangial cells (MCs) provide structural support to the glomerular tuft, in part by the secretion and maintenance of the extracellular matrix. There is very little MC turnover in the healthy adult kidney, suggesting that under normal circumstances MCs are either not exposed to mitogens or protected from them by inhibitory factors. Similar to podocytes, mature MCs are kept in this quiescent state by up-regulation of p27 [19]. The onset of MC proliferation on

mitogen stimulation or upon injury is associated with a decrease in p27 levels.

### Kidney injury

**Podocytes.** Intrinsic renal cell injury can promote either cell proliferation, hypertrophy or cell death. Podocytes as post-mitotic cells do not readily proliferate upon injury; however, in certain diseases, such as collapsing focal segmental glomerulosclerosis (FSGS), the podocytes stain positive for cell cycle markers and even binuclear podocytes are often seen [20]. Cyclins and CDKs are altered in collapsing FSGS and human immunodeficiency virus-associated nephropathy (HIVAN), where p27, p57 and cyclin D are absent in podocytes, and p21, cyclin A and Ki-67 are induced [15, 21, 22]. This implies a dysregulated podocyte phenotype characterized by bypassing cell cycle restriction points and podocyte loss via podocyte mitosis, i.e. mitotic catastrophe [23]. In the setting of adriamycin-induced podocyte injury, the presence of CDK inhibitor p21 is protective for podocytes in this model of toxic podocytopathy [24]. In other glomerular diseases, such as membranous nephropathy, podocytes upon immune-mediated injury increase DNA synthesis in S-phase and up-regulation of cyclin A and Cdk2 and finally enter mitosis. Nevertheless, the lack of podocyte proliferation in this model indicates that although podocyte is able to enter mitosis, it is unable to successfully complete it, as indicated by the occurrence of multinucleated podocytes and absence of cytoplasmic division (cytokinesis). Diabetic nephropathy is characterized by podocyte hypertrophy. In various experimental models of diabetic nephropathy, such as Zucker diabetic rats and *db/db* mice, both models of type 2 diabetes, or type 1 model, induced by streptozotocin administration, the increased expression of p27 and p21 is identified [25–27]. Diabetic *p21<sup>-/-</sup>* as well as *p27<sup>-/-</sup>* mice are protected from glomerular hypertrophy and development of progressive renal failure [26, 28]. All these above described studies indicate that the proliferative or quiescent phenotype of injured podocytes is probably given by up-regulation or down-regulation of chronic kidney disease (CKD) inhibitors p21, p27 and p57, respectively. When challenged, podocytes react to the injury by re-entry into the cell cycle, it results in either (i) dedifferentiation, hyperplasia, dysfunction and podocyte death, like in HIVAN, (ii) G1/S cell cycle arrest to allow compensatory podocyte hypertrophy or (iii) aberrant mitosis and podocyte cell death by catastrophic mitosis. Hypertrophy is the only cellular strategy, which allows podocyte to partially and temporarily compensate injury-mediated podocytopenia. When mature podocytes are forced to override this cell cycle restriction point, they fulfil an aberrant mitosis followed by detachment and death, i.e. catastrophic mitosis. Such podocytes appear multinucleated with aberrant mitotic spindles or micronuclei and are found in several human and experimental glomerular diseases, such as HIVAN, FSGS, Minimal change disease (MCD), Immunoglobulin A (IgA) nephropathy or adriamycin-induced nephropathy [23, 29].

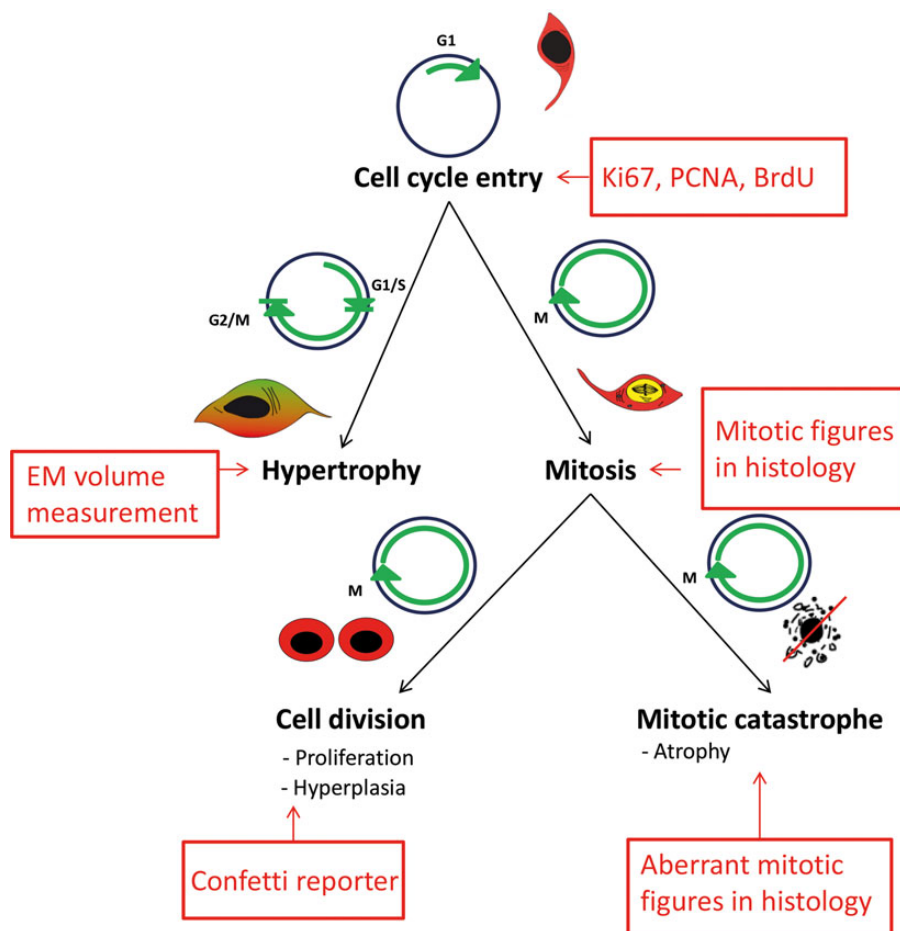
**Mesangial cells.** In response to a variety of injurious stimuli, the quiescent phenotype of MC changes, leading to an increase in proliferative rate and progressive matrix accumulation,

which can lead to glomerulosclerosis. These features are seen in diseases such as IgA nephropathy, lupus, membranoproliferative glomerulonephritis and diabetic nephropathy. In experimental model of mesangial proliferative glomerulonephritis, Thy1 nephritis, the levels of cyclin D, E, A and CDK2 and CDK4 all increase during the phase of marked mesangial proliferation [30]. Blockade of CDK kinase activity by specific purine analogue roscovitine inhibited substantially MC proliferation in Thy1 model [31]. At the onset of MC proliferation in the Thy1 model, the levels of p27 decrease dramatically. If experimental nephritis is induced in *p27<sup>-/-</sup>* mice, the mesangial proliferation starts earlier and the magnitude of the proliferative response, coupled with extracellular matrix (ECM) expansion, is greater [32]. T-type calcium channels control progression through the G1/S checkpoint in proliferating MC. Pharmacological inhibition of these channels reduces MC proliferation by their arrest in G1 phase and ameliorates glomerular injury in Thy1 model [33].

**Tubular epithelial cells.** In acute kidney injury (AKI), the tubular epithelial cells react to the ischaemic or toxic insult by a massive necrotic and apoptotic cell death coupled with immediate onset of cell cycle activation of surviving epithelial (progenitor) cells. When cells of the injured kidney enter into cell cycle, there is rapid and massive induction of cyclin inhibitor p21 but not of p27 or p57, in several AKI models [34]. *p21*-deficient mice show more cells entering the cell cycle but develop more severe damage after ischaemia-reperfusion or toxic injury in comparison to *p21<sup>+/+</sup>* animals. Moreover, *p21* induction ameliorates AKI. [35]. The protective effect of *p21* in AKI is coupled with its binding and inhibiting CDK2. The CDK2 dependence was confirmed *in vivo* by pharmacological inhibition of CDK2 and resulted in less severe nephrotoxicity after cisplatin treatment [36]. Also, inhibition of CDK4/6 ameliorates AKI after ischaemia-reperfusion injury despite reduced proliferation of tubular epithelial cells which are arrested in G0–G1 phase. The notion of lack of epithelial cell proliferation being beneficial for kidney regeneration seems to be counter-intuitive; nevertheless, extended cycle arrest would allow more time for DNA repair. Vice versa, early tubular cell mitosis leads to mitotic catastrophe, which deletes cells with DNA damage. This way proliferation is not regenerative but rather contributes to cell death, kidney injury and dysfunction [37].

### Regeneration and repair

**Tubular epithelial cells.** Mammalian adult kidneys, unlike fish kidneys, are not capable of *de novo* nephrogenesis. Therefore, upon kidney injury, renal cells need to be recovered to avoid nephron loss and renal atrophy. Upon tubular injury ~70% of the surviving tubular cells stain positive with ‘proliferation’ markers that simply indicate cell cycle entry. Concluding on subsequent cell division from the positivity of these markers is incorrect and may lead to entirely erroneous disease concepts (Figure 2). Regenerative tubular cell or progenitor cell proliferation can only be validated with lineage tracing using a progenitor- or differentiated tubular cell-specific marker and the multicolour rainbow reporter, evidence still pending to date (Figure 2). Cell cycle entry involves an



**FIGURE 2:** Markers of cell cycle entry, hypertrophy, mitosis and mitotic cell death. This figure depicts different detection markers that can distinguish whether the cell cycle entry will lead to hypertrophy (cell cycle arrested at restriction point) or to cell division into two living cells (mitosis, cell proliferation) or to cell death during/upon cell division (mitotic catastrophe). Markers like Ki67, PCNA and BrdU indicate only entry in the cell cycle. PCNA, proliferating cell nuclear antigen; BrdU, 5-bromo-2'-deoxyuridine. Confetti reporter can be used for detection of clonal proliferation of single cells and thus for simultaneous lineage tracing in multicolour confetti mouse models. Confetti-mouse is a stochastic multicolour Cre-reporter expressing ubiquitously Brainbow-2.1 construct encoding four fluorescent proteins. Upon cre activation, the Brainbow-2.1 recombines in a random fashion to four possible outcomes, allowing a way to label and distinguish individual/adjacent cells with fluorescent proteins in *cre* recombined cells.

induction of cyclin D, A, CDK2 and CDK4/6 and p21 [38]. Until recently, AKI was considered a reversible process leading to complete kidney recovery if the patient survived the acute phase. However, recently it has been shown that AKI can lead to the development of fibrosis and to chronic renal failure via maladaptive repair. Indeed, during the maladaptive repair, many tubular cells that are undergoing cell division spend a prolonged period in the G2/M phase of the cell cycle and begin to secrete profibrotic factors, such as TGF- $\beta$ , which leads to increased production of interstitial matrix. Thus, the tubule cells assume a senescent secretory phenotype. [39]. Moreover, the senescence of tubular cells mediated by increased p21 expression is associated with early stage of diabetic nephropathy in streptozotocin-induced diabetes 1 model [40]. On the contrary to protective effect of p21 in AKI, the long-term p21 activation may contribute to progression of CKD [41]. The p21 knock-out mice did not develop chronic kidney failure after 5/6 nephrectomy. It has been suggested that lack of p21 allows hyperplastic compensatory proliferation of

remaining kidney tissue while preventing maladaptive hypertrophy [41].

**Podocytes.** Unlike tubule cells, podocyte regeneration cannot derive from surviving post-mitotic podocytes and therefore involves the differentiation of podocyte progenitor cells that retain their capacity for cell cycle completion and cell division [42]. Experimental podocyte ablation demonstrated podocyte regeneration from progenitor cells other than the neighbouring podocytes [43]. In fact, this study ultimately disproved the concept that podocyte de-differentiation could be a source of podocyte regeneration. In contrast, aberrant proliferation of parietal epithelial cells produces hyperplastic lesions in crescentic glomerulonephritis [44]. Notch, Wnt and microRNAs regulate the accurate proliferation and differentiation of podocyte progenitor cells. Notch activates renal progenitor cells to enter the S-phase of the cell cycle and successive cell division, while Notch suppression is essential for their differentiation into podocytes [45]. Impaired silencing of Notch at the right stage forces podocytes

to pass the G2/M checkpoint leading to podocyte loss by mitotic catastrophe [45].

**Mesangial cells.** In contrast, MC can be readily replaced by proliferation of their local MC progenitors. After glomerular injury, cytokines such as platelet-derived growth factor (PDGF), interleukin-1 (IL-1) or TGF- $\beta$  may initially stimulate appropriate re-population of the glomerular tuft with MCs, but in disease they seem to be promoting ongoing MC proliferation and ECM production leading to progressive glomerular damage. Nevertheless, PDGF signalling through CDK4/6 is crucial for MCs renewal by proliferation in Thy1 rat model, while PDGF inhibition results in a significant MC proliferation reduction and ECM deposition in this model [46]. The source of proliferating cells was identified as subpopulation of cells residing in extraglomerular mesangium in juxtaglomerular area, which migrate and repopulate the glomerular tuft in the anti-Thy 1 model of mesangial proliferative glomerulonephritis [47]. Moreover, recently also extraglomerular renin lineage cells were suggested to represent a major source of repopulating cells for reconstitution of the intraglomerular mesangium after injury [48]. Dysregulated MC proliferation in disease has pathological effects but a degree of MC proliferation is beneficial under certain conditions, for example, in allowing resolution of human transient glomerulopathies such as post-streptococcal glomerulonephritis or experimental Thy-1 nephritis in rats which, if left to run its course, resolves within a few weeks. Any therapy designed to inhibit MC proliferation may potentially limit these beneficial healing processes.

### BALANCING THE CELL CYCLE—P53 AND MDM2

P53 is a guardian of cell cycle. It is up-regulated upon genotoxic stress and initiates cell cycle arrest in G1/S restriction point via induction of p21, allowing thus repair of damaged DNA. P53 can also block the progression of the cell cycle in G2/M point, preventing mitosis and division of cells with aberrant, genetically unstable DNA thus avoiding cancerogenesis or massive death by mitotic catastrophe. When the damage of the cells is too severe, p53 can drive their exit from cell cycle to senescence or to cell death. MDM2, E3-ubiquitine ligase, is a major negative regulator of p53 and as such MDM2 suppresses coordinated cell cycle arrest or cell death and promotes cell survival and growth. Vice versa, *MDM2* is a p53 target gene that forms together with p53, a tightly regulated negative feedback loop in which activated p53 upregulates MDM2 expression, which in turn will target p53 for degradation. MDM2 deficiency leads to p53-driven, uncontrolled cell death and its up-regulation or p53 deficiency results in uncontrolled proliferation and malignancy. Genetic deletion of the *Mdm2* gene in mice results in embryonic lethality from massive p53-dependent cell death, while concomitant *p53* deficiency completely rescues this phenotype [49]. But how does the MDM2-p53 signalling regulate cell cycle in kidney? In developing kidney, specific deletion of MDM2 from ureteric bud cells or from nephron progenitor cells in the embryonic kidney compromises

ureteric bud growth and branching and stem/progenitor cell renewal and differentiation. This mutant phenotype is mediated by aberrant p53 activity [50, 51].

Deletion of MDM2 in adult mouse kidney is associated with cell atrophy and damage in the tubular compartment. This phenotype is mediated by p53 and its effector gene p21, which is 190-fold up-regulated in affected kidney [52]. In lupus nephritis model blocking MDM2 suppresses the abnormal expansion of immune cells and ameliorates disease [53]. Podocyte-specific MDM2 deficiency in quiescent, unchallenged kidney results in p53 overactivation-related cell death of healthy podocytes and glomerulosclerosis (own unpublished data). In contrast, the MDM2 inhibition in damaged podocytes in adriamycin nephropathy mouse model promotes podocyte viability and reduces proteinuria and glomerulosclerosis. MDM2 blockade in this model induces G2/M arrest through increase of p53 and p21 protein levels to prevent aberrant nuclear divisions and detachment of dying aneuploid podocytes, a feature of mitotic catastrophe [29]. In ischaemia-reperfusion mouse model, we detected a dual effect of MDM2-p53 signalling in injury and regeneration phase of AKI model. MDM2 inhibition impaired tubular cell regeneration during post-ischaemic AKI in wild-type mice in a p53-dependent manner; however, MDM2 blockade also prevented tubular necrosis by suppressing sterile inflammation during the early post-ischaemic phase, but also possibly by promoting cell cycle arrest and DNA repair [54]. Moreover, inducing MDM2 by Nrf2 activation can enhance renal cell survival and tubular repair *in vitro* [55]. Together, balance of MDM2-p53 cell cycle regulators is essential for kidney development and homeostasis of renal intrinsic cells. Nevertheless transient MDM2 blockade and p53 increase seem to be beneficial in both podocytes and tubular cells in a specific temporal setting and in specific injury models.

### THERAPEUTIC OPTIONS TO MODULATE THE CELL CYCLE IN KIDNEY DISEASE

#### Current therapeutics

How do frequently used drugs modulate the cell cycle? ACE inhibitors, statins, anticoagulants, glucocorticoids, cyclophosphamide, azathioprine and mechanistic target of rapamycin (mTOR) inhibitors all have unspecific anti-proliferative properties, but how exactly they block cell cycle progression in specific renal cells is often not so clear. Statins limit MC proliferation by inhibiting the Rho and Ras pathway [56]. mTOR inhibitor rapamycin attenuates the hypertrophy in diabetes model via down-regulation of p70S6kinase signalling [57]. ACE inhibitors can reduce aberrant proliferation of renal progenitor cells via deactivation of NCAM+ and thus reduce development of hyperplastic lesions in podocytopathies, but can also foster glomerular regeneration via the transcription factor C/EBP $\delta$  [58]. Moreover, anti-proliferative properties of drugs can be detrimental in specific disease settings, such as ischaemia-reperfusion injury after renal transplant, as they impair re-epithelialization as part of the regeneration process.

Furthermore, immunosuppressive drugs elicit their effects by inhibiting lymphocyte proliferation but also impair wound healing as a side effect. In crescentic glomerulonephritis, however, the anti-proliferative effect of cyclophosphamide also helps to limit crescent formation from hyperplastic of parietal epithelial cells, which is another rationale for the use of this drug for induction therapy.

### Novel therapeutic targets

Following this concept, CDKs are considered as promising targets and small molecule inhibitors were developed and tested in clinical trials. Previous preclinical studies had primarily tested CDK2 inhibitors in polycystic kidney disease, mesangial proliferative glomerulonephritis, crescentic glomerulonephritis and collapsing glomerulopathy with positive outcomes. Nevertheless, the clinical trial to treat IgA nephropathy with CDK2 inhibitor roscovitine was stopped due to unexpected severe side effects associated with prolonged treatment. While CDK2 inhibitors can reduce cisplatin toxicity in the AKI model, existing CDK2 inhibitors have important off-target effect, inhibiting cSrc and other molecules, which are activated in tubular cells after ischemia-reperfusion injury. They also inhibit other CDKs, involved in other cellular homeostatic processes, therefore causing more adverse effects. Another group of CDK inhibitors, CDK4/6 inhibitors, have been tested in an ischaemia-reperfusion injury experimental model and ameliorated AKI. This effect was reached by transient arrest in G1 phase, allowing improved DNA repair and thus decreasing epithelial cell death by mitotic catastrophe and apoptosis [37]. CDK/GSK-3 inhibitors belong to combined group of cell cycle modulators and apoptosis inhibitors. They were tested in a variety of renal parenchymal diseases and their efficacy was reviewed previously [59].

Furthermore, G2/M arrested cells were identified as a novel therapeutic target in kidney disease. G2/M arrest correlates with the development of fibrosis and provides thus pathophysiological link between AKI and CKD [39]. The therapeutic strategies targeting the G2/M arrest involve inhibition of ATM pathway, histone deacetylase and p53 inhibition, blocking of JNK pathway and depletion of senescent cells [17].

Another new therapeutic target is the MDM2-p53 pathway. Small molecule MDM2 inhibitors, such Nutlin-3a, are tested in clinical cancer trials. In kidney disease, they could be used to avoid cell death by mitotic catastrophe and to diminish injury in early injury phase of AKI [29, 37, 54]. However, such drugs should be used with caution in the regeneration phase of AKI due to their anti-regenerative effects [54]. Pharmacologic inhibition or genetic suppression of p53 was also found to improve AKI in various experimental models [60, 61]. Nevertheless, it is important to emphasize that ~50% of human cancers harbour p53 deletions and mutations and that p53 deficiency in mice is associated with a high frequency of spontaneous cancers [62], therefore such treatments could be used only with great caution and for a limited time interval. However, it has been reported that short-term inhibition of p53 with subsequent restoration of its normal function is safe even from the standpoint of potentially increased carcinogenicity known to be associated only with complete and permanent p53 loss [63, 64].

**Table 1. Key points about the cell cycle in the kidney**

- Regulation of the cell cycle is essential for maintaining homeostasis. Upon injury activation of the cell cycle may be needed to replace lost cells, e.g. by hypertrophy or cell division of surviving cells
- Cell cycle entry may lead to
  1. Hypertrophy (cell cycle arrested at restriction point)
  2. Cell division into two living cells (mitosis, cell proliferation)
  3. Cell death during/upon cell division (mitotic catastrophe)
- Markers like Ki-67, PCNA or BrdU indicate only cell cycle entry and, hence, cannot tell whether the cell undergoes hypertrophy, it will divide or rather die
- Clonal proliferation of single cells can be proven by multicolour lineage tracing
- Mitotic catastrophe assures that cells with persistent DNA damage are eliminated during mitosis, a mechanism that prevents the persistence or expansion of potentially malignant cells. Mitotic catastrophe also occurs, when cells that cannot undergo cytokinesis are forced to complete the cell cycle. This mechanism may contribute to podocyte loss in HIVAN
- Drugs that affect the cell cycle can be curative only in particular disease contexts. Cell cycle inhibitors prevent abnormal cell hyperplasia but inhibit wound healing. Cell cycle activators may enforce tissue regeneration but also promote hyperplasia, cancer growth and fibrosis

PCNA, proliferating cell nuclear antigen; BrdU, 5-bromo-2'-deoxyuridine; HIVAN, human immunodeficiency virus-associated nephropathy.

### FUTURE PERSPECTIVES

The cell cycle is an important component of renal tissue homeostasis, injury and repair (Table 1). In homeostasis it assures maintenance of post-mitotic cells (podocytes) and appropriate basal cell turnover in the kidney. Upon injury cell cycle activation has three possible outcomes: cell hypertrophy or mitosis, whereby mitosis can lead to cell division (proliferation) or cell death. Traditional cell cycle markers are often erroneously taken as proliferation markers, although cell cycle entry alone does not imply which of the three possible outcomes may occur. Dysregulation of cell cycle programme can result in cell loss and tissue atrophy, insufficient regeneration, premature senescence and thus progression of AKI in CKD, or to pathological hypertrophy and hyperplasia and scarring. The MDM2-p53 balance is a central element of cell cycle control and, hence, an attractive novel therapeutic target also in renal disease.

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### CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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