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Tubular epithelial cells (TCs) compose the majority of kidney parenchyma and play fundamental roles in maintaining homeostasis. Like other tissues, mostly immature TC with progenitor capabilities are able to replace TC lost during injury via clonal expansion and differentiation. In contrast, differentiated TC lack this capacity. However, as the kidney is frequently exposed to toxic injuries, evolution positively selected a response program that endows differentiated TC to maintain residual kidney function during kidney injury. Recently, we and others have described polyploidization of differentiated TC, a mechanism to augment the function of remnant TC after injury by rapid hypertrophy. Polyploidy is a condition characterized by >2 complete sets of chromosomes. Polyploid cells often display an increased functional capacity and are generally more resilient to stress as evidenced by being conserved across many plants and eukaryote species from flies to mammals. Here, we discuss the occurrence of TC polyploidy in different contexts and conditions and how this integrates into existing concepts of kidney cell responses to injury. Collectively, we aim at stimulating the acquisition of novel knowledge in the kidney field as well as accelerating the translation of this basic response mechanism to the clinical sphere.

Kidney International (2024) **105,** 709–716; https://doi.org/10.1016/j.kint.2023.10.036

KEYWORDS: hypertrophy; kidney injury; polyploidy; stress response; tubular epithelial cells

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Received 17 August 2023; revised 26 September 2023; accepted 5 October 2023; published online 9 January 2024

The kidney is a highly specialized organ with a complex architecture and a great diversity of functions and cell types. The kidney parenchyma is largely composed of tubular cells (TCs) that play a critical role in maintaining kidney function. Importantly, the number of nephrons is determined during prenatal kidney development and does not change even if the hemodynamic and metabolic workload increases during body growth and eventually with obesity or pregnancy or upon nephron loss during kidney injury.[1] However, kidney function can spontaneously recover after acute injury, depending on the degree of damage, which has long been interpreted as a sign of "kidney regeneration"[2] (see definitions in Box 1). Nevertheless, even stages 1–2 episodes of acute kidney injury (AKI) predispose to chronic kidney disease (CKD), although the risk of progression among individuals is heterogenous for reasons remaining largely obscure.[1,3,4] These observations suggest that kidney regenerative potential is rather limited, implying additional mechanisms drive spontaneous recovery of kidney function after AKI.[3,5,6] Accordingly, recent studies suggested that TC proliferation after injury is mostly attributable to a subpopulation of progenitor cells.[3] By contrast, in differentiated TC, entry into the cell cycle is frequently not followed by cell division, but rather triggers polyploidization,[3,5] suggesting a more complex kidney injury response than previously believed. Here, we review the evolving data on this novel response mechanism spanning from basic biological meaning up to the clinical implication of TC polyploidy in the kidney.

Polyploidization: a conserved stress response throughout evolution

Polyploid cells are defined as cells with >2 complete sets of chromosomes. Cells can become polyploid through different types of cell cycle–dependent mechanisms such as endocycle, endomitosis, mitotic slippage, and cytokinesis failure[7] (Figure 1) or via cell cycle–independent mechanism such as cell fusion[7] (not discussed in this review). However, polyploidy can also be a prerogative of entire organisms that are mostly generated via meiotic nonreduction.[8] In mammals, cell polyploidy has been traditionally considered the result of an abnormal DNA synthesis associated with DNA damage and potentially with cancer.[9] However, evidence obtained in plants and animal species indicate that polyploidy (at both the cellular and organism levels) was positively selected during evolution because it confers specific advantages under

Box 1 | Definitions

Regeneration: Structural restoration after injury, for example, via proliferation and differentiation of stem cells.
→The process is driven by renal progenitors replacing lost parenchymal cells, but this process requires time and is rarely able to fully restore all lost cells. Kidney regeneration is uncoupled from functional recovery early after acute kidney injury that involves different mechanisms (polyploidy and renal reserve).
Repair: Mechanical adaptation and stabilization involving tissue scarring and fibrosis.
→Focal segmental glomerulosclerosis lesions are the result of glomerular repair. Interstitial fibrosis can be the result of tubular repair or can be developed to replace lost nephrons.
Maladaptive repair: A process that leads to CKD development.
→The term suggests a dysregulated and incidental process that is corrupted or insufficient. On the contrary, the capacity to increase TC ploidy represents a strategy of kidney adaption to injury, resulting in CKD that was likely evolutionarily selected to avoid worst consequences such as death and irreversible function loss.
Dedifferentiation/EMT: Epithelial cells acquiring mesenchymal markers.
→The process occurs during the differentiation from epithelial progenitors to mature TCs or upon polyploidization, when the expression of all genes is enhanced, including mesenchymal genes.
Proliferation: Cells that are able to successfully complete the cell cycle, resulting in cell division/mitosis and hence cell population expansion.
→The cell of origin and the daughter cells have the same diploid DNA content (2C). Proliferation is the response pattern of immature cells that do not yet contribute to specific organ functions.
G2/M arrest: Cells that enter the cell cycle but do not complete nuclear division or mitosis.
→They were shown to be profibrotic and senescent, driving maladaptive repair. However, methods routinely used to detect cell cycle arrested cells cannot discriminate them from polyploid cells.
Polyploidization: Cells undergoing alternative cell cycles skipping (nuclear division and/or) mitosis. They have multiple DNA content (4C, 8C…).
→Polyploidization increases cell size (hypertrophy) and functional capacity without mitosis interruption, which is lifesaving in acute organ failure. Polyploidy is the response pattern of highly specialized differentiated cell types that would lose their specific functions if being forced to undergo mitosis.
Karyomegaly: Enlargement of nuclei that underwent cell cycle entry for DNA synthesis but did not undergo cell division, that is, polyploidization.
→These nuclei are hyperchromatic and often with multiple nucleoli. Identification of karyomegalic nuclei relies on standard microscopy investigation, limiting its recognition only to cell where this phenomenon becomes extreme (up to 30 μm in humans).
Multinuclear cells: Cell characterized by ≥2 nuclei, that is, polyploid cells, that underwent nuclear division but not cytokinesis, that is, cell division.
→Historically, the definition of polyploidy in the kidney was assimilated with the concept of multinucleation. Nevertheless, the advent of new technologies proved that mononucleated cells that are not karyomegalic can also be polyploid.

CKD, chronic kidney disease; EMT, epithelial to mesenchymal transition; TC, tubular epithelial cell.

challenging conditions.[10] Indeed, emergence of polyploid organisms during history overlaps with periods of dramatic climate change.[11] Nowadays, although polyploid organisms are spread all over the world, they are frequently observed in extremely dry and cold environments,[10,11] confirming the close relationship between stress and polyploidy.[12,13] The increased genetic variation and the buffering effect of duplicated genes are considered the key to the short-term adaptive potential of polyploids in harsher environments,[10,11] particularly by affecting the expression of genes involved in stress pathways.[14] In the context of adaptation to drought stress, genome doubling can lead to changes in water use efficiency and antioxidant response.[15] For example, tetraploid *Arabidopsis thaliana* and other polyploid plants or yeasts exhibit increased tolerance to salt compared with diploids.[16] Among animals, frog species of the genus tetraploid *Neobatrachus* resist better to harsher environments than the diploid counterparts.[14] Delving deeper, a challenging condition can also occur at a cellular level in organs after an insult. In the zebrafish heart, tissue repair is promoted by polyploid epicardial cells arising upon mechanical tension caused by direct tissue damage.[17] Among insects, polyploidization is crucial in the *Drosophila* gut to achieve wound healing by increasing cell size and function, filling the gap left by the injured tissue, that is, cell hypertrophy.[18–21] Moreover, polyploid cells accumulate over the course of a life span in the adult *Drosophila* brain protecting neurons against DNA damage–induced cell death.[22] In mammals, polyploidy was identified in 1940s in the liver, where binuclear polyploid hepatocytes can be easily recognized by standard microscopy.[7,23,24] Since then, polyploidy has also been identified in the heart, brain, placenta, blood vessels, skin, megakaryocytes, and other organs.[23] Like in lower species, polyploid cells in these organs protect against genotoxic stress, compensate for cell loss, and provide enhanced cell function.[7,13,17,24,25] For example, in the mammary gland, epithelial cells undergo hypertrophy, becoming polyploid during lactation to cope with the increased function needed for milk production and secretion.[25] In the liver, increased hepatocyte size and genetic diversity promote better adaptation to chronic injury.[7,17,24] Collectively, these observations suggest that polyploidy in cells and organisms confers resilience during stressful conditions.[3,4,26–29]

Polyploidy in the kidney tubule: hypertrophy and adaptation to injury

Unlike in the organs described in the previous section, where polyploidy is an accepted and well-studied mechanism of adaptation, in the kidney the presence of polyploid cells has been considered a rare event. Indeed, up until recently the description of polyploidization in the kidney has relied simply on light and electron microscopy, which

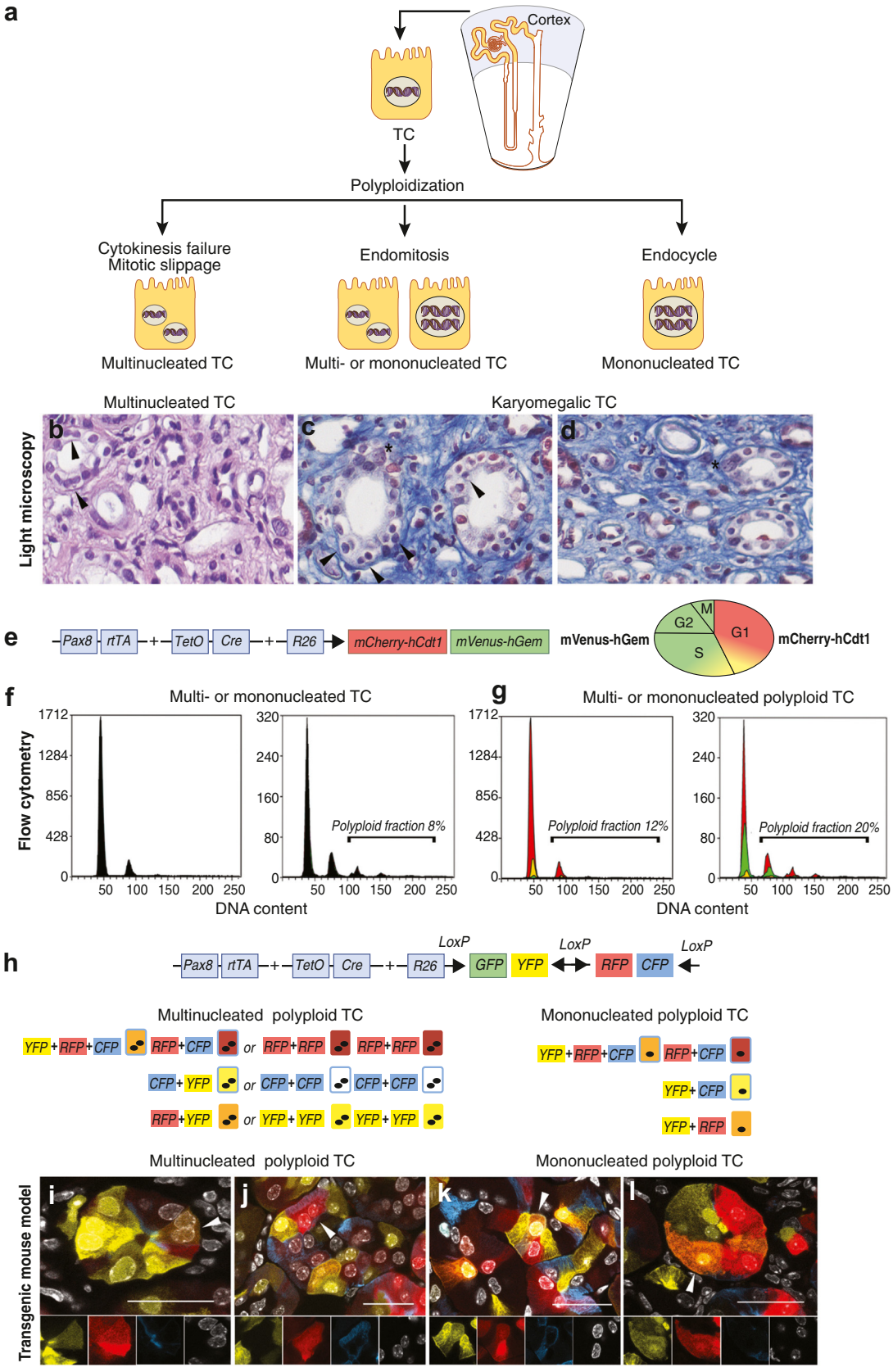

Figure 1 | Identification of polyploid tubular cells (TCs) in the kidney tubule. (**a**) Schematic representation of the nephron, showing tubular segments where polyploid TCs have been described, and schematic representation of cell cycle–regulated mechanisms of polyploidy, which can result in either multi- or mononucleated polyploid TC. Representative pictures of (**b**) hematoxylin and eosin and (**c,d**) (continued)

Table 1 | Pathology definitions to describe polyploidy in the kidney and the associated conditions

Multinucleation	Reference	Karyomegaly	Reference
HIV-associated nephropathy	31, 32, 33	Chemotherapy-induced damage or nephrotoxic drugs	5, 39
Cystinosis	34	Viral infection	5, 39
Polycystic kidney disease	35	Polycystic kidney disease	35
Kidney transplantation	36	Kidney transplantation	40, 41
Healthy kidney tissue	29, 30	BK virus–associated nephropathy	40
		Karyomegalic interstitial nephritis	5, 37, 39, 41
		DNA damage repair defects (FAN1 and ERCC1)	5, 26, 38, 42

ERCC1, ERCC excision repair 1; FAN1, FANCD2/FANCI-associated nuclease 1; HIV, human immunodeficiency virus.

incidentally reported TC multinucleation (i.e., polyploidy) in association with various pathologies and disease conditions since 1949.[30] Multinucleated TC had been observed in kidney biopsies in human immunodeficiency virus–associated nephropathy,[31–33] cystinosis,[34] and polycystic kidney disease.[5,35] In kidney transplantation, the frequency of multinucleated TC associated with tubular inflammation and T cell–mediated rejection.[36] The kidney biopsies of healthy subjects were also reported to present multinucleation in the absence of specific pathologies[29] (Table 1). In addition, standard microscopy in human biopsies sporadically described the occurrence of another pathology lesion associated with polyploidization, which is karyomegaly. Karyomegalic TC are cells with a giant nucleus (where the nuclei can be up to 30 μm in diameter)[37] containing a large amount of DNA with hyperchromatic chromatin distribution.[38] Scattered karyomegalic TC are observed in kidney biopsies associated with many conditions, such as tissue damage related to chemotherapy or nephrotoxic medications,[5] viral infections,[5,39] polycystic kidney disease[35] (in these 3 conditions they have also been observed in mouse kidneys),[5] BK virus–associated nephropathy,[40] and transplantation.[5,40,41] Sometimes, in patients with symptoms of interstitial nephritis, this phenomenon becomes so extreme that it deserves the specific pathology definition of karyomegalic interstitial nephritis (KIN). KIN is a progressive disease characterized by tubulointerstitial fibrosis and associated with oxidative stress[26] and with defects in the DNA damage repair pathway, such as those caused by FAN1 (FANCD2/FANCI-associated nuclease 1)[26] or ERCC1 (ERCC excision repair 1) mutations[42] (Table 1). Using specific transgenic mouse models, different groups demonstrated that such defects promote the progressive accumulation of polyploid TC with giant nuclei and high DNA content.[26] Overall, the use of different terms ("multinucleation" and "karyomegaly") to describe cells with increased DNA content (i.e., polyploid cells) has generated the wrong perception that TC polyploidization is a sporadic event associated with rare conditions. On the contrary, their appearance in different contexts, diseases, and even species rather suggest that polyploid TC, detected as multinucleated or karyomegalic cells, are not an occasional manifestation and that the term "polyploidization" can be (and should be) used to refer to all the aforementioned conditions to avoid confusion (see definitions in Box 1). Moreover, recognition of a TC as karyomegalic requires a significant increment of nuclear dimension associated with multiple rounds of polyploidization, such as those observed in the context of DNA repair defects. This suggests that standard pathology investigation in humans and mice largely overlooks the occurrence of karyomegaly in TC that underwent a single round of polyploidization, implying polyploidy is much more common than previously believed.

Figure 1 | (continued) Masson's trichrome staining on a kidney biopsy, showing polyploid TCs identified by standard light microscopy. The black arrowheads indicate multinucleated cells; asterisks indicate karyomegalic cells. Bar = 25 μm. (**e**) The inducible Pax8.rtTA;TetO.Cre;R26.FUCCI2aR mouse is produced by crossing Pax8.rtTA transgenic mice with TetO.Cre and Rosa26-FUCCI2aR transgenic mice. Recombination is triggered upon doxycycline administration. mCherry-hCdt1 is expressed in the gap 1 (G1) phase, whereas mVenus-hGem is expressed in the synthesis/gap 2/mitosis (S/G2/M) phase of cell cycle phases. Cells at the G1/S boundary express both mCherry-hCdt1 and mVenus-hGem, resulting in yellow cells. (**f**) Representative flow cytometry plots of cell cycle analysis by DNA content (C). DNA content quantification can identify polyploid cells only after they have undergone multiple rounds of DNA replication (≥8C). (**g**) Combination of DNA content analysis with FUCCI2aR technology permits the discrimination of polyploid cells (red peaks with ≥4C) and proliferating polyploid cells (green peaks with ≥8C) from cells actively entering the cell cycle (green peaks up to 4C). The red peaks represent mCherry-hCdt1–positive cells, that is, cells in the G1 phase. The green peaks represent mVenus-hGem–positive cells, that is, cells in the G2/M phase. The yellow peaks represent cells at the G1/S boundary (i.e., coexpressing both mCherry-hCdt1 and mVenus-hGem). The percentages reported in (**g**) are calculated on the total number of FUCCI2aR-positive cells. (**h**) The inducible heterozygous Pax8.rtTA;TetO.Cre;R26.Confetti (Pax8/Confetti) mouse is produced by crossing Pax8.rtTA transgenic mice with TetO.Cre and Rosa26-Confetti transgenic mice. Recombination is triggered upon doxycycline administration. The scheme represents the possible fluorescence color combinations that would allow the identification of multi- and mononucleated polyploid TC. Green fluorescent protein (GFP) is not included, as it is rarely expressed because of the intrinsic toxicity of the fluorochrome. (**i–l**) Representative picture of the kidney cortex of heterozygous Pax8/Confetti mice. The confetti reporter is driven by the Pax8 promoter, expressed by all TCs. The arrowheads indicate polyploid (**i**) multinucleated TC with 3 colors (red, yellow, and blue), (**j**) multinucleated TC with 2 colors (red and blue), (**k**) mononucleated TC with 3 colors (red yellow, and blue), and (**l**) mononucleated TC with 2 colors (red and yellow). Bar = 25 μm. CFP, cyan fluorescent protein; RFP, red fluorescent protein; YFP, yellow fluorescent protein. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Strategies to detect mononucleated polyploid TCs

Another issue associated with difficulties in identifying polyploid cells in the kidney tubule is represented by the analysis of cell cycle markers and measurement of DNA content alone in human or animal tissues that are routinely used to detect proliferating cells. However, these techniques cannot identify mononucleated polyploid TC or discriminate them from proliferating TC[3,4] because of their identical DNA content (Figure 1 and definitions in Box 1). In fact, only with the advent of novel technologies, more articles revealed the occurrence of mononucleated polyploid TC[3,4,26–28] in animal models of tissue injury and human kidney biopsies,[29] raising the question of their pathophysiological role. To resolve this issue and address the pathophysiological role of polyploid TC, we used the fluorescent ubiquitination–based cell cycle indicator (FUCCI2aR).[3,4,27] Thanks to this technology, cells in the gap 1 (G1) phase can be recognized for their specific expression of the G1-specific protein Cdt1 fused with the mCherry fluorochrome while those in the gap 2/mitosis (G2/M) phase express Geminin fused with the fluorescent protein mVenus. Combining FUCCI2aR reporter analysis with DNA content measurement can distinguish mononuclear polyploid TC in G1 from proliferating diploid TC in G2/M[3,4] (Figure 1). Another strategy that allows the identification of mononucleated polyploid TC is based on the multicolored reporter Confetti.[5,43] Using mice heterozygous for the *Confetti* allele (i.e., only 1 of the 2 sets of chromosomes harbors a *Confetti* allele) permitted the identification of polyploid TC as cells labeled by >1 fluorochrome, which is possible only when multiple alleles carrying the transgene are present[5,43] (Figure 1). Using these technologies, we demonstrated that up to 20% of TC become polyploid after AKI.[4] Their widespread occurrence suggests that polyploid TC have a fundamental role after AKI. Accordingly, the block of polyploid response via Yes-associated protein 1 (YAP1) inhibition in pharmacological and transgenic models of AKI causes death from severe uremia within 24 to 48 hours of injury, implying that when numerous TC are lost, polyploidization of survived TC is a quick way to achieve cellular hypertrophy, sustain kidney function, and avoid death due to acute kidney failure[4] (Figure 2). This observation is in agreement with studies performed in *Drosophila*, which demonstrated that polyploid cells arise to promote tissue repair and restore tissue mass[21,44] via the Yorkie ortholog of YAP1.[21,44] Collectively, these studies suggest that although polyploidy in TC has been historically associated with pathological conditions and considered a rare event, it is rather a widely observed crucial mechanism to quickly adapt to stressful conditions and guarantee organism survival, in analogy to other organs and species.

Polyploidy in the kidney tubule: from adaptation to injury to CKD

Although TC polyploidization is required to survive AKI by rapidly sustaining residual function of the injured kidney, a continuous and repeated induction of polyploidization, as observed in conditions of YAP1 overactivation, is sufficient to promote CKD development[5,45] even in the absence of tissue injury. This suggests that as many other lifesaving acute stress responses, TC polyploidy comes with the long-term trade-off of organ failure. CKD and kidney failure involve tubulointerstitial fibrosis triggered by polyploid TC that become senescent, likely to avoid further cycles of DNA synthesis that would lead to genomic instability and high risk of cancer transformation, as shown for the liver[24] (Figure 2). The role of senescent cells in driving chronic organ failure is already proven not only in the liver[46] but also in the heart[47] and the skeletal muscle.[48] In the mouse kidney, senescent polyploid TC are initiators of fibrosis by secreting transforming growth factor β1 and other profibrotic cytokines, hence promoting tubulointerstitial crosstalk among polyploid TC, fibroblasts, and macrophages.[49] The profile of these profibrotic TC has been repeatedly reported using different definitions as "partial epithelial to mesenchymal transition," "dedifferentiated," or "G2/M arrested" cells, but these definitions likely describe different phases of the same phenomenon (see definitions in Box 1). For example, recent studies implicated cyclin G1 as a pivotal driver of "dedifferentiation" in TC, promoting senescence and CKD transition in humans and mice.[50] However, cyclin G1 controls and promotes polyploidization during development and under conditions of cardiac overload in cardiomyocytes.[51] In addition, the identification and definition of "G2/M arrested" TC is largely based on the use of cell cycle markers or measurement of DNA content,[52,53] which are unable to distinguish them from polyploid TC. After AKI,

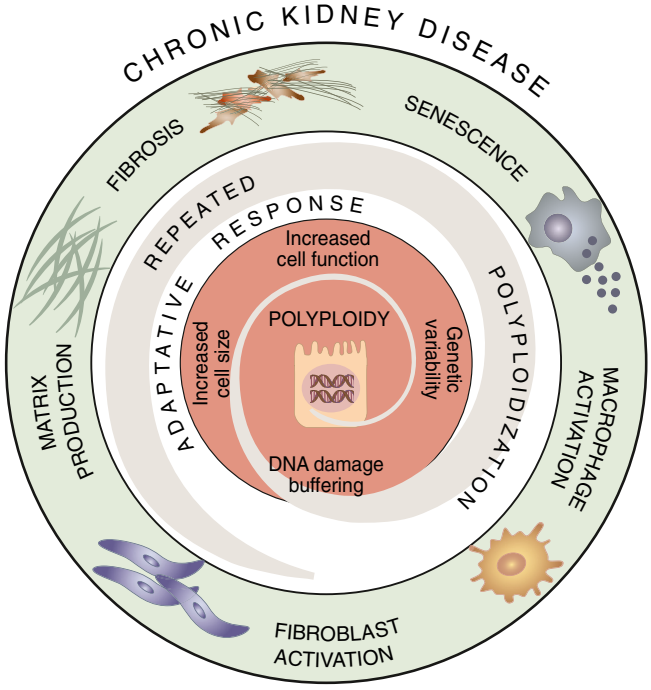

Figure 2| Central role of polyploidy in adaptation and chronic kidney disease. Tubular cell polyploidy, displayed in the center of the spiral, has a central role in providing specific advantages in different conditions. Elucidating the consequences of polyploidy at multiple levels is pivotal to understand its role in chronic kidney disease and prevent chronic kidney disease progression.

many TC were believed to arrest in the G2/M phase because of unrepaired DNA damage, becoming senescent and therefore driving CKD progression—a process termed "maladaptive repair"[52,53] (see definitions in Box 1). However, recent reports based on single-cell RNA-sequencing and cyclin G1 knockout models questioned the presence of G2/M arrested TC in kidney tissue after AKI.[50,54] Using specific transgenic mouse models, Airik *et al.* further provided direct evidence that in response to persistent DNA damage, TC undergo cell cycle activation and enter the G2/M phase while expressing the cell cycle inhibitor p21, which prevents mitosis completion and triggers polyploidization. Therefore, DNA damage directly results in polyploidy stimulation.[26] Given that DNA damage activates senescence to prevent errors from being passed on to daughter cells, DNA damage–stimulated polyploid cells may acquire senescence as a ploidy-limiting mechanism, as shown for the human placenta.[55] In line with this hypothesis, patients with FAN1 mutations diagnosed with KIN do not have an increased incidence of kidney cancers, although deficiencies of other components of the Fanconi anemia pathway can lead to a significantly increased risk of malignant tumor.[56] Finally, p21 expression that characterizes DNA-damaged TC in the context of KIN[26] was shown to activate the immune system to destroy DNA-damaged cells and prevents cancer development.[57] Taken together, these observations suggest that polyploidy, as many lifesaving acute stress responses, also comes with the long-term trade-off of promoting chronic organ failure (Figure 2).

Potential therapeutic implications of polyploid TCs

Progressive kidney function loss and CKD are highly prevalent conditions that contribute to a substantial proportion of disease burden globally. Yet over the past 30 years, the burden of CKD has not declined to the same extent as many other noncommunicable diseases (i.e., cardiovascular and cancer-related diseases), implying a substantial gap in the understanding of mechanisms underpinning the disease progression. Recent evidence showed that the regenerative capacity of the kidney tubule involves proliferation of tubular progenitors, aimed at replacing lost TC and reconstituting tubular integrity,[3] while polyploidization-mediated hypertrophy of remnant TC aimed at rapidly sustaining kidney function.[3,4] Experimental studies demonstrate therapeutic potential on short-term outcomes by targeting both mechanisms. In mouse studies, pharmacological stimulation of progenitor proliferation accelerates the intrinsic kidney capacity, enforcing function recovery by preventing irreversible loss of injured nephrons.[3] However, enhancing progenitor proliferation implies a higher risk of subsequent kidney cancer,[58] suggesting that an approach that targets tubular progenitor proliferation is not feasible and safe. Likewise, even if immediately after AKI, TC polyploidy can rescue kidney failure on the long-run, enhancing polyploid TC formation negatively influencing kidney function.[4,28] Therefore, continuous TC polyploidization may significantly impair long-term prognosis in those patients who survive the acute phase, favoring the progression to CKD (Figure 2).

Can polyploid TC represent a valid alternative target to block CKD? Our recent *in vivo* study supports this hypothesis, suggesting that polyploid TC that kept increasing their DNA content are the primary trigger of CKD progression.[4] Thus, YAP1 inhibition initiated right after the early injury phase of AKI can attenuate ongoing TC polyploidization, which is sufficient to prevent the long-term trade-off of this survival mechanism, that is, AKI-CKD transition.[4] Importantly, senolytic treatment was also able to effectively prevent CKD development by blocking continuously cycling polyploid TC, further reinforcing the concept that polyploid TC acquire a senescent phenotype driving CKD.[4] This suggests that the use of this treatment in the context of kidney function loss may be beneficial, if administered within the correct window of opportunity.[4,59] Accordingly, emerging efforts to pharmacologically targeting senescent and polyploid tumor cells might pave the way toward the incorporation of senolytic agents into cancer therapeutic strategies.[60] Another line of therapeutic intervention may come from the observation of a direct link between DNA damage and polyploidy. The treatment with compounds that block the activation of the DNA damage pathway and cell cycle activation prevented the formation of polyploid cells in the mouse models of KIN.[26] The key appears to be the block of repeated polyploidization after its lifesaving effect in an injury event. This is a potentially interesting therapeutic intervention in patients with AKI as well as in those undergoing chemotherapy or kidney transplantation.

Conclusions and perspectives

In recent years, the use of more sophisticated transgenic strategies has redefined our understanding of the true prevalence of polyploidy and challenged long-held views of the kidney response to injury. However, there is still much to be learned about the functional significance of polyploidy in the kidney. As with many other important adaptive responses, it is likely that TC polyploidy can be both beneficial and detrimental depending on the context and its continued long-term presence, for instance, during kidney aging.

In light of recent evidence pointing to the critical importance of polyploidization in facilitating cell survival and adaption to stressful conditions, the term maladaptive is likely not appropriated when describing CKD development in different (physiological and pathological) contexts. Indeed, the term maladaptive has a negative connotation, suggesting a dysregulated and incidental process that is corrupted or insufficient. On the contrary, learning from the other organs and species,[47] the capacity to increase TC ploidy represents a strategy of kidney adaption to injury, resulting in CKD that has been likely evolutionarily selected to avoid worst consequences such as death, cancer, and irreversible function loss. Finally, polyploidization of TC represents a potential window of opportunity to develop promising therapeutic strategies to halt the AKI-CKD transition, but its harmful effects warrant caution and deserve further investigation.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This research was supported by the European Union's Marie Skłodowska-Curie fellowship program (grant agreement no. 845774, to LDC). This research was also funded by PNRR (Piano Nazionale di Ripresa e Resilienza) - Young Researchers - MSCA grant dm247_msca from MUR (Ministero dell'Università e della Ricerca) to LDC and by PRIN (Progetti di Ricerca di Interesse Nazionale; 2017T95E9X, to PR).

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