Epithelial proliferation and cell cycle dysregulation in kidney injury and disease

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Various cellular insults and injury to renal epithelial cells stimulate repair mechanisms to adapt and restore the organ homeostasis. Renal tubular epithelial cells are endowed with regenerative capacity, which allows for a restoration of nephron function after acute kidney injury. However, recent evidence indicates that the repair is often incomplete, leading to maladaptive responses that promote the progression to chronic kidney disease. The dysregulated cell cycle and proliferation is also a key feature of renal tubular epithelial cells in polycystic kidney disease and HIV-associated nephropathy. Therefore, in this review, we provide an overview of cell cycle regulation and the consequences of dysregulated cell proliferation in acute kidney injury, polycystic kidney disease, and HIV-associated nephropathy. An increased understanding of these processes may help define better targets for kidney repair and combat chronic kidney disease progression.

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The ability to repair and regenerate damaged tissue is essential for the restoration of functional integrity of tissues and organs and ultimately the survival of complex organisms. However, complete recovery and regeneration of tissues can be observed during prenatal development in humans, but it is largely lost during adult life.[1] In contrast, various nonmammalian vertebrates, such as certain salamanders (e.g., newts and axolotl), retain the capacity in adulthood to fully regenerate various organs and appendages and serve as model organisms to gain insights into near-perfect regeneration and scar-free healing.[2,3] In the context of kidney injury and repair, it has long been held that a complete recovery was possible following an acute kidney injury (AKI), because proximal tubular cells, as the major target of AKI, are endowed with the capacity to proliferate and replenish the lost cells. However, recent evidence indicates that the repair is often incomplete, leading to maladaptive responses that promote the progression toward chronic kidney disease (CKD).[4,5] Recent studies in experimental models of AKI have highlighted the cell cycle arrest of renal tubular epithelial cells (RTECs) as a key component in fibrosis development following AKI.[6,7] A similar cell cycle arrest of RTECs has been observed in the setting of HIV-associated nephropathy (HIVAN) that features a prominent tubulointerstitial injury that accompanies the collapsing variant of glomerulosclerosis.[8] In the contexts of polycystic kidney disease (PKD), such as autosomal dominant PKD (ADPKD), an unchecked tubular proliferation is a characteristic feature and a driver of disease pathogenesis. Thus, dysregulated cell cycle and proliferation of RTECs may underlie various modes of kidney injury and disease, whether in growth-arrested RTECs in AKI or hyperproliferative RTECs in ADPKD. Therefore, in this review, we provide a brief overview of the mammalian cell cycle and discuss the current understanding of the shared and nonoverlapping mechanisms of cell cycle dysregulation in RTECs in AKI, ADPKD, and HIVAN.

Canonical and noncanonical mitotic cell cycle

The mitotic cell cycle enables cell proliferation and division to occur with precise genome replication and duplication of its cellular components and machinery in the daughter cells necessary to repeat the process. The mammalian cell cycle consists of the S phase, in which genomic DNA is replicated; the M phase, in which the nuclear division (mitosis/karyokinesis) and cell division (cytokinesis) occur; and the 2 gap

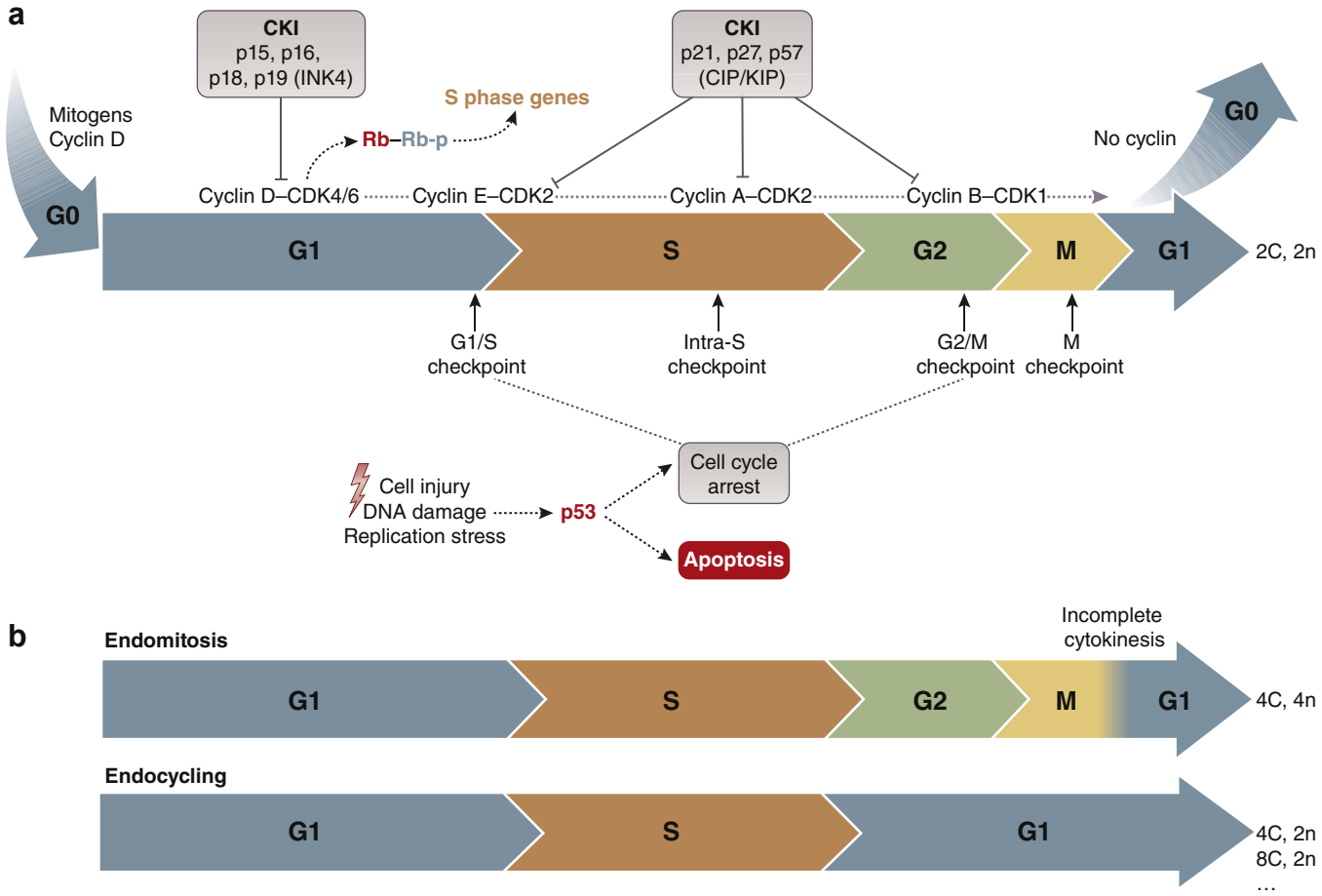

Figure 1 | Canonical and noncanonical cell cycle in eukaryotic cells. (**a**) The schematics of the canonical mitotic cell cycle with specific expression patterns of cyclins and cyclin-dependent kinases (CDKs) are shown above the phases of the cell cycle with a dashed line. Cell cycle checkpoints are indicated below with arrows. (**b**) The schematics of endoreplication showing endomitosis (top) and endocycling (bottom). In endomitosis, cells undergo incomplete cytokinesis and generate a polyploid binucleate or polyploid mononucleate cell. In endocycling, cells increase their DNA by alternating between G and S phases without cell division. Polyploid cells can also reduce ploidy through multipolar spindle formation, a process known as ploidy reversal. During this ploidy reversal process, chromosome segregation errors are common, resulting in the formation of aneuploid daughter cells with a gain or loss of ≥1 individual chromosomes. C, total number of pairs of chromosomes in the cell, chromatin amount or DNA content as a multiple of haploid genome (e.g., 4C = chromatin amount of a diploid cell in the G2 phase of the cell cycle); CIP/KIP, CDK-interacting protein/Kinase inhibitory protein; CKI, cyclin-dependent kinase inhibitors; INK4, inhibitors of CDK4; n, number of sets of chromosomes as a multiple of the haploid genome (e.g., 2n = chromosomal content of a diploid cell); Rb, retinoblastoma.

phases (G1 and G2) that separate the S and M phases. G1 is a particularly important regulatory period, as once the cell passes the G1 checkpoint and enters the S phase, it becomes irreversibly committed to progression through the cell division cycle. The fidelity and the coordination of each of these events are tightly regulated through the sequential activation of cyclin-dependent kinase (CDK) complexes, composed of cyclins that are expressed and degraded at specific cell cycle phases and CDKs whose activities are regulated by interactions with activators and inhibitors to ensure that the cell cycle progresses in one direction only.[9,10] As outlined in Figure 1a, mammalian cells enter the cell cycle in response to cyclin D expression, which is required for CDK4 and CDK6 activation. Cyclin E accumulates in the late G1 phase and specifically activates CDK2. Together with cyclin D–CDK4/6 complex, cyclin E–CDK2 is responsible for passage through the late G1 and transitioning into the S phase, which involves the inhibitory hyperphosphorylation of retinoblastoma, allowing for the expression of genes necessary for DNA replication and further cell cycle progression. Among these genes is cyclin A that will complex with CDK2 and replace cyclin E that is degraded during the progression of S phase. The cyclin A–CDK2 remains active through S and early G2 phases. With the subsequent degradation of cyclin A during G2, the activation of a cyclin B–CDK1 complex drives the G2/M transition and commencement of mitosis. Concomitant with cyclin B/CDK1 degradation, mitotic exit and cytokinesis occur. In addition to these tightly coordinated activations of cyclin-CDK complexes, cell cycle progression is regulated by CDK inhibitors comprising the inhibitors of CDK4 (INK4) and CDK interacting protein/Kinase inhibitory protein (Cip/Kip) family members. The INK4 proteins ($p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$) suppress the cyclin D–CDK4/6 complex during G1, whereas Cip/Kip family members

($p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$) restrain the activities of a broader set of cyclin-CDK complexes[11] (Figure 1a). These cell cycle regulators in the context of kidney cell homeostasis and injury have been widely studied and summarized in excellent reviews.[12–14] Several quality-control checkpoints are also placed during the cell cycle to guard against damaged or incompletely replicated DNA (between G1/S, G2/M, and intra-S) and to monitor chromosome alignment for proper segregation of the sister chromatids (mitotic checkpoint, also called spindle assembly checkpoint). As cells exit the M phase at the end of the cell cycle, they commit to the next round of cell cycle progression or cell cycle exit (G0). G0 is a reversible quiescent nondividing state, which is nevertheless actively maintained by continual repression of genes necessary for cell division and activation of genes to block apoptosis and senescence.[15–17] Terminal differentiation is coupled with a permanent exit from the cell cycle, where cells are refractory to proliferative signals and associated with low expression of cyclins and CDKs, hypophosphorylation of retinoblastoma, and upregulation of CDK inhibitors to ensure the inhibition G1 progression.[18,19]

The noncanonical mitotic cell cycle (*i.e.*, endoreplication) is also observed in mammalian cells, including the mouse kidney RTECs.[20,21] Although a strict consensus in the nomenclature is not yet found, endoreplication comprises *endocycling*, in which cycling cells with fully replicated DNA (from S or G2) bypasses the M phase entirely and re-enters the next G1; and *endomitosis*, in which cells that have entered M phase re-enter the G1 before completion of karyokinesis or cytokinesis[22–24] (Figure 1b). As a result, endoreduplication results in polyploidization (duplicated chromosomes, e.g., 4C, 8C, or 16C). Although commonly observed in invertebrates and plants, the occurrence of endoreplication was considered to be rare in mammalian cells, with the exception of few specialized cell types, such as trophoblasts, megakaryocytes, and liver hepatocytes, as a part of development and differentiation programs.[22] However, recent studies show more widespread occurrences of endoreplication in terminally differentiated mammalian cells. Endoreplication has been observed in epidermal differentiation,[25] vascular smooth muscle cells in response to various hypertensive stimuli,[26–28] mammary epithelial cells in lactation,[29] and liver development[30–32] and regeneration.[33] The emerging theme is that within the correct timing and context, endoreplication in differentiated cells, particularly those with high metabolic activity, serves to promote mammalian tissue growth and repair.[22–24] It is hypothesized that endoreplication, rather than mitotic cell division, would provide a homeostatic advantage in situations where energy sources are limiting and rapid growth is required to sustain mass production of proteins and high metabolic activity during tissue growth and repair. However, polyploidization and subsequent aneuploidy and genomic instability are also hallmarks of oncogenic tumors that drive cancer progression.[34] Thus, it remains to be shown how the polyploid cells in tissue repair can remain mitotically active and accumulate genomic instability without becoming tumorigenic. In addition, molecular mechanisms coordinating proliferation and endoreplication in tissue adaptation, particularly in the kidney, remain to be better elucidated.

RTEC proliferation and cell cycle dysregulation

Acute kidney injury. Under the normal condition in the adult kidney, cells are mainly quiescent, with proliferation occurring in <1% of tubular cells.[35,36] It is notable that a sizable portion of the proximal tubular cells, particularly in the S3 segment, expresses markers consistent with being in G1, rather than in G0, quiescence.[37,38] This is thought to poise the S3 proximal tubular cells, most susceptible to acute kidney insult, to be primed for rapid cell division for efficiency in recovery, as cells entering from G0 have a significantly longer G1 phase than actively cycling ones.[39] On AKI, such as those induced by toxic or ischemic insults, heavily injured cells are lost through apoptosis or necrosis, but surviving tubular cells rapidly re-enter the cell cycle and proliferate to restore the nephron function[40–42] (Figure 2). Interestingly, concomitant with cell cycle entry, rapid induction of cell cycle inhibitor $p21^{Cip1}$ (p21) has been observed in RTECs in various experimental models of AKI.[43] The activation of p21 to put the brakes on the cell cycle progression seem counterproductive in RTECs in AKI, but p21 (and $p27^{Kip1}$) has been shown to participate in the assembly and nuclear import of cyclin D/CDK4 complex during the early G1 progression, in addition to their cell cycle inhibitory roles.[44,45] Thus, it is presumed that p21/p27's participation in G1 progression while delaying the G1/S transition is an adaptive mechanism that allows sufficient time for proper DNA repair in injured RTECs. Such protective role of p21 in RTEC repair is in accordance with the observation that p21 knockout mouse kidneys displayed worsened tubular damage and increased mortality following cisplatin exposure or ischemia-reperfusion injury.[46,47] Loss of p21 also mitigated the beneficial effects of renal ischemic preconditioning on ischemia-reperfusion injury in mice.[48] Similarly, the delay of S phase entry by CDK4/6 inhibitors, palbociclib and ribociclib, reduced tubular cell apoptosis in experimental models of AKI.[49,50] Because the inhibition of CDK4/6 by palbociclib and ribociclib is transient and reversible,[49–51] it is thought that their actions sufficiently slow down the G1/S transition to mitigate cell death but allow subsequent cycling and division necessary for recovery, further supporting the notion that appropriately regulated G1 progression is essential in proper kidney repair after AKI. Paradoxically, in contrast to the protective role in the setting of AKI, p21's actions are associated with the worsening of CKD progression.[52] In the murine remnant kidney model, the genetic ablation of p21 attenuated the histologic lesions and prevented the progressive kidney decline in kidney function.[52] These contrasting observations of p21 inhibition suggest that transient cell cycle arrest may be a protective mechanism in acute injury, whereas prolonged cell cycle arrest is deleterious. Moreover, well beyond the cell cycle regulatory role, p21 is now recognized as

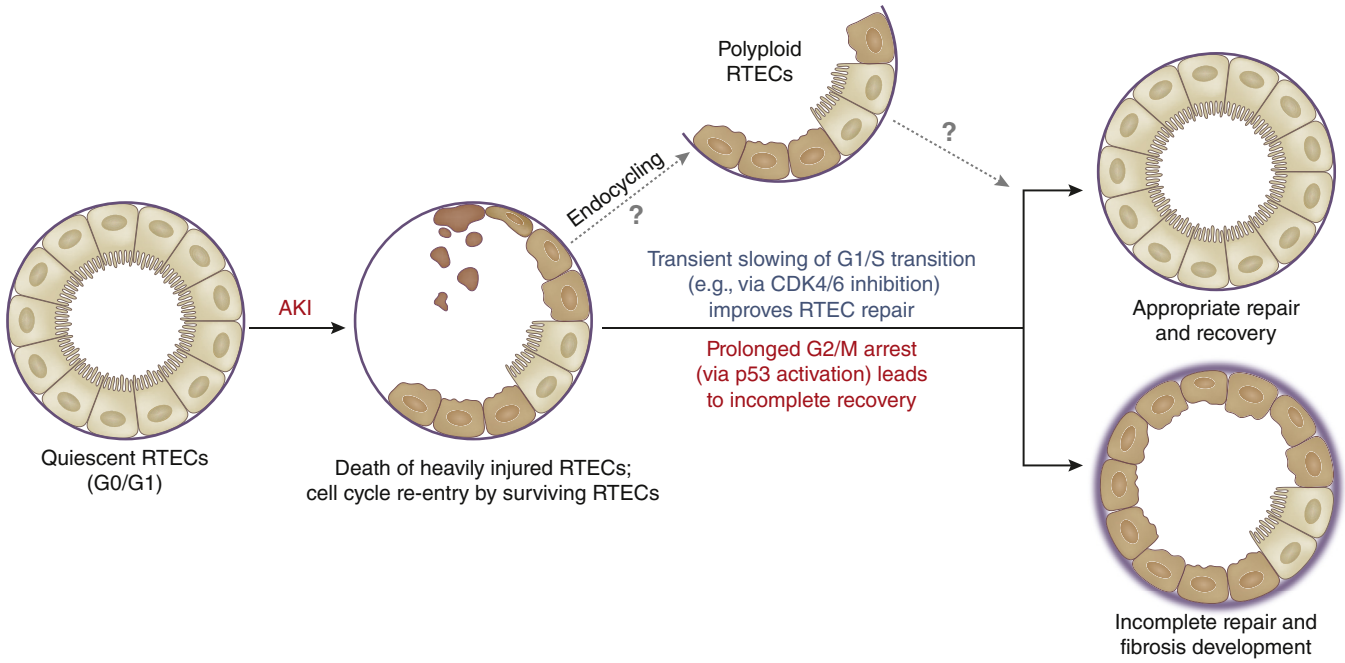

Figure 2 | Renal tubular epithelial cell (RTEC) repair following acute kidney injury (AKI). On AKI, normally quiescent RTECs in G0 or G1 re-enter the cell cycle and proliferate. Slowing the G1/S transition aids in proper RTEC repair, but prolonged G2/M arrest leads to premature senescence and incomplete recovery. CDK, cyclin-dependent kinase.

a multifunctional protein whose actions include regulation of apoptosis, differentiation, transcription, and migration.[53–55] Thus, it is likely that the actions of p21 beyond the cell cycle regulation contribute to RTEC impairment and recovery after AKI.

The importance of proper cell cycle regulation in RTECs in kidneys recovering from AKI is further underscored by the work of Yang *et al.*, which showed that the proliferating proximal tubular cells after AKI are often arrested in G2/M checkpoint and that the ensuing fibrosis development correlates with the extent of G2/M arrest.[7] G2/M arrest typically triggered by DNA damage is a reversible process, following DNA repair and downregulation of p53. p53, as one of the key guardians of genome integrity, provides surveillance against various cellular insults, including missegregated chromosomes, by inducing cell cycle arrest and senescence.[56] However, irreversible cell cycle arrest by p53 leads to premature cellular senescence.[57,58] Consistent with prolonged and irreversibly arrested cells, G2/M-arrested proximal tubular cells in response to AKI assumed a senescent-associated secretory phenotype, thereby producing increased amounts of profibrotic factors.[7] The heterogeneous composition of senescent-associated secretory phenotype molecules that are released into the surrounding microenvironment includes growth factors, inflammatory cytokines, and modulators of extracellular matrix, such as insulin-like growth factor 1, interleukin-6 and interleukin-8, plasminogen activator inhibitor-1, and transforming growth factor beta.[59] Interestingly, in obstructed kidneys, enhanced transforming growth factor beta signaling in tubules also triggered p21-mediated G2/M arrest in RTECs,[60,61] suggesting a vicious cycle between cell cycle arrest–mediated senescent-associated secretory phenotype and irreversible RTEC cell cycle arrest.

Although p53-mediated cell cycle arrest is an essential trigger of senescence induction, the activities of other pathways converge to make the process irreversible, such as those induced by $p16^{INK4a}$/retinoblastoma, nuclear factor-κB, and mechanistic target of rapamycin.[62–64] In oncogene-induced senescent cells, senescent-associated secretory phenotype production is further boosted by a reorganization of endomembrane compartments to coordinate protein synthesis and autophagy by mechanistic target of rapamycin, termed target of rapamycin–autophagy spatial coupling compartments.[65] In line with these observations, Canaud *et al.* demonstrated the increased formation of target of rapamycin–autophagy spatial coupling compartments in G2/M-arrested proximal tubular cells during kidney fibrosis progression, in part mediated by p53-induced expression of atypical cyclin, cyclin G,[6] such that genetic deletion of Raptor, a major component of mechanistic target of rapamycin complex 1, or pharmacologic inhibition of p53 reduced the target of rapamycin–autophagy spatial coupling compartment formation and ensuing renal fibrosis progression after AKI.[6] However, analogous the opposing role of p21 in AKI and in CKD, although the inhibition of p53 has shown to promote better recovery after AKI recovery in rodent models,[66–71] the long-term inhibition of p53 in kidneys after AKI is also associated with augmented renal inflammation and fibrosis.[72–74] These observations are not entirely surprising, given the multifaceted role of p53 that can at times exert dichotomous effects on biological processes in that in

contrast to its well-defined antiproliferative and proapoptotic functions, p53's role is also implicated in promoting cell survival[75] and reducing inflammatory responses and levels of intracellular reactive oxygen species.[76–79] Thus, collectively, these studies underscore the complexities and importance of canonical cell cycle regulation in RTECs in the setting of AKI and CKD progression (Figure 2) and the need to consider the spatiotemporal inhibition of key cell cycle regulators in therapeutic approaches to AKI and CKD.

In addition to the canonical cell cycle entry following AKI, a recent study by Lazzeri *et al.* demonstrated that both the canonical (mitotic) cell cycle and noncanonical endoreplication (endocycling) occur in mouse RTECs following ischemia-reperfusion injury.[21] Interestingly, only a small subset of cells that were of paired box (Pax)2^+ progenitors proliferated via mitotic cell cycle, whereas the majority of tubular Pax8^+ cells, particularly in the S1 and S2 segments of the proximal tubules, underwent endocycle-mediated hypertrophy.[21] Although both Pax2 and Pax8 transcription factors are coexpressed at the onset of kidney development and together are critical regulators of nephric lineage specification,[80–82] Pax2 expression becomes downregulated in most nephron segments in the adult kidneys except in the collecting duct and renal papilla, whereas Pax8 expression continues to be widespread in all epithelial cells. However, high expression of Pax2 has been noted in renal cancer[83–85] and hyperproliferative cyst-lining cells in PKD.[86–88] Congruent with the findings by Lazzeri *et al.*, the study by Manolopoulou *et al.* also reported an increased number of polyploid proximal tubular cells following the aristolochic acid–mediated kidney injury.[89] However, in another study examining the proliferating RTECs following ischemia-reperfusion injury in mice by lineage tracing of kidney injury molecule-1 (KIM1)–positive cells, the authors did not observe increased DNA content that would be consistent with endoreplication in this cell population.[90] Moreover, their findings support the clonal expansion of injured KIM1–positive cells as mainly responsible for recovery after AKI, rather than proliferation of a subset of fixed progenitor cells.[90] Thus, it remains to be further clarified whether the endoreplication of RTECs in addition to the canonical mitotic division and expansion occurs as a means of adaptive and reparative mechanism in response to AKI (Figure 2). If so, it also remains to be determined whether the polyploid RTECs in recovered kidneys will eventually undergo ploidy reversal to reduce the chromosome content, as observed in other organs, and what impact, if any, such reduction in ploidy would subsequently yield in RTECs and in the susceptibility of CKD progression.

Polycystic kidney disease. PKD is a common genetic disorder characterized by progressive growth and expansion of fluid-filled cysts in the renal parenchyma and progressive development of tubulointerstitial fibrosis, leading to renal insufficiency.[91] Notably, gene products whose mutations result in PKD are involved in the structure or function of the primary cilium. ADPKD, the most common form of PKD, is caused by a mutation in *PKD1* and *PKD2* genes that encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively.[92] It is believed that cyst initiation occurs when the level of functional polycystins falls below the "cystogenic threshold," as a consequence of the germline mutation, somatic mutation ("second hit"), and/or genetic modifiers that affect PC1/PC2 expression[93] (Figure 3). Nevertheless, the molecular mechanisms governing the cystogenic conversion as a consequence of lowered polycystin expression remain elusive, despite a large body of information that has been acquired over the past years on the genetics and cellular functions of cystic kidney cells. One of the hallmark features of ADPKD and a determinant of kidney failure is the hyperproliferation of RTECs with the continuous expansion of the cysts.[94,95] Diverse signaling mechanisms are implicated in hyperproliferation and cell cycle dysregulation of cystic PKD cells.[96] The first direct evidence linking PC1 and cell cycle regulation came from the observation that PC1 overexpression induces G1 arrest through the Janus kinase (JAK)–signal transducer and activator of transcription (STAT)–dependent expression of p21.[97] Conversely, knockdown of *Pkd1* lowered the fraction of cells in G1/S, suggesting that loss of PC1 may drive premature G1/S transition.[98,99] PC2 overexpression similarly arrested RTECs in G1/S, which occurred through the cytosolic sequestration of inhibitor of DNA binding 2 (Id2),[100] a member of the helix-loop-helix family of transcription regulators that enables cell cycle progression by repressing p21 and activation of various genes involved in G1/S transition by binding to retinoblastoma.[101] Notably, the cytosolic sequestration of Id2 by PC2 was also shown to be PC1 dependent,[100] suggesting that under basal conditions, both polycystins participate in keeping RTEC proliferation in check, in part through increased p21 expression. Indeed, increased nuclear Id2 expression was observed in cyst-lining epithelia in the *Pkd1*-null kidneys, and deletion of *Id2* attenuated cyst formation in *Pkd1*-null mice.[102] In line with cell cycle dysregulation and hyperproliferation as a major component driving PKD pathogenesis, roscovitine, a potent inhibitor of CDKs,[103] was shown to effectively reduce the cystic volume and improve kidney function in *jck* and *cpk* PKD mice (characterized by the mutation of the *Nek8* and *Cystin* genes, respectively),[104,105] and more recently in collecting duct-specific cilia lacking *Cep164*-deficient PKD mice[106] and in *Pkd1*-deficient mice.[107]

Associated with the hyperproliferative phenotype of PKD RTECs is the reactivation of developmentally regulated genes, such as *Cux1* and *Pax2*.[80–82,108] The homeodomain protein Cux1, which promotes cell cycle progression by suppressing the transcription of the G1-checkpoint CDK inhibitors p21 and p27,[109,110] was shown to be highly expressed in PKD kidneys.[111,112] The loss of *Cux1* in a murine model with conditional inactivation of *Pkd1* in collecting ducts increased the expression of p27, reduced the severity of the cystic disease, and prolonged survival.[113] Persistent Pax2 expression is also observed in proliferating cystic RTECs of human juvenile cystic and ADPKD kidneys, and in various murine models of PKD.[86,87,114]

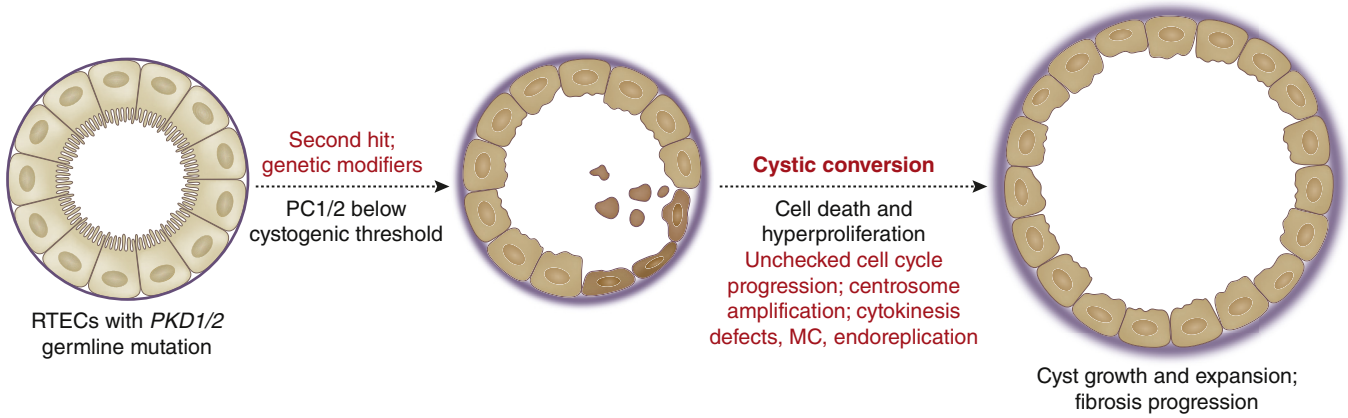

Figure 3 | Renal tubular epithelial cell (RTEC) proliferation in autosomal dominant polycystic kidney disease. Loss of polycystin-1 (PC1) or polycystin-2 (PC2) proteins beyond the "cystogenic threshold" in RTECs with the *PKD1* or *PKD2* germline mutation through the genetic mutation alone, an acquired somatic mutation ("second hit"), and/or genetic modifiers results in cystogenesis and hyperproliferative RTECs marked with cell cycle anomalies. MC, mitotic catastrophe.

Reduction in *Pax2* gene expression attenuated the cystic severity in the *cpk* mouse model (that resembles autosomal recessive PKD)[86] and in the mouse model of ADPKD.[87] The interference with growth-promoting pathways has shown to be similarly effective in reducing the cystic burden in various experimental PKD models. Although signaling by cyclic adenosine monophosphate in RTECs tends to have an inhibitory effect on proliferation, its high accumulation in cystic cells was shown to promote proliferation in a B-Raf and mitogen-activated protein kinase dependent manner.[115,116] Cyclic adenosine monophosphate also contributes to cystic growth by stimulating chloride and fluid secretion.[117] Pharmacologic antagonism of the vasopressin V2 receptor that attenuates cyclic adenosine monophosphate signaling was shown to be effective in various PKD animal models.[118,119] Tolvaptan, a select V2 receptor antagonist, was shown to slow the kidney function decline in ADPKD patients,[120] although its effect on estimated glomerular filtration rate was moderate, and a considerable proportion of patients reported polyuria and polydipsia among the adverse events. It is the first Food and Drug Administration–approved drug for slow kidney function decline in adults at risk of rapidly progressing ADPKD.[121]

Interestingly, many of the cellular pathways dysregulated in PKD cells bear much resemblance with those in cancer cells, including cell cycle dysregulation, even though PKD does not lead to the formation of spontaneous renal tumors.[122] Similar to tumorigenesis, renal cysts are thought to arise from clonal populations that have undergone additional "hits" beyond the genetic mutation.[123–130] Other intriguing parallel features noted between malignant lesions and renal cystic cells are the genomic instability and the presence of supernumerary centrosomes as a result of aberrant cell division and endomitosis. Battini *et al.* showed that the knockdown of *Pkd1* expression in cultured collecting duct cells induced centrosome amplification, leading to multipolar spindle formation and defective cytokinesis that subsequently led to mitotic catastrophe (MC).[131] MC is distinct from programmed cell death as a result of prolonged arrest at G2/M checkpoint.[34] But it is functionally defined, as widely studied in the context of neoplastic transformation, as "an oncosuppressive cascade that precedes but operates through apoptosis, necrosis, or senescence for the avoidance of genomic instability in cells sensing mitotic failure."[34,132] Although the precise mechanisms that trigger MC in cells are not well defined, it is triggered during the prolonged mitotic arrest and results in gross nuclear changes, such as multinucleation, macronucleation, or micronucleation.[132] Interestingly, extensive studies in cancer cells with antimitotic agents have shown that during a prolonged mitotic arrest, a subset of cells can escape the demise of MC through endomitosis and exiting the cell cycle prematurely by cyclin B degradation.[133,134] Similarly, although PKD1 loss resulted in MC in cultured collecting duct cells, a subset of hyperproliferative cells with abnormal ploidy was also observed.[131] Interestingly, the surviving polyploid cells over time underwent ploidy reversal, converging toward a parental ploidy and establishing a relatively stable cell population, although cytologic abnormalities, such as micronucleation and aneuploidy, remained common in the proliferating population of cells.[131] Similar observations of defective cytokinesis and polyploidy were made in endothelial cells with *Pkd1* or *Pkd2* loss[135] and in capillary endothelial cells from ADPKD patients.[136] A common aberration in cells with abnormal ploidy is centrosome amplification in both cancer and in renal cysts,[137–139] as duplication of centrosomes is tightly coordinated with cell cycle and division. Mahjoub *et al.* demonstrated that the overduplication of centrosomes leads to disrupted primary cilium signaling in epithelial cells,[140] and Dionne *et al.* further demonstrated that centrosome amplification *in vivo* predisposed kidneys in adult mice to cystic development following ischemic kidney injury, without alteration in established cystic genes.[141] These studies highlight the role of centrosome and ciliary signaling in cell cycle

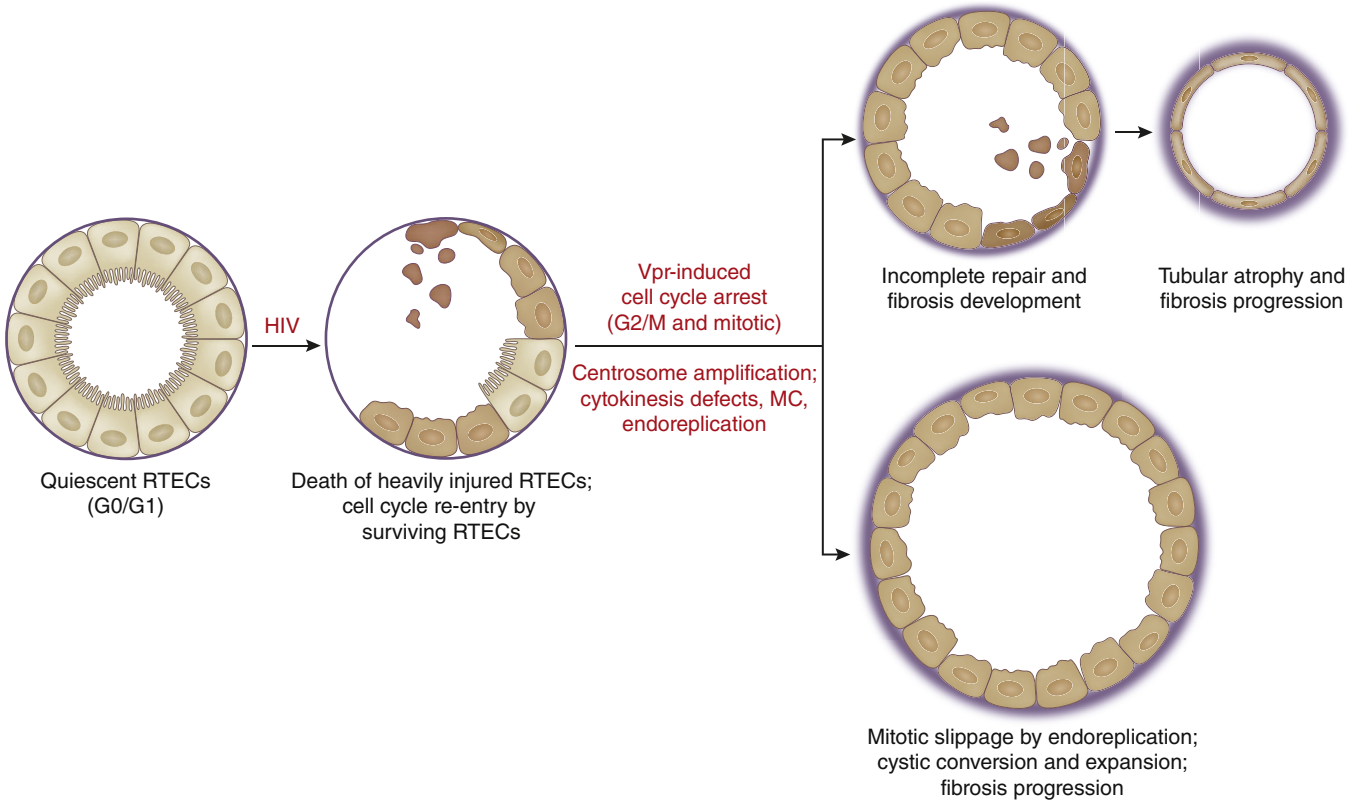

Figure 4| Renal tubular epithelial cell (RTEC) proliferation and injury in HIV-associated nephropathy (HIVAN). HIV infection of RTECs (and Vpr expression) results in ongoing RTEC injury, marked by cell cycle arrest, tubular atrophy, cystic development, and fibrosis. The 2 distinct fates of RTEC injury due to Vpr injury in HIVAN are hypothesized from *in vitro* and *in vivo* findings. MC, mitotic catastrophe.

aberration in cystic cells[142] and suggest that it may be a common denominator in renal cystic diseases (Figure 3). Together, these observations indicate that although proliferation is not per se sufficient to cause cystogenesis, targeting the hyperproliferation and aberrant cell cycle and division may be a promising approach to slow down the cystic growth and expansion, and thereby the disease progression.

HIV-1–associated nephropathy. Classic HIVAN, caused by HIV-1 infection of renal epithelial cells, including the podocytes,[143–145] is characterized histologically by the collapsing form of glomerulosclerosis, tubular microcystic dilatation and degeneration, interstitial inflammation, and fibrosis[8,146] (for recent comprehensive reviews on HIVAN, see these studies[147–149]). HIVAN patients develop nephrotic-range proteinuria with renal insufficiency, which often rapidly progresses to end-stage kidney disease. Characterization of HIVAN pathology in experimental mouse models has suggested that the glomerular injury precedes and likely disposes the tubular epithelial cells to subsequent inflammation and apoptosis.[150,151] Nevertheless, early histologic findings of HIVAN kidneys have noted a significant tubular injury that appeared disproportionate to the glomerular pathology,[8] suggesting that some tubular injury may arise directly through HIV infection. Indeed, HIV-1 gene expression and ensuing microcyst development were observed in multiple segments of the nephron.[144,152]

The pathogenic mechanisms of HIVAN have been significantly aided by the use of transgenic mouse models, which have shown that the renal expression of HIV genes, particularly the genes that encode accessory proteins Negative Regulatory Factor (Nef) and Viral Protein R (Vpr), are directly responsible for HIVAN pathogenesis.[150,153–155] HIV-1 encodes 4 accessory proteins, namely Nef, Vpr, Viral Infectivity Factor (Vif), and Viral Protein U (Vpu), that are dispensable for replication in some cell types *in vitro*, but are essential for viral pathogenesis.[156] Although the full function of the accessory proteins is not entirely understood, the emerging theme from various studies is that the accessory proteins serve to counteract the host antiviral defense mechanisms through the sequestration and recruitment of multiple host proteins for the cellular degradation machinery.[156,157] Interestingly, a recent study using unbiased quantitative proteomics showed that almost all proteomic changes as a result of the HIV accessory protein-mediated degradation in HIV-1–infected T cells were mediated by Vpr.[158] Vpr is a multifunctional protein whose role includes the transactivation of the viral promoter, nuclear import of HIV preintegration complexes, activation of DNA damage response, cell cycle arrest, and apoptosis.[159] In HIVAN, various studies have also demonstrated Vpr as a major culprit in the demise of RTECs. Vpr expression in human RTECs (HK-2 cells) induced DNA damage response[160] and apoptosis that was dependent on sustained extracellular signal-regulated kinase

Table 1 | Cell cycle dysregulation in RTECs and podocytes in kidney injury and disease

Cell cycle regulation/impairment	Mediators	Results	References
RTECs in AKI-CKD			
G1/S transition delay	↑p53/p21 (and p27) and ↓CDK2/cyclin E activity	Improved RTEC recovery after AKI	46–48
	Transient CDK4/6 inhibition	Improved RTEC recovery after AKI	49, 50
G2/M checkpoint arrest	Cyclin G → ↑p53/p21	SASP, TASCC formation, and fibrosis	6, 7
	TGF-β → ↑p21	Tubular damage, EMT, fibrosis	60, 61
Endocycling ($Pax8^+$ RTECs)	Not identified	Polyploidy and hypertrophy	89
PKD (ADPKD)			
Unchecked cell cycle progression	Loss of PC1 → ↓p53/p21	RTEC hyperproliferation	97, 172, 173
	Loss of PC1 → ↑Cux1, ↑Pax2		108, 111, 112
			86, 87, 114
Premature G1/S transition	Loss of PC1 → ↓STAT1 activation → ↓p21	Cell cycle progression and hyperproliferation	97
	Loss of PC1 → ↑cyclin A		98, 99
	Loss of PC2 → ↑Id2 nuclear translocation and gene expression		100
Cytokinesis defects	Loss of PC1	Centrosomal amplification, MC	131, 141
		Mitotic slippage/endomitosis, aberrant ploidy	
RTECs in HIVAN			
G2/M checkpoint arrest	Vpr → DNA damage response (ATM/ATR)	Caspase-mediated cell death	160, 161, 171
Cytokinesis defects	Vpr→ FAT10	Centrosome amplification, MC	162–166, 171
	Vpr → DNA-PK	Mitotic slippage/endomitosis, aberrant ploidy	

ADPKD, autosomal dominant polycystic kidney disease; AKI, acute kidney injury; ATM, Ataxia telangiectasia mutated; ATR, ATM and RAD3-related; CDK, cyclin-dependent kinase; CKD, chronic kidney disease; Cux, Cut-like homeobox; DNA-PK, DNA-dependent protein kinase; EMT, epithelial-to-mesenchymal transition; FAT10, HLA-F–adjacent transcript 10; HIVAN, HIV-associated nephropathy; MC, mitotic catastrophe; Pax, paired box; PC1, polycystin-1; PC2, polycystin-2; PKD, polycystic kidney disease; RTEC, renal tubular epithelial cell; SASP, senescence-associated secretory phenotype; STAT, signal transducers and activators of transcription; TASCC, target of rapamycin–autophagy spatial coupling compartment; TGF-β, transforming growth factor-β; Vpr, Viral Protein R.

activity and caspase-8– and caspase-9–mediated apoptosis.[161] In addition to Vpr's well-established role of inducing G2/M arrest, cell cycle anomaly by Vpr extends to aberrant mitosis due to centrosome amplification, aberrant mitotic spindles, and cytokinesis failure, which result in multinucleated cells and/or mitotic cell death in various cells *in vitro*.[162–164] *In vivo*, hypertrophic multinucleated tubular cells were observed in kidneys of Tg26 HIVAN transgenic mice, and increased chromosome content in human HIVAN kidney biopsies by *in situ* hybridization.[164] One mechanism by which Vpr induces RTEC apoptosis and polyploidy was shown to be mediated through HLA-F–adjacent transcript 10 (FAT10), a ubiquitin-like modifier that targets proteins for proteasomal degradation. FAT10 was shown to be one of the most highly upregulated genes in HIV-infected RTECs,[165] and the suppression of FAT10 expression in cultured RTECs prevented Vpr-induced apoptosis and polyploidy.[166] Although the exact mechanism of FAT10 in Vpr-induced RTEC cell cycle regulation requires further analysis, these observations in RTECs are consistent with the recent findings showing the involvement of FAT10 in the regulation of mitotic progression.[167] Interestingly, a recent study has implicated common variants in *UBD*, encoding FAT10, as a genetic modifier of Apolipoprotein-L1 (APOL1)–mediated CKD risk, and showed the protective effects of FAT10 in cells overexpressing *APOL1* risk variants in cultured cells.[168] Given that *APOL1* risk variants are strongly associated with HIVAN,[169,170] whether FAT10 is also involved in Vpr-mediated cell cycle deregulation and injury in glomerular epithelial cells (i.e., podocytes) remains to be explored.

More recently, work by Payne *et al.* has unraveled the molecular mechanism of RTEC cell cycle deregulation by Vpr.[171] They demonstrated that although the Vpr expression in RTECs initially results in G2 arrest, many cells eventually enter mitosis and the majority of the cells succumb to MC and death as a result of supernumerary centrosomes and multipolar spindle formation.[171] Notably, a smaller fraction of Vpr-expressing RTECs overcame MC and death by exiting mitosis via endomitosis and polyploidization.[171] The induction of Vpr expression in RTECs under the control of *Pax8* promoter-driven reverse tetracycline transactivator transgene in mice (Pax8-Vpr mice) resulted in overt tubulointerstitial injury, cystic development, and renal failure (Chen Y, D'Agati V, Lee K, He JC, manuscript in preparation, personal communication, 2021). Consistent with *in vitro* observations of cell cycle arrest, MC, and polyploid RTEC fates by Vpr, multinucleated RTECs were readily visible in Pax8-Vpr kidneys as well as atrophic tubules. In addition, Pax8-Vpr mice manifested overtly cystic kidneys over time with increased proliferation in cyst-lining cells. The eventual fate of Vpr-induced polyploid RTECs that escape MC *in vivo* remains to be determined, and at present, it is unclear what molecular pathways dictate the fates of Vpr-expressing RTECs to cell death and tubular atrophy versus cystic conversion and hyperproliferation. Intriguingly, the observations of centrosomal amplification, MC, and endoreplication observed in the subset of Vpr-expressing RTECs are redolent of cystic cells in PKD and raise the speculation that RTECs that escape the MC and death in Pax8-Vpr kidneys may acquire changes toward cystic conversion that is associated with PKD (Figure 4). Future investigations to explore different cell fates after Vpr-induced RTEC injury may shed

additional insights into the initial molecular signals initiating cystic conversion in PKD that remains yet to be defined.

Concluding remarks

Irrespective of the etiology of RTEC injury and whether the injury is acute or persistent, it is clear that cell cycle dysregulation underlies the fate of RTECs in progression to CKD (Table 1[6,7,46–50,60,61,86,87,89,97–100,108,111,112,114,131,141,160–166,171–173]). Rapid progression through phases of the cell cycle with improper checkpoints, as well as prolonged cell cycle arrest, are both deleterious in RTECs. Although considerable insights have been gained in the cell cycle dysregulation in the respective renal epithelial cells, much remains to be explored for a broader molecular insight in the complete cell cycle–associated pathways (canonical and noncanonical) for appropriate therapeutic interventions (accounting for both duration and intensity) in the treatment of renal diseases.

DISCLOSURE

All the authors declared no competing interests.

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