Review

Surviving Acute Organ Failure: Cell Polyploidization and Progenitor Proliferation

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In acute organ failure, rapid compensation of function loss assures survival. Dedifferentiation and/or proliferation of surviving parenchymal cells could imply a transient (and potentially fatal) impairment of residual functional performance. However, evolution has selected two flexible life-saving mechanisms acting synergistically on organ function recovery. Sustaining residual performance is possible when the remnant differentiated parenchymal cells avoid cell division, but increase function by undergoing hypertrophy via endoreplication, leading to polyploid cells. In addition, tissue progenitors, representing a subset of less-differentiated and/or self-renewing parenchymal cells completing cytokinesis, proliferate and differentiate to regenerate lost parenchymal cells. Here, we review the evolving evidence on polyploidization and progenitor-driven regeneration in acute liver, heart, and kidney failure with evolutionary advantages and trade-offs in organ repair.

How to Survive Acute Organ Failure?

Acute injury-related organ failure is a global healthcare challenge with a high risk of mortality, even in settings of intensive care and availability of artificial organ replacements [1–3]. In those surviving an episode of **acute organ failure** (see Glossary), the related tissue remodeling often results in subsequent chronic organ disease, significantly affecting long-term morbidity, mortality, and healthcare costs (see Clinician's Corner) [1–3]. A better understanding of the cellular and molecular mechanisms responsible for acute organ failure may help to limit its devastating short- as well as long-term consequences in the future.

Evolution has provided a series of compensatory mechanisms to minimize the risk of fatal acute organ failure due to infections, poisoning, or hypovolemia, which include immunoregulatory signaling pathways limiting overshooting inflammation, antioxidant signaling pathways reducing cell stress, and neurohumoral responses maintaining blood pressure and organ perfusion, respectively [4]. Moreover, injured tissues must also recover lost functional performance by compensating for parenchymal cell loss. For example, injury-related necrosis of cardiomyocytes, hepatocytes, and tubular epithelial cells directly cause acute cardiac, hepatic, or renal failure, respectively (see Clinician's Corner) [1–3]. How do these organs sustain residual function during acute failure and which mechanisms promote the recovery of lost function at the cellular level?

In this review, we discuss the pathophysiology of acute organ failure focusing on tissue **regeneration** via cell **proliferation** and cell **polyploidization** via **endoreplication** as the main mechanisms to restore tissue integrity and to rapidly recover organ function [5–8]. We will limit our discussion to heart, liver, and kidney injury, which present themselves clinically as diverse syndromes, although they share numerous pathophysiological mechanisms (see Clinician's Corner), and for which data on

Highlights

Acute injuries of heart, liver, and kidney, which present clinically as diverse syndromes, share numerous pathophysiological mechanisms. Survival of dedifferentiated parenchymal cells and/or widespread proliferation is often spoken of as a potential mechanism of repair for acute injuries. Recently, two types of responses after acute organ failure have appeared to be shared in the liver, heart, and kidney: (i) surviving differentiated parenchymal cells undergo cell hypertrophy via polyploidization; and (ii) a population of progenitors, mostly identified as resident, more immature diploid parenchymal cells, self-renew and differentiate to replace lost cells.

In order to guarantee uninterrupted life-saving functional performance after acute failure, essential organs such as the heart, liver, and kidney avoid widespread proliferation that would be largely counterproductive because it implies a transient loss of the specialized cellular functions. Therefore, these organs require polyploidization in order to sustain organ function.

In the liver, polyploidization after acute failure is the predominant but not the only response. Indeed, the increase in hepatocyte ploidy precedes the proliferation of diploid hepatocyte progenitors. Polyploidization of hepatocytes drives functional recovery, increasing the metabolic function in output, whereas, hepatocyte progenitor proliferation drives the restoration of liver mass by generating new hepatocytes.

In the heart, it is still debated whether newly generated cardiomyocytes originate from pre-existing cardiomyocytes that retain their proliferative capacity throughout adulthood or from a population of cardiomyocyte

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both regeneration and polyploidization have recently become available. We envision that a better understanding of the functional contribution of polyploidization and regeneration could help to refine potential targets for therapeutic interventions that aim at improving the short- and long-term outcomes of acute organ failure.

Parenchymal Cell Loss Induces Two Types of Responses in Surviving Cells

Heart, liver, and kidney require **repair** strategies that guarantee uninterrupted life-saving functional performance such as blood circulation and metabolic homeostasis. The life-saving functions of these organs rely on highly differentiated parenchymal cells that usually no longer divide, or, if they do, may succumb to mitotic catastrophe [9,10]. It is interesting to note that surviving parenchymal cell **dedifferentiation** and/or widespread proliferation were often spoken of as potential repair mechanisms for acute injury [11–15].

However, widespread parenchymal cell dedifferentiation and/or proliferation would be largely counterproductive in acute organ failure because they imply a transient loss of specialized cellular functions. If the remnant parenchymal cells that sustain minimal residual function of an organ such as the heart, liver, or kidney were to undergo widespread **mitosis**, this would further reduce functional performance, which might become critical and, in the worst case, lead to death [16–18]. Indeed, cytoskeleton structure [16,17] and cell polarity [18] can be reiteratively lost during mitosis and **cytokinesis**, correlating with transient adhesion disengagement and dramatic deformation of organism morphology [18]. However, evolution has selected alternative solutions and minimized the mitotic ability of differentiated parenchymal cells [19,20]. In recent years, evolving evidence has revealed that two main response programs sustain residual functional performance and enforce recovery upon **acute organ injury**.

Local Progenitor Cells Regenerate Lost Parenchymal Cells by Proliferation and Differentiation

Progenitors are a small subset of parenchymal cells that reside inside adult organs in a more immature structural and functional differentiation state, and hence, do not contribute to organ function, and maintain the capacity to quickly undergo mitosis and to self-renew by asymmetric division (Figure 1) [6,21–24]. In adult liver, heart, and kidney such progenitors have been identified as resident diploid parenchymal cells with the capacity to self-renew and differentiate in order to replace lost cells (Figure 1) [5,7,8]. Progenitor cells start to contribute to organ function only once mitosis and the differentiation process have been completed.

Polyploidization and Hypertrophy of Remnant Parenchymal Cells Recover Lost Organ Function

Surviving differentiated parenchymal cells, facing increasing functional demands during organ failure, cannot go through cell division and instead increase their functional capacity by undergoing **hypertrophy** via polyploidization while maintaining functional performance (Figure 1) [5,7,8,25]. Polyploidization is a consequence of the alternative cell cycle; a process known as endoreplication that can generate either mononucleated polyploid cells via the **endocycle** [26] or mono/multinu- cleated polyploid cells via **endomitosis** (Figure 1) [27]. Polyploid cells also occur in the absence of injury, at least in the liver and heart [21,28]. Therefore, there is a normal or healthy degree of polyploidy in the tissues, and the capacity for additional polyploidization provides unique opportunities in response to injury [21,28]. Indeed, polyploidization allows an increase in the gene copy number in response to a need to quickly support an increased functional request for a higher metabolic output while persistently maintaining differentiated and specialized cell functions [25]. Strikingly, there is a proportional tissue injury response-ploidy that increases with cell number loss, suggesting that cells respond to injury, and according to its severity, by restoring the genomic

progenitors. Cardiomyocyte progenitors can proliferate following injury but this ability declines with aging in the mammalian heart. In the mammalian neonate the heart exhibits a high regenerative capacity due to the presence of diploid cardiomyocyte progenitors. In the mammalian adult heart, the regenerative capacity is almost lost and an increase of polyploidy results in scars in the long run.

In the kidney, polyploidization in response to injury, was known solely in podocytes. However, recent findings have demonstrated that injured tubules respond to acute tubular necrosis through two main mechanisms: (i) endoreplication-mediated hypertrophy of remnant tubular epithelial cells, aiming to sustain renal function despite significant epithelial cell necrosis; and (ii) survival and proliferation of tubular progenitors, aiming to replace tubular cell loss. Since polyploid cells mostly maintain a single nucleus, their identification has become feasible thanks to an advanced technology of cell cycle phase live imaging combined with DNA quantitation.

Benefits of polyploidization and proliferation associate with distinct trade-offs, including fibrosis, senescence, and cancer. Evolution has favored the coexistence of both mechanisms in the same organs, ensuring a correct self-balance of these responses.

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content rather than cell number. This response is highly conserved across evolution [29–31]. Finally, polyploidization permits genomic buffering against DNA-damaging agents or mutations and provides a more efficient protective barrier owing to a lower density of cell–cell junctions that helps to deal with exposure to toxic or infectious agents [8].

Cell Cycle Regulation towards Either Polyploidization or Proliferation

The diversity of cell cycle programming in response to acute tissue injury is increasingly appreciated [5]. Although the cell cycle regulators in endoreplication and mitosis are mostly the same, two essential cell cycle modifications are needed in order to undergo endoreplication instead of the mitotic cell cycle, that is, downregulating the activity of those cyclin-dependent kinases normally driving G2/M phase progression (M-CDK) and maintaining the activity of the CDK responsible for S-phase entry (S-CDK) (Figure 1). First, inhibition of the mitotic machinery occurs by downregulating M-CDK complex activity, in which CDK1 is bound and activated by cyclin B or cyclin A, and by sustaining the S-CDK complex activity, in which CDK2 is bound and activated by cyclin E (Figure 1) [5,25]. E2F transcription factors are divided into activator (E2F1–3) and repressor (E2F4–6) subclasses. In the latter subclass, two new repressors were recently added (E2F7–8) and were shown to be critical regulators of the transition from G to S phase by the transcriptional inhibition of cyclin E, impairing the formation of the CDK2–cyclin E complex (Figure 1) [32]. By the balancing of E2F proteins, mammalian cells can regulate the oscillation of CDK2–cyclin E activity, from low-to-high in the G to S phase, enabling cells to regulate mitosis as well as transition from mitosis to endocycle in cells that are unable to proliferate (Figure 1) [32,33]. Indeed, the activity of atypical E2F8 repressor drives hepatocyte binucleation and polyploidization, whereas its deficiency leads to an increase in the expression level of E2F target genes promoting cytokinesis (Figure 1) [32]. The second modification requires inhibition of the mitotic machinery by targeting mitotic cyclins for proteolytic degradation via the anaphase-promoting complex/cyclosome (APC/C) E3 ligase (Figure 1). The Fizzy-related receptor (Fzr), the *Drosophila* homolog of mammalian FZR1/CDH1, a binding partner of the APC/C, is a regulator of cell cycle programming in adult differentiated cells, and dictates the decision between mitosis and endocycle by facilitating degradation of cyclin B or cyclin A, preventing the formation of the M-CDK complex (Figure 1) [29]. Another regulator of polyploidization is the Hippo/Yap signaling pathway (Figure 1) [34,35]. Yap promotes polyploid cell growth by regulating Skp2, an E3 ligase that targets p27 for proteolytic degradation [35]. Indeed, the accumulation of p27 causes hepatocytes to move towards endoreplication and to boost **ploidy** [35].

Although polyploidization and proliferation require distinct cell cycle machineries, cells can occasionally be forced to reprogram their cell cycle in response to stress. However, switching from polyploidization to mitosis is not feasible for differentiated parenchymal cells with an endoreplication machinery without entering an aberrant mitosis and mitotic catastrophe. The consequences are further parenchymal loss and organ function impairment [9,10,17].

Polyploidization and Progenitor Proliferation in the Liver

Postnatal hepatocyte polyploidization increases with aging. In human adults, polyploidization starts slowly at an early age but intensifies after 50 years when 20–30% of hepatocytes are polyploid [36,37] compared with >70% of polyploid hepatocytes in adult rodents [37]. The predominant mechanism leading to polyploidy in rat hepatocytes is endoreplication generating a range of hepatocyte ploidy from diploid cells (2n, 2C) to polyploid ones (mono or binucleated cells) with a tetraploid DNA content (4n, 4C), with an octaploid DNA content (8n, 8C), or with an hexadecaploid DNA content (16n, 16C) (Figure 2;**C ploidy values, n ploidy values**) [5,37]. Hepatocytes are gradually replenished during homeostasis and robustly after liver injury [38]. In adults, new hepatocytes originate from an existing

Glossary

Acute organ failure: acute loss of organ function to such a degree that physiological homeostasis cannot be maintained.
Acute organ injury: acute harm to an organ that causes loss of parenchymal cells.
Cytokinesis: division of the cytoplasm of a cell following the division of the nucleus.
C ploidy values, Cytokinesis: C indicates chromatin amount or DNA content, as a multiple of the haploid genome.
Dedifferentiation: reversion of specialized postmitotic cells to a more primitive phenotype or differentiation status. Dedifferentiated cells are unable to perform the specialized function but that can complete mitotic cell divisions.
Endocycle: alternative cell cycle passing once – or even multiple times – through G and S phases while skipping M phase in its entirety and resulting in the formation of polyploid mononuclear cells.
Endomitosis: alternative cell cycle passing once – or even multiple times – through G1, S, and G2/M phases without nuclear division (chromatids are separated but in a single nuclear envelope) or with nuclear division but no cytokinesis, resulting in the formation of mono or polynuclear cells, respectively.
Endoreplication: incomplete cell cycle that replicates DNA during the S phase without the completion of mitosis or cytokinesis.
Hypertrophy: increase in size (the term can be used for a cell or an organ). Hypertrophy usually results from an increased workload due to some physical defect, such as when an organ is partially damaged or nonfunctional.
Mitosis: act of cell division that results in two daughter cells with a DNA content of 2n.
n ploidy values: n indicates the number of sets of chromosomes, as a multiple of the haploid genome: 1n is the chromosome content of a germinal cell, 2n is the chromosomal content of a somatic diploid cell.
Polyploid cell: cell that has more than a diploid copy of its genome. Polyploid cells arises from cell cycle dependent mechanisms (endocycle and endomitosis), thereby generating

hepatocyte pool [39–41]. Whereas some studies have suggested that, regardless of ploidy, the capacity of hepatocytes to divide would be randomly distributed [41], recent evidence demonstrates the existence of a distinct progenitor population [21,38]. In particular, a population of diploid Wnt-responsive progenitor cells, adjacent to the central vein in the liver lobule, contributes to hepatocyte maintenance by self-renewing and differentiating into mature hepatocytes, that gradually lose the capacity to divide and undergo polyploidization [14]. In addition, by using a lineage tracing mouse model, a subset of hepatocytes that expresses high levels of telomerase displaying high self-renewal capacity, has been identified [38]. These cells, distributed throughout the liver lobule, repopulate the liver during homeostasis, thus behaving as progenitors [38].

Acute Liver Injury

Some clues to establishing the role of polyploidization and proliferation of hepatocytes following acute liver injury, may be deduced from data originating in hepatectomy experiments in mice [28]. After hepatectomy, organ function recovery and restoration of the original liver mass depend on a sophisticated balance between polyploidization (endoreplication-mediated hyper-trophy) and proliferation of hepatocytes, respectively [28]. Indeed, functional recovery in a mouse model with 70% partial hepatectomy was achieved by an increase of ploidy of hepatocytes (Figure 2) [28]. However, this response is not the only one, but rather precedes the wave of proliferation of diploid hepatocytes (hepatocyte progenitors) that provide the true restoration of liver mass (Figure 2) [28]. Accordingly, blocking mitosis through the conditional knockout of CDK1 in a mouse liver did not impair recovery of liver function mediated by polyploidization upon 70% partial hepatectomy, indicating that **polyploid cells** are crucial players in supporting and maintaining almost normal liver function [42]. In contrast, by using a model of polyploidy knockout (E2F7/E2F8 knockout mice) with 70% partial hepatectomy, Wilkinson *et al*. demonstrated that a true mitotic cell division occurs and is driven mostly by a small population of diploid hepatocyte progenitors, representing about 10% of all hepatocytes [20]. Moreover, proliferation of hepatocyte progenitors occurs sooner and faster than poly-ploidization in differentiated hepatocytes and is responsible for the true structural recovery of the organ [20]. Consistently, hepatocyte progenitors also clonally expand after acute toxic injury by CCl$_4$ injection in order to maintain liver mass [38]. A comparative genome-scale analysis demonstrated that gene expression varies in diploid, tetraploid, or octaploid hepatocytes [43,44]. Compared with diploid hepatocytes, polyploid hepatocytes revealed a tendency to switch from fatty acids to glucose oxidation, in order to increase anaerobic energy production and to obtain ATP in long-term energy starvation periods such as in acute organ injury [43,44]. Overall, polyploidy increases tissue-specific transcription and function, and promotes adapta-tion to energy depletion and to stressful environments [43,44]. This scenario confirms that only a small population of hepatocyte progenitors invests energy in cell division, whereas the majority of hepatocytes become polyploid and invests energy in sustaining liver function. Thus, the functional recovery and restoration of liver mass after acute injury is achieved by selection of several ploidy classes endowed with specialized functions inside the liver. In summary, whereas polyploid differentiated hepatocytes drive the functional recovery, increasing the output of metabolic function, hepatocyte progenitors drive the restoration of liver mass by generating new hepatocytes by a process of proliferation and differentiation.

Polyploidization and Progenitor Proliferation in the Heart

The adult mammalian heart has long been considered an organ lacking any regenerative capacity due to the inability of terminally differentiated cardiomyocytes to divide [19,45]. Indeed, the muscular pump must continuously operate and hence it invests all its energy into contractile activity [43,45]. Mitosis and cytokinesis would require disintegration of sarco-meres, implying a potentially fatal temporary loss of contractile function [43]. Alternatively,

both mono- or multinucleated cells, and by cell cycle independent mechanisms (cell fusion).

Polyploidization: mechanism to increase DNA content with or without increasing nuclear number.

Progenitor: a cell that can self-renew and differentiate into one or more specific cell type(s) to replace cell loss, during homeostasis and/or after injury.

Proliferation: mechanism to increase cell number via cell division with mitosis.

Regeneration: process leading to complete restoration of tissue integrity after injury by replacement of lost cells with new cells, re-establishing the organ normal state and function.

Repair: process leading to complete or partial restoration of tissue architecture integrity and function after injury that can occur by regeneration and/or by scarring (i.e., laying down of connective tissue to heal the gap).
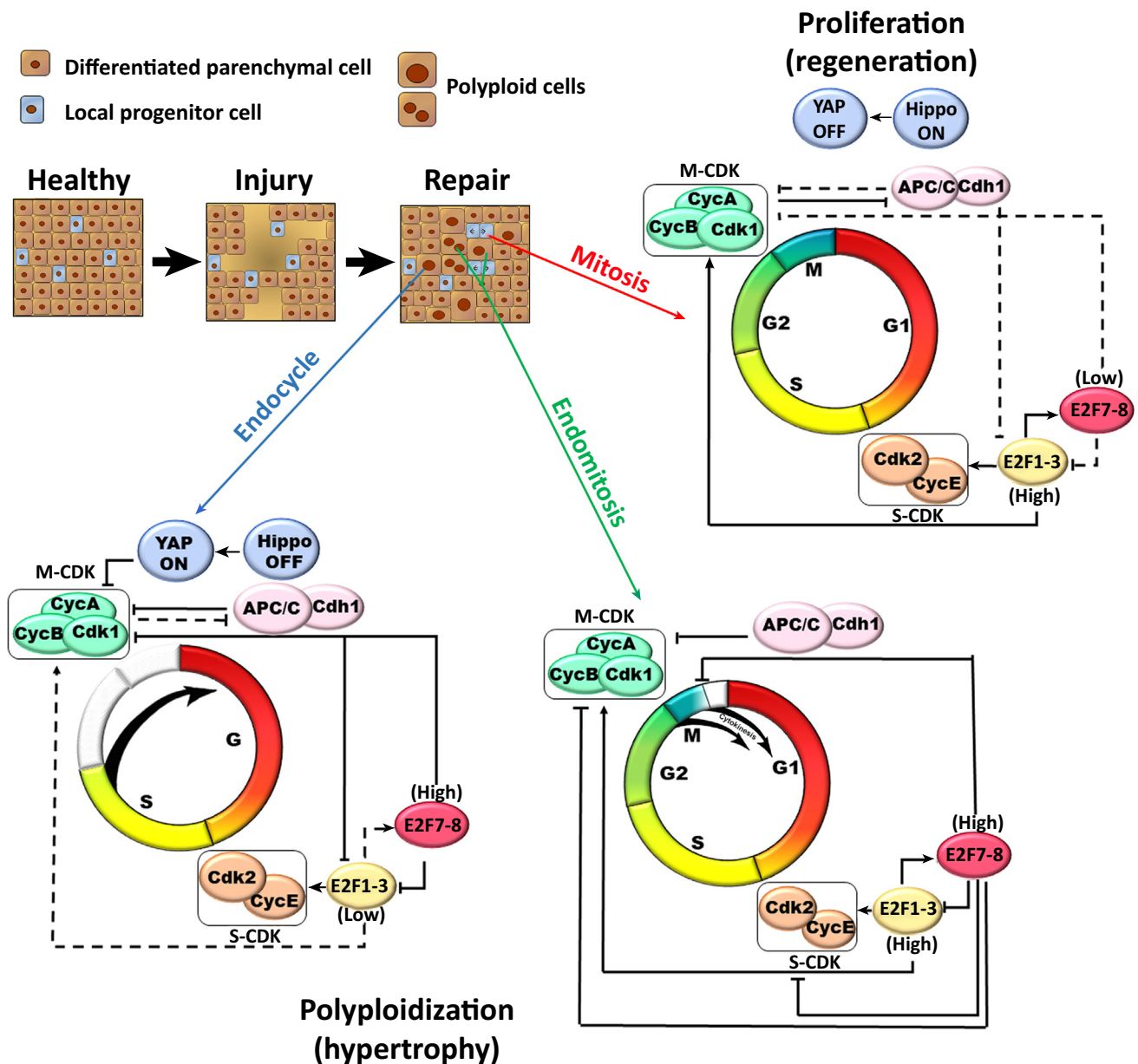
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Figure 1. Two Ways of Compensating Parenchymal Cell Loss: Polyploidization via Endocycle and Endomitosis of Differentiated Cells and Regeneration via Proliferation of Local Progenitors. Note that endoreplication via endomitosis or endocycle results in mono/multinuclear polyploid cells. Mitosis requires activation of the transcription factors E2F1–3. E2F1–3 control G1/S phase through transcriptional activation of the complex S-CDK composed of cyclin E (CycE), which binds to and activates cyclin dependent kinase (Cdk)2. E2F1–3 also activate the expression of E2F7–8, which in turn repress expression of E2F1–3, thus forming a negative feedback loop. E2F1–3 is also required for the expression of cyclin A and B (CycA and CycB) which activate Cdk1, forming the mitotic cyclin dependent complex M-CDK responsible for G2M-phase progression. The activity of M-CDK is modulated by the complex anaphase-promoting complex/cyclosome (APC/C) E3 ligase and Cdh1. The switch to polyploidization (endocycle or endomitosis) requires: a high expression of E2F7–8, which inhibits M-CDK and cytokinesis, the strong activity of APC/C Cdh1, and the inhibition of the Hippo signaling pathway.

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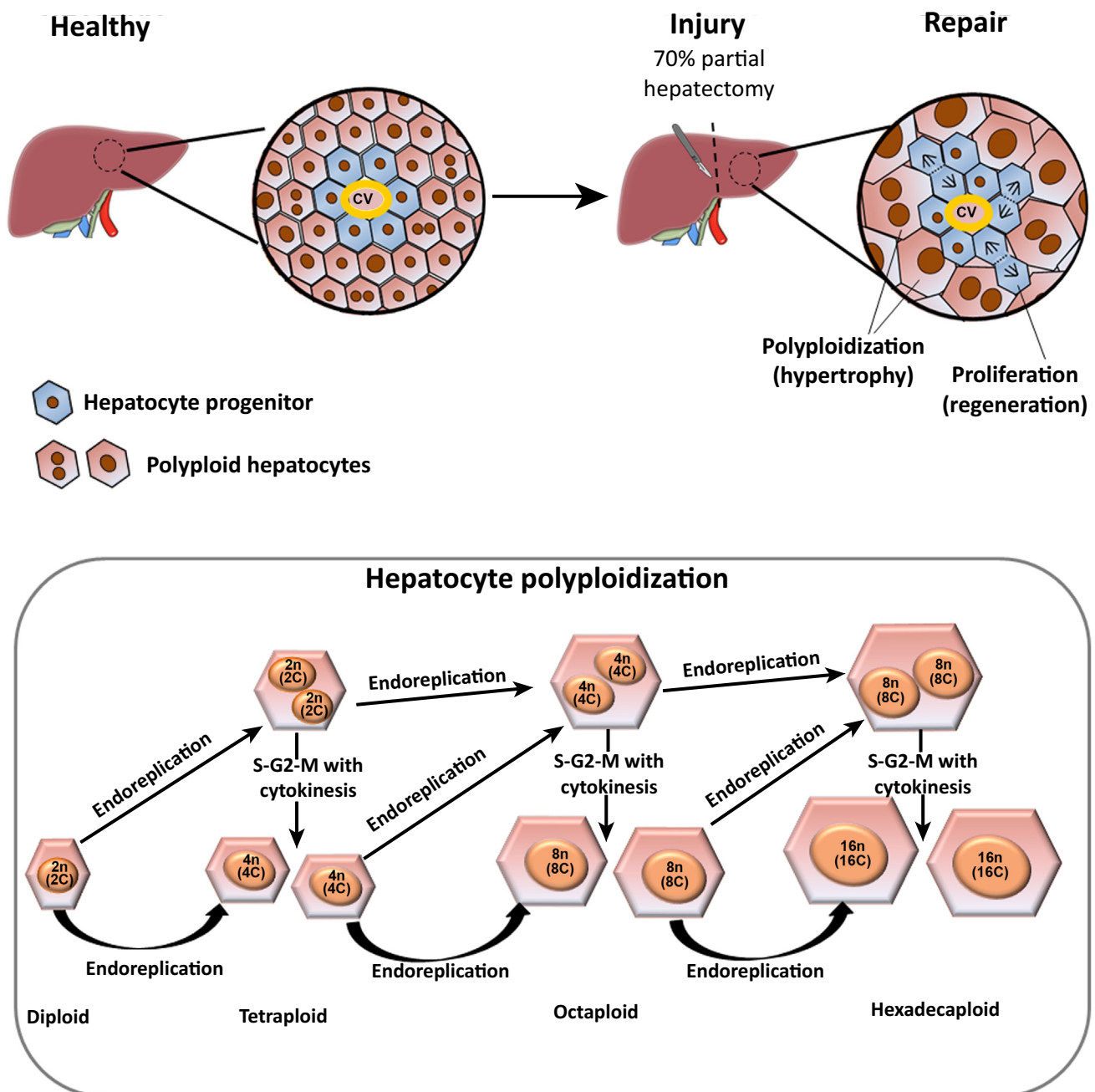
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Figure 2. Hepatocyte Polyploidization and Progenitor Proliferation Repair of Acute Liver Injury. The response to liver injury after a 70% partial hepatectomy is achieved by polyploidization which facilitates the preservation of organ function, but precedes the wave of hepatocyte progenitor proliferation that restores liver mass. Postinjury livers are, thus, characterized by division of diploid cells and by subsequent division of polyploid cells via endoreplication, generating tetraploid, octaploid, and hexadecaploid cells and providing heterogenous cellular patterns of DNA content and numbers of nuclei. Abbreviations: CV, central vein.

polyploidization allows adding more sarcomeres while maintaining contractile performance upon injury-related cell loss [43,45].

However, recent studies have demonstrated a low but constant rate of cardiomyocyte turnover in the adult mammalian heart [46–49]. Newly generated cardiomyocytes originate from a subpopulation of pre-existing cardiomyocytes that retain their proliferative capacity throughout adulthood [19,22,46–50]. Several studies have suggested that these may represent a population of cardiomyocyte progenitors [19,22,51,52]. Cardiomyocyte progenitors were identified in the adult mouse and human heart by using a variety of approaches: (i) the expression of surface markers such as c-Kit [51,53] or Sca1 (even if no human ortholog exists) [54]; (ii) the demonstration of physiological properties such as the ability to efflux Hoechst dye [55] or to form cardiospheres in culture [56], and (iii) by lineage tracing at a single-cell level [22,52].
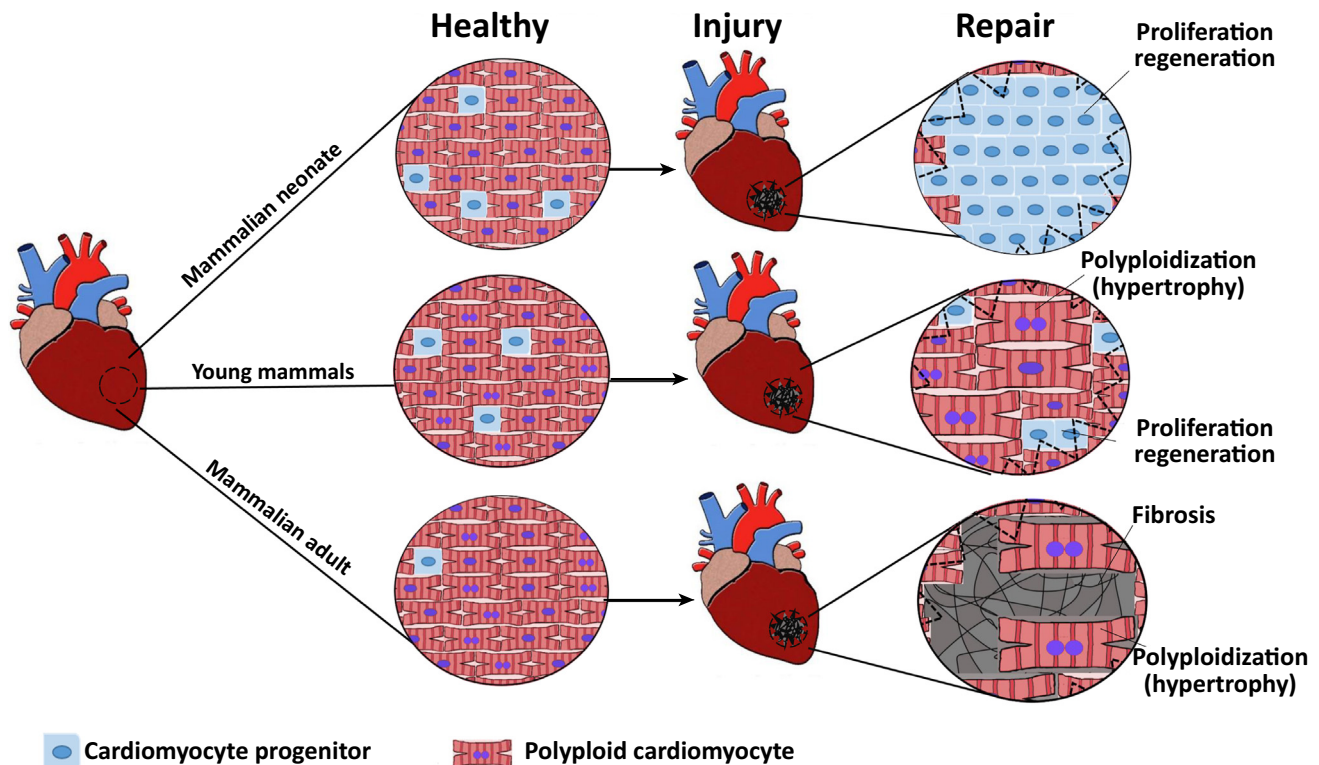
Acute Heart Injury

When acute ischemic injury occurs in the heart, cardiomyocyte progenitors can proliferate but this ability declines with age in mammalian hearts (Figure 3) [19,22,52–54,57,58]. Indeed, neonatal murine hearts can regenerate efficiently after injury [59], mirroring what happens in lower vertebrates (Figure 3) [60–62]. Of note, the main marker of nonregenerative versus regenerative heart is the DNA content of cardiomyocytes [62]. Indeed, heart aging is characterized by a lower regenerative capacity and increased polyploidy, even resulting in cardiac hypertrophy in the long run [62]. Specifically, nonregenerative hearts such as mammalian adult hearts, contain a majority of polyploid cardiomyocytes whereas highly regenerative hearts, such as mammalian neonate and zebrafish hearts, contain a majority of diploid cardiomyocytes, regarded as progenitors (Figure 3) [62]. By using a transgenic strategy in zebrafish to resemble the behavior of mammalian adult hearts, the increment of myocardial polyploidization above 50% is sufficient to dampen regeneration and promote scarring (Figure 3). This suggests that polyploid cardiomyocytes create a barrier to heart regeneration, which is detrimental in the long term and only rare cardiomyocyte progenitors proliferate and provide tissue regeneration [62]. Similar to hepatocyte polyploidy, cardiomyocyte polyploidy is associated with a switch of tissue-specific functions to an energy-saving mode [43]. This switch is demonstrated by the substitution of highly effective and energy-intensive proteins with economic energy-saving proteins at the expense of velocity of contraction [43]. For example, in polyploid cells, the α-myosin heavy chain (fast and adult) is substituted by the β-myosin heavy chain (slow and embryonic) [43]. β-Myosin heavy chains require 3–5-fold less ATP for contraction than α-myosin heavy chains require [43]. Therefore, the overexpression of slow (β) myosin heavy chains is a commonly used marker of cardiac energy starvation in experimental models of heart disease and human hypertensive heart disease, dilated cardiomyopathy, myocardial infarction, and hypoxia [43]. The evidence that all these diseases are accompanied by cardiomyocyte polyploidy suggests a tight link between polyploidization and changes in myosin heavy chain isoforms [45].

Overall, in the early phase of injury, polyploidization of cardiomyocytes and regeneration driven by cardiomyocyte progenitors are critical to maintain heart function and to restore tissue integrity, respectively. However, polyploidization can be detrimental in the long term, as it is associated with scarring and chronic heart failure.

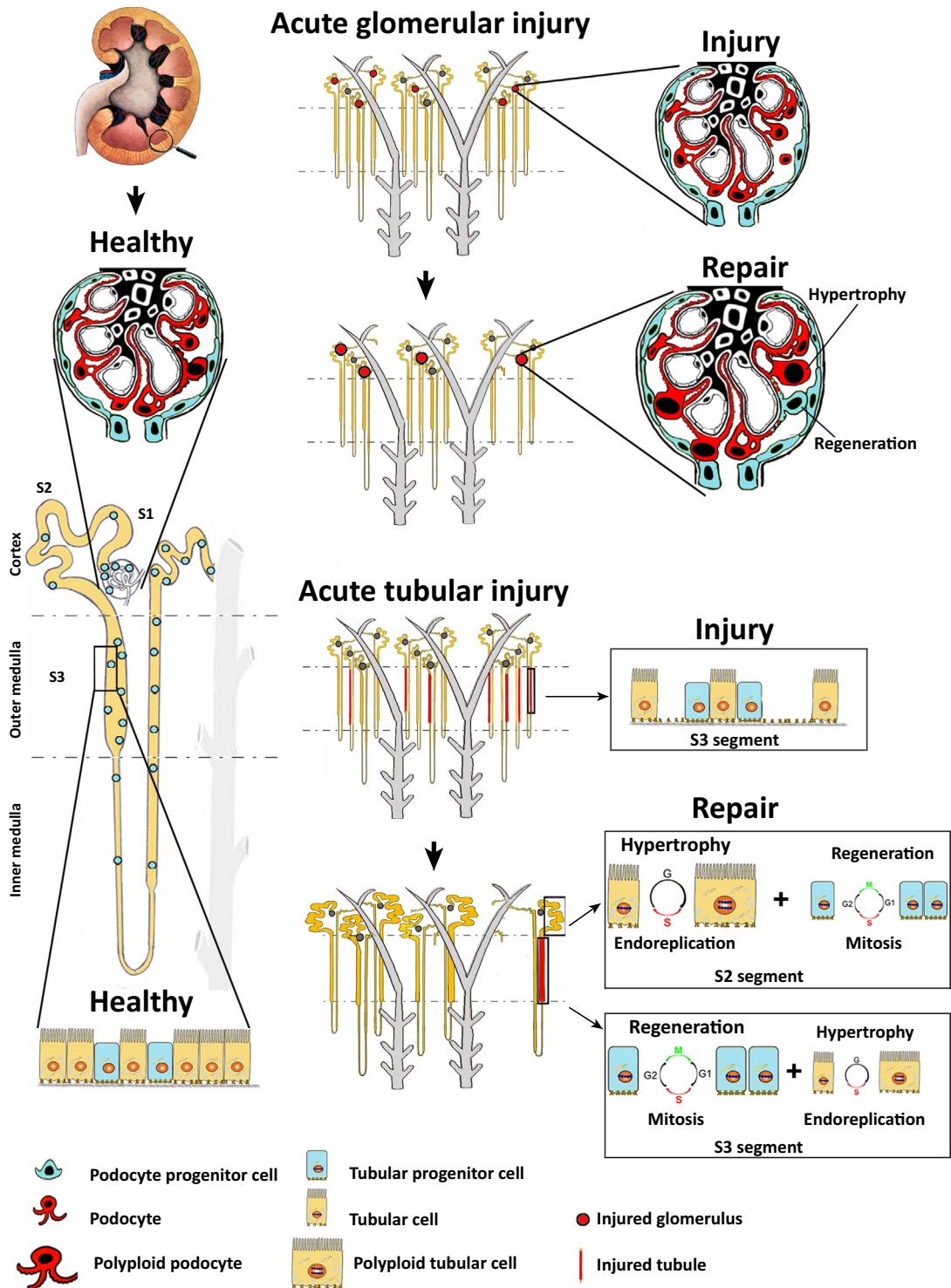## Polyploidization and Progenitor Proliferation in the Kidney

Every kidney consists of several hundreds of thousands of independent functional units, that is, nephrons. Kidney injury does not affect all nephrons equally, hence uninjured nephrons have to compensate for the function of lost or severely injured nephrons. Each nephron is composed of

Figure 3. Cardiomyocyte Polyploidization and Progenitor Proliferation Repair Acute Heart Injury. The most prevalent type of cellular response can be different in different species and at different phases of life. In neonates, the high number of cardiomyocyte progenitors (light blue cells) guarantees regeneration of injured areas by proliferation. In young mammals, the number of cardiomyocyte progenitors decreases and organ repair is guaranteed by both mechanisms: proliferation of a subset of cardiomyocytes endowed with progenitor capacity and polyploidization of cardiomyocytes. In adults, the lack of sufficient numbers of progenitors in the heart impedes sufficient regeneration. Therefore, mesenchymal healing remains the only viable option beyond cardiomyocyte hypertrophy which leads to focal or diffuse scar formation, depending on the nature of the injury. The polyploidization strategy is sufficient to dampen regeneration and promote scarring, suggesting that polyploid cardiomyocytes create a barrier to heart regeneration.

two morphologically and functionally distinct compartments, that is, the glomeruli and tubuli, built by unique, highly specialized epithelial cells. The glomeruli contain two types of epithelial cells, parietal epithelial cells located along the Bowman's capsule and podocytes located along the glomerular filtration barrier (Figure 4). Podocytes are highly differentiated epithelial cells that form unique cytoplasmic extensions, foot processes, interdigitating with those of neighboring podocytes (Figure 4). Maintaining these sophisticated structural elements requires an intricate actin cytoskeleton [63,64]. Therefore, podocytes cannot complete cytokinesis [17,65]. However, parietal epithelial cells along the Bowman's capsule harbor resident podocyte progenitors that can provide new podocytes during kidney growth [66–69], but not in aging mice under physiological conditions [66]. Tubular epithelial cells were considered a homogeneous population of diploid cells showing the same potential to re-enter the cell cycle [70]. However, several studies have suggested the existence of a specific subset of more immature tubular cells displaying higher self-renewal potential, referred to as tubular progenitors (Figure 4) [23,71,72]. Proliferation markers used as indirect indicators of cell division suggest a constant tubular turnover from resident tubular epithelial cells (Box 1) [70]. However, a low percentage of polyploid tubular cells have recently been demonstrated in healthy adult mice [23]. Since glomeruli and tubuli are differently affected by specific disorders we discuss them separately.

Acute glomerular injury

Injury

Repair

Hypertrophy

Regeneration

Acute tubular injury

Injury

S3 segment

Repair

Hypertrophy

Regeneration

Endoreplication

Mitosis

S2 segment

Regeneration

Hypertrophy

Mitosis

Endoreplication

S3 segment

Healthy

Healthy

Cortex

Outer medulla

Inner medulla

S1

S2

S3

- Podocyte progenitor cell
- Podocyte
- Polyploid podocyte
- Tubular progenitor cell
- Tubular cell
- Polyploid tubular cell
- Injured glomerulus
- Injured tubule

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Box 1. Distinguishing Polyploidization from Proliferation

A major challenge in the tissue growth and regeneration field has been to faithfully distinguish between polyploidization and proliferative cell cycle events. Sophisticated strategies are now being used to conclusively detect a complete mitotic cell cycle resulting in cytokinesis and generation of two diploid daughter cells versus endoreplication generating polyploid cells.

Cell Cycle Markers
S-phase markers [proliferating cell nuclear antigen (PCNA), 3H-thymidine, 5-ethynyl-2′-deoxyuridine (EdU), or 5-bromo-2′-deoxyuridine (BrdU)] do not distinguish endoreplication from a mitotic cell division, since in both cases cells will enter S phase and express cell cycle markers. Likewise, the G2/M phase marker, phospho-histone H3 (pH3), does not differentiate between endomitosis and mitotic cell division, as chromosomes condense during metaphase and are readily labeled with pH3 in both cell cycles [106]. The Ki67 marker labels all phases of the cell cycle and only distinguishes cycling cells from postmitotic cells [107]. No cell cycle marker exists that can distinguish cell division that results in a diploid versus a polyploidy cell.

DNA Content Analysis
This strategy allows the measurement of cell nuclear DNA content (ploidy) in order to distinguish between diploid (4C, C ploidy value) and polyploid ($\geq$4C) cells with a rigorous method. DNA content analysis can be performed with several methods: (i) by imaging fixed cells in which DNA is stained with 4′,6-diamidino-2-phenylindole (DAPI) and a quantitative analysis of the fluorescence intensity is performed [30]; (ii) by cell cycle analysis with fluorescence-activated-cell sorting [108]; and (iii) by single-cell DNA sequencing [109].

Cell Cycle Live Imaging
Cell cycle live imaging in combination with fluorescent reporters allows us to detect all phases of the cell cycle to follow cell division in transgenic mice *in vivo*. This strategy based on fluorescent ubiquitination-based cell cycle indicator (FUCCI) technology, allows us to distinguish nuclei of cells in G1 phase, expressing the fluorescent protein mCherry, from nuclei of cells in S/G2/M phase, expressing the fluorescent protein mVenus [110]. As a result of this technology, cell cycle phase analysis performed contemporarily with the measurement of DNA content, can distinguish between diploid (4C) and polyploid ($\geq$4C) cells in cell cultures or in organs [110]. This emerging technique has led to a significant recognition of the presence of polyploidy in several organs, and puts into question the classic concept about tissue growth during development and repair after injury [23,50,86].

Acute Glomerular Injury

After acute glomerular injury, podocytes undergo hypertrophy by DNA synthesis and G2/M arrest and eventually polyploidization in an attempt to cover the underlying glomerular basement membrane in exposed areas from where neighboring cells have detached or died (Figure 4) [63,64,73–75]. However, if the injury exceeds a certain threshold, podocyte hypertrophy becomes an unfit strategy over time and, by forcing mitosis, podocytes usually generate aneuploid cells that detach and die by mitotic catastrophe (Figure 4) [17,65]. In addition, lost podocytes can be replaced by podocyte progenitors that contribute to the restoration of the leaky filtration barrier and recover kidney function, improving outcome (Figure 4) [69,76–79]. Podocyte progenitors are not equally distributed across nephrons. While superficial cortical nephrons are smaller, have fewer podocytes, and are well endowed with podocyte progenitors, juxtamedullary nephrons, in contrast are larger, have more podocytes but fewer progenitors, hence more frequently succumb to scarring upon glomerular injury [79].

Figure 4. Renal Epithelial Cell Polyploidization and Progenitor Proliferation Repair of Acute Kidney Injury

For a Figure360 author presentation of Figure 4, see the figure legend at https://10.1016/j.molmed.2019.02.006

The kidney responds to acute injury with two separate strategies: local progenitor cells produce new podocytes on the glomerular tuft or new tubular cells depending on the site of injury and epithelial cell loss. In the glomeruli, differentiated podocytes cannot undergo cytokinesis and increase their working capacity by entering the cell cycle to increase their DNA content and become polyploid. At the same time, podocyte progenitors localized among parietal epithelial cells of the Bowman's capsule differentiate and replace lost podocytes. Tubular epithelial cells that are already fully engaged in essential organ functions increase their working capacity without any interruption by entering the cell cycle to increase their DNA content; a form of alternative cell cycle named endoreplication. This process allows differentiated cells in uninjured S2 segments a compensatory hypertrophy to take over the filtration load of nonfunctioning injured or lost nephrons, while tubular progenitors scattered along the nephron proliferate, completing cell division and drive regeneration in necrotic S3 segments of the proximal tubule of affected nephrons.

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REVIEWS

Acute Tubular Necrosis

In the kidney, the term polyploidy has long been associated solely with podocytes. Acute tubular necrosis (ATN) has long been considered largely reversible based on the capacity of surviving tubular cells to dedifferentiate and replace lost cells via cell division [70,80]. Staining for classical proliferation markers has suggested that fully differentiated tubular cells act as a reserve that promptly re-enters the cell cycle upon injury (Box 1) [70]. However, recent studies have demonstrated that tubular regeneration may involve tubular progenitors [23,71,72]. In particular, a combination of fluorescent ubiquitination-based cell cycle indicator (FUCCI) transgenic labeling of the cell cycle phase with DNA content analysis, has demonstrated that injured tubules respond to ATN through two main mechanisms (Box 1) (Figure 4): (i) endoreplication-mediated hypertrophy of remnant tubular epithelial cells, aimed at sustaining renal function despite significant epithelial cell necrosis; and (ii) survival and proliferation of tubular progenitors, aimed at replacing lost tubular cells. Tubular progenitors represent a subset of scattered immature epithelial cells that retain the capacity for clonal cell division and can regenerate necrotic tubule segments [23]. The recognition of polyploid tubular cells in the kidney was particularly challenging because polyploid cells mostly maintain a single nucleus (Box 1) [23]. Tubular cell-endoreplication after ATN compensates for the decline of renal function associated with tubular cell necrosis and the irreversible demise of some nephrons. Although it may not restore tubular integrity in injured nephrons, this mechanism is certainly of importance when handling the sudden increase in filtration load in uninjured nephrons. Nevertheless, lineage tracing experiments in mice have demonstrated that tubule progenitors undergo clonal expansion in order to replace lost tubular cells in the necrotic S3 segments of the proximal tubule in affected nephrons, contributing to the structure recovery (Figure 4) [23]. In contrast, tubular cell endoreplication is frequently observed in other nephron segments, in particular the proximal convoluted tubule (S2 segment), that is not directly injured during ATN (Figure 4). Likewise, this response probably occurs in uninjured nephrons even if dissecting affected nephrons from unaffected nephrons is technically challenging due to their complex 3D structure [23].

In summary, podocytes and tubular cells share similar mechanisms of response to injury. Indeed, polyploidization-induced hypertrophy and progenitor proliferation contribute to the recovery of glomerular as well as tubular injuries in analogy to what was previously described with regard to the heart and liver.

Polyploidization and Proliferation in Other Solid Organs

In other organs, evidence of a combined contribution of proliferation and polyploidization in repairing acute injury is still limited; mostly because new techniques that could allow us to distinguish mononuclear polyploid cells from diploid ones have yet to be applied.

In the lung, response to acute tissue injury is mediated by different types of progenitor cells, that proliferate and migrate to more peripheral regions. Although polyploidization has not yet been described in the lung, it has been reported in the bronchi [81].

In the pancreas, endocrine and exocrine cells undergo programmed polyploidization during weaning resulting in cells with different ploidy status [82]. However, in the exocrine pancreas, polyploidization mildly contributes to function recovery after injury [82], while new acinar cells are mostly generated by proliferation of survived acinar cells [83], or through proliferation of the metaplastic duct-like cells [83]. In the endocrine pancreas, the response to acute injury involves modest proliferation of existing cells and/or putative progenitors as well as hypertrophy [84].

Clinician's Corner

Acute Heart Injury

Acute, focal cardiac injuries, for example, due to regional ischemia, can lead to infarction areas. Heart injury is indicated by an increase in serum levels of troponins or creatinine kinase [102]. Acute heart failure is indicated by an acute drop in ejection fraction [102]. Current treatments include fluid control, vasopressors, inotropic medications, antiarrhythmic drugs, or ultimately ventricular assist devices [102]. Shifting from polyploidization to proliferation can improve heart function after acute myocardial infarction in animal models suggesting this may represent a potential target for future treatments [100,101].

Hypertrophy/polyploidization of cardiomyocytes in the unaffected regions of the left ventricle reduces short-term mortality by sustaining cardiac output [103]. In contrast to other organisms, adult mammalian hearts lack a major proliferative response involving cardiomyocyte progenitors and their contribution to sustained cardiac function is still debated. After the acute phase, current treatment protocols aim to reduce left ventricular wall stress as a stimulus for persistent cardiomyocyte hypertrophy and cardiac remodeling, which otherwise drives morbidity and mortality from subsequent heart failure.

Acute Liver Injury

Acute liver injury most commonly involves diffuse hepatocyte necrosis secondary to toxins or bacterial and viral infections, congestion (right heart failure or liver vein thrombosis), as well as autoimmunity. Liver injury is indicated by an increase in serum levels of liver enzymes. Liver failure is indicated by a reduction of coagulation factors, cholinesterase or albumin. Current treatment targets the specific cause of liver failure if possible and it includes supportive management of secondary complications. Ultimately, liver assist devices and liver transplantation are options where available [104]. Polyploidization and hepatocyte (progenitor) proliferation both occur in hepatocytes in injured adult mammalian liver and seem to synergistically

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In the bladder and excretory system, polyploidization and progenitor proliferation were recently described in the urinary epithelium (urothelium) during homeostasis and after acute injury. A population of diploid progenitors replaces binucleated superficial cells as they die off after injury, thereby maintaining the urothelium barrier [85].

Polyploidization and progenitor proliferation were recently described also in the epicardium. Mechanical tension drives both endocycle and endomitosis for generation of polyploid epicardial cells. After heart repair, the polyploid cells are cleared by apoptosis and replaced by epicardial progenitors [86].

Polyploidization and Proliferation: Trade-offs and Therapeutic Windows

The survival benefits of polyploidization and proliferation are associated with distinct trade-offs [8]. For example, excessive polyploidization due to repetitive injury or aging can lead to fibrosis. Indeed, cell cycle arrest upon polyploidization and cellular hyperfunction represents a hypersecretory state that is also associated with a persistent secretion of profibrotic mediators driving fibrogenesis, that is, cell senescence [87]. The association of tissue polyploidization, fibrosis, and senescence, has been demonstrated for the liver [88,89], heart [89,90], and is likely to account also for kidney fibrosis [91]. In all of these organs, polyploidization may also lead to fibrosis and organ dysfunction through another mechanism. Indeed, podocytes and cardiomyocytes cannot efficiently complete mitosis and when polyploidization does not involve growth arrest, cells can be forced to pass the M phase and die through mitotic catastrophe, implying further cell loss [9,17]. Examples of podocyte polyploidization followed by mitotic catastrophe and further podocyte loss have been reported in most glomerular disorders. Likewise, when cardiomyocytes are forced to re-enter the cell cycle, they die through mitotic catastrophe, resulting in cardiomyocyte loss and impaired cardiac function as in dilated cardiomyopathy and heart failure [9].

Vice versa, progenitor cell proliferation prevents fibrosis and cell senescence. For example, in the liver, organ aging is characterized by a lower regenerative capacity due to the decline of progenitor proliferation and/or progenitor loss and increase of polyploid hepatocytes [37]. In addition, fibrosis is rare in high turnover organs such as the skin or the intestinal tract. These organs are more often affected by cancer. Solid organs utilizing progenitor-driven repair can develop cancer, for example, the liver and possibly the renal tubules. Indeed, in mutagen- and high-fat-induced mouse models, tumorigenesis was accelerated by proliferation of diploid hepatocytes [92], and accordingly, human data show that, for example, hepatocarcinomas are predominantly diploid [93–95]. Consistently, in mouse models of a stable polyploidy knockout, hepatocyte progenitors proliferate faster than in wild-type mice and become highly tumorigenic when challenged with tumor-promoting stimuli, suggesting that tumors are driven by rapid progenitor proliferation [20]. Preliminary evidence indicates that renal tumors, such as angiomyolipoma [96], papillary carcinoma, and clear cell carcinoma, may derive from tubular progenitors [97,98]. By contrast, the glomeruli and the heart, in which polyploidization predominates, are largely devoid of cancerogenesis, demonstrating a tumor-suppressive function by polyploidy [92,93]. However, it is still debated if the increased ploidy or the aneuploidy cause genome-destabilizing effects and provide another avenue for tumorigenesis [99]. Indeed, hepatocytes with a specific aneuploid karyotype can be differentially resistant to toxic injury [99]. A similar mechanism might also be valid for the adaptation of tumor cells, in which loss or gain of chromosomes provides a growth advantage [99].

The same mechanisms and their trade-offs also represent potential windows of opportunities to develop promising therapeutic strategies for acute organ failure. In fact, overexpression of

contribute to the recovery of liver function and mass. Polyploidization is obvious in multinucleated hepatocytes (endomitosis) and it is also present in humans. Whether interfering with one of the two or both mechanisms could improve outcomes in patients with acute liver injury is still unknown.

Acute Kidney Injury

Acute kidney injury often involves diffuse necrosis of S3 segments of proximal tubules due to toxins, ischemia, sepsis (often all in combination), alloimmunity, or autoimmunity. Urinary kidney injury markers include insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP2). Renal failure is indicated by a decrease in urinary output and/or an increase in serum creatinine levels [105]. Current treatments include abrogating the causative stimulus, control of fluids, electrolytes and metabolic acidosis, eventually employing peritoneal or hemodialysis [105]. Proliferation of tubular progenitors and polyploidization of differentiated tubular epithelial cells synergize to rapidly recover kidney function. In mice, pharmacological stimulation of progenitor proliferation can enforce function recovery by preventing irreversible loss of injured nephrons. Whether this approach is feasible and safe also in humans is currently unknown.

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CDK1, CDK4, cyclin B1, and cyclin D1 significantly improved heart function after acute myocardial infarction by inducing efficient division of cardiomyocytes [100]. Likewise, cardiomyocyte proliferation is promoted during pregnancy and after myocardial infarction by factors produced by T-regulatory cells, thus improving outcomes, suggesting that it could represent a potential therapeutic target [101].

In the kidney, stimulating proliferation of podocytes or tubular progenitors has been proposed as a new strategy to regenerate injured nephrons. Stimulating podocyte progenitors from along the Bowman's capsule to migrate to the glomerular tuft and to differentiate into new podocytes is efficiently achieved by the inhibition of GSK3β (BIO) or CXCL12 [69,79]. Strikingly, both treatments attenuate proteinuria and chronic kidney disease (CKD) progression, highlighting the critical role of podocyte progenitors for either resolution or progression of glomerular diseases [69,77,78]. Likewise, drugs that target tubular progenitor proliferation, such as histone deacetylase inhibitors (HDACi), trichostatin, (TSA) and 4-phenylbutyrate (4-PBA), accelerate the intrinsic capacity of the kidney for tubular regeneration upon ATN [23]. Their effect results from a selective expansion of tubular progenitors, restoring tubular cell number and reconstituting tubular integrity. Tubular progenitor proliferation results in sustained recovery of renal function, avoiding development of tissue fibrosis and CKD [23]. It remains to be explored if enhancing kidney regeneration implies a higher risk for subsequent kidney cancer.

Concluding Remarks and Future Perspectives

Acute solid organ injury can present itself with or without organ failure. In the life-threatening setting of organ failure, survival depends on the maximal working capacity of the remnant parenchymal cells, which are also highly differentiated postmitotic cells. These two considerations argue against the previous concept of dedifferentiation and proliferation. New data can now resolve this conflict. Only a small subpopulation of more immature parenchymal cells, referred to as local progenitors, proliferate and differentiate to replace at least some of the irreversible loss of parenchymal cells during injury. The vast majority of the differentiated parenchymal cells also enter the cell cycle (explaining the widespread positivity of cell cycle markers often miscalled proliferation markers) but do not undergo cell division. The structural consequence of polyploidization is cell hypertrophy. The functional consequence of polyploidization is increased working capacity. Both mechanisms together allow context-dependent and dynamic responses to different extents and possibly also to different types of injury. Experimental studies demonstrate therapeutic potential on short-term outcomes by targeting both mechanisms, but possible harmful effects warrant caution and deserve further investigation. Progenitor-related tumorigenesis and polyploidization-related senescence and tissue fibrosis may significantly impair long-term prognosis in those who survive the acute phase. These and other important questions for further research are highlighted in the Outstanding Questions. Another important conclusion, expanding the view from within each research domain to other research domains that are tackling similar problems, may reveal unexpected similarities in the available data and provide a conceptual framework providing guidance in all the fields. Evolutionary pressure has been similar for the heart, liver, and kidney, as well as for other solid organs. It is more than likely that the cellular constituent, that is, postmitotic cells and resident progenitors, albeit of different shapes, are similar, as are the responses to injury.

Outstanding Questions

Both mechanisms, polyploidization and proliferation, have their pros and cons and it may be difficult to avoid the consequences of their dysregulation. Can/could pharmacological modulation of the balance between polyploidization and proliferation improve acute organ failure outcomes without increasing the risk of the associated trade-offs such as cancer or fibrosis?

The possibility to modulate the intrinsic regenerative potential by acting on the balance between polyploidization and proliferation could be a new therapeutic window but stimulating one side may increase the risk of the related trade-offs. Can/could we identify windows-of-opportunity in terms of dosing or treatment intervals to minimize the risk of adverse effects from such therapeutic interventions?

Polyploidization is associated with fibrosis. This suggests that a quantification of polyploid cells could predict the development of fibrosis. Could diagnostic evaluation of polyploidization of parenchymal cells in the heart, liver, and kidney, be considered as a new predictor of subsequent organ fibrosis? Could polyploidization be a molecular target to prevent organ fibrosis in the long run?

Solid organs involving progenitor-driven repairs can develop cancer, suggesting that tumors are driven by rapid proliferation of progenitors, for example, in the liver and renal tubules. Which types of cancer may evolve from local tissue progenitors and their regenerative response after a previous episode of organ failure?

In mouse models of a stable polyploidy knockout, it has been demonstrated that polyploidy is a protective barrier against excessive proliferation. Indeed, if there is a lack of polyploid response, progenitors are forced to proliferate, becoming tumorigenic when challenged with tumor-promoting stimuli. What determines whether polyploidy is a barrier or driver for tissue repair? How does the extent of damage determine the balance between endoreplication or proliferation? Does the extent of polyploidization influence the proliferation rate of progenitors?

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Evolution has provided a series of compensatory mechanisms to minimize the risk of fatal acute organ failure. Heart, liver, and kidney share two types of responses after acute organ failure: regeneration via cell proliferation to restore tissue integrity, and cell hypertrophy via polyploidization to quickly restore organ function. Could the same synergy also be applied to the lung, pancreas, thyroid, and other solid organs or low turnover tissues?

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