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Cell-cycle Alterations in Post-mitotic Cells and Cell Death by Mitotic Catastrophe

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Abstract

Mitotic catastrophe (MC) has long been accounted as a cell death path activated by premature or inappropriate entry of cells into mitosis following chemical or physical stresses. Although various possible explanations related to MC have been formulated, no general accepted definition of this phenomenon has been found yet. Recent evidences, however, demonstrate that MC is not a distinguished way of cell death, rather a "prestage" anticipating cell death, taking place in mitotically disrupted cells, which later occurs via necrosis or apoptosis. Moreover, even though it is widely accepted that MC is the main outcome after ionizing radiation treatment or treatment with drugs that influence microtubule assembly/stability inducing mitotic failure, the final cell death pathway and the final outcome of MC, which strongly depend on the cell type and its related molecular profile, still need to be fully elucidated. Post-mitotic cells, like neurons in the central nervous system, and podocytes or tubular cells in the kidney, are particularly susceptible to MC. In the central nervous system, MC has been claimed as the cause of neuronal death in many neurologic disorders, while MC in podocytes and tubular death is connected with the development of progressive glomerulosclerosis.

Keywords: Mitotic catastrophe, neuron, podocyte, tubular cell, cell-cycle reentry

1. Introduction

Cell cycle is as old as life itself. In most situations, it is a generative force that creates new cells from old. It is a tightly regulated process whose misregulation can lead to unchecked proliferation and neoplastic disease. Moreover, a decade ago, it was hypothesized that cell-cycle abnormalities may be intimately connected with the death of terminally differentiated cells, such as neurons. In this case, the consequence of cell-cycle alterations is loss of cells, and this phenomenon has been postulated as a mechanism of pathogenesis in several neurodegener-

ative disorders. Recently, the link between aberrant cell-cycle reentry, cell death, and degenerative diseases has been observed also in other post-mitotic cell types, such as podocytes and tubular cells in the kidney. In this kind of process, post-mitotic cells enter into the cell cycle in response to stress signals in order to substitute death cells, but the absence or malfunction of a specific array of cell-cycle proteins may not allow for its completion. The final result is that the cells can neither reverse the course of the cell cycle or complete division, remaining locked in a non-functional state that push them to trigger a programmed cell death response. Interestingly, in *in vitro* and animal models of neurodegenerative diseases, the presence of active apoptotic pathways has been observed and reported, while there are conflicting data on the activation of classic apoptotic pathways in the human damaged tissues. Thus, it remains possible that the cell-cycle-linked cell death response may occur through different still not completely understood pathways. Mitotic catastrophe (MC) has long been accounted as a cell death path activated by premature or inappropriate entry of cells into mitosis following chemical or physical stresses. Although various possible explanations related to MC have been formulated, no generally accepted definition of this phenomenon has been found yet. Recent evidence, however, demonstrate that MC is not a distinguished way of cell death, rather a pre-stage anticipating cell death, taking place in mitotically disrupted cells, which later occurs via necrosis or apoptosis. Moreover, even though it is widely accepted that MC is the main outcome after ionizing radiation treatment or treatment with drugs that influence microtubule assembly/stability inducing mitotic failure, the final cell death pathway and the final outcome of MC, which strongly depend on the cell type and its related molecular profile, still need to be fully elucidated. Post-mitotic cells, like neurons in the central nervous system, and podocytes in the kidney, are particularly susceptible to MC. In the central nervous system, MC has been claimed as the cause of neuronal death in many neurologic disorders, while MC in podocytes is connected with the development of progressive glomerulosclerosis.

2. Regulation of the cell cycle

The cell cycle of eukaryotic cells comprises four main successive phases: G1 phase (first gap), S phase (DNA synthesis), G2 phase (second gap), and M phase (mitosis) (Figure 1). The orderly transition from one phase to the following and subsequent progression through the mitotic cycle is controlled by a group of protein kinases whose activity is central to this process, the cyclin-dependent kinases (CDKs). Their levels in the cell remain fairly stable, but each must bind with their activating partners, cyclins, whose levels of expression fluctuate throughout the cycle.

Mitogenic signals, such as soluble growth factors or cell-to-cell contact, stimulate the activation of D-type cyclins and their connection with CDK4 or CDK6. Cyclin D-CDK4 and cyclin D-CDK-6 complexes phosphorylate the retinoblastoma protein (Rb) and inhibit its affinity to bind the transcription factor E2F-1. Thus, E2F-1 is free to induce the transcription of specific genes involved in DNA replication. Moreover, in late G1, inhibition of Rb activates the expression of cyclin E that binds with CDK2. The cyclin E-CDK2 complex ensures the G1/S transition to occur by fully inactivating Rb by hyperphosphorylation. Thus, CDK2 to regulate progression

from G1 into S phase. Cyclin A binds with CDK2 that phosphorylates various substrates allowing DNA replication. The formation of cyclin A/CDK2 complex is required during S phase. After completion of S phase, DNA replication ceases and cells enter the G2 phase of the cycle. The cyclin A-CDK1 complex plays a central role in the transition from S to G2/M phase of the cell cycle by regulating the phosphorylation of specific substrates necessary for the completion of the G2 and M phases of the cell cycle. Mitosis is further regulated by cyclin B–CDK1 complex, which appears in late G2 and triggers the G2/M transition. Cyclin A is degraded and the system is reset. In this way, cells are now ready to start a new cell cycle when the presence of mitogenic stimuli induces the upregulation of D-type cyclins.
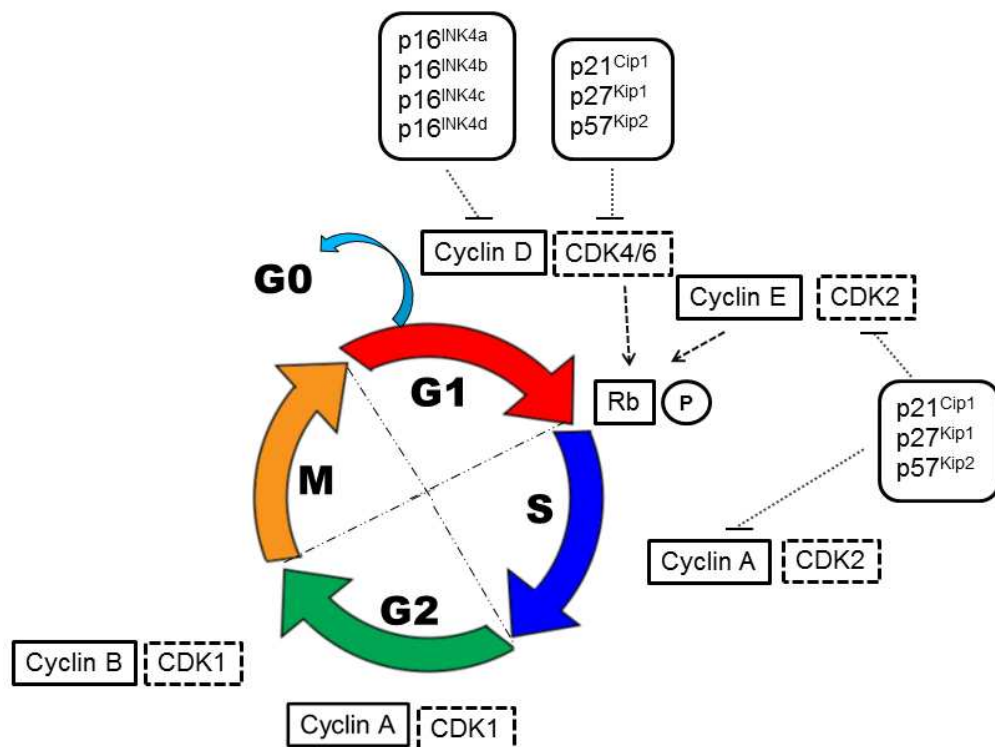
Figure 1. Schematic representation of the eukaryotic cell cycle.

CDK activity can be counteracted through post-translational modifications and subcellular translocations of specific CDK inhibitors (CDKIs), which bind with CDK alone or to the CDK–cyclin complex. CDKIs are organized in two families: INK4 and Cip/Kip. The INK4 family (inhibitors of cyclin D-dependent kinases) includes four members—p16^{INK4a}, p15^{INK4b}, p18^{INK4c}, and p19^{INK4d}—which specifically inactivate G1 CDK (CDK4 and CDK6) and the Cip/Kip family (inhibitors of cyclin D-, cyclin E-, and cyclin A-dependent kinases) comprises p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}. CDKIs are regulated by both internal and external signals. The intracellular localization of different cell-cycle-regulating proteins also contributes to a correct cell-cycle progression.

Two important checkpoints (G1/S and G2/M) coordinate CDKs activity and ensure that each stage of the cell cycle is correctly completed before allowing further progress through the cycle.

If conditions are inadequate, the cell will not be allowed to progress through the cell cycle and be both arrested, until conditions are favorable, or induced to die through apoptosis (reviewed in reference [2]). The G1/S checkpoint, also known as the restriction point in mammalian cells, is defined as a point of no return in G1, following which the cell is committed to enter the cell cycle. It is necessary to control the progression of cell cycle in the presence of DNA damage. At this checkpoint, p53 activity arrests cell cycle induced by DNA damage, stimulating the transcription of different genes including p21. At the G2/M checkpoint, mitotic entry is prevented in response to DNA damage by mechanisms similar to those in the G1/S checkpoint. An additional checkpoint, the mitotic spindle checkpoint, occurs at the point in metaphase where all the chromosomes should have aligned at the mitotic plate and be under bipolar tension.

3. Molecular basis of mitotic catastrophe

"Mitotic catastrophe" has been reported, for the first time, in a temperature-sensitive lethal phenotype of *Schizosaccharomyces pombe* in 1989.[3] The first hallmark that induced researchers to distinguish this cell-death modality from others already characterized (i.e., apoptosis) was the macroscopic alteration in chromosome segregation of some mutant strains.[3,4] Nevertheless, alterations in sister chromatids division during mitosis can result from a vast range of factors. Between these, alterations that perturb the structure and/or the high dynamicity of microtubules are key factors able to impair chromosomes segregation, mainly because of the subsequent inability to form the mitotic spindle.[5] Given the central role that microtubules play in the MC process, it has to be pointed out that the term "catastrophe" associated with a phenotype of cellular death reported 5 years before in 1989, when McIntosh JR. proved that microtubule can undergo a process of disintegration that he called "microtubule catastrophe". [6] What is then mitotic catastrophe? According to what was demonstrated in references [3,4], some thought this process happens to mammalian cells unable to fulfill a complete mitotic process , which would result in tetraploidy, a double chromosome quantities (or 2N, after a single cell cycle),[7] or endopolyploidy,[8] a situation characterized by multiple chromosome settings in a single cell (or XN, several than a cell cycle). Actually, even if exact definitions of MC are still missing, MC is prevalently defined as a cell death path that is activated by premature or inappropriate entry of cells into mitosis, and that can be activated upon chemical or physical stresses.[9] Recent evidence demonstrates that MC is not a distinguished way of cell death, but rather represents a "*pre-stage*" anticipating cell death, which later occurs via necrosis or apoptosis.[9] Moreover, even though it is widely accepted that MC is the main outcome after ionizing radiation treatment or administration of drugs that influence microtubule assembly and stability, thus inducing mitotic failure, the final cell death pathway and the final outcome of MC (Figure 2) strongly depend on the cell type and its related molecular profile.[9,10] These molecular profiles still need to be fully elucidated and classified. However, some key features of MC are already established, as failure in DNA repair mechanisms, genomic instability, chromosome segregation impairment, and microtubules destabilization. [6,11] But how these factors can drive through MC? It is well established that to preserve genome integrity, DNA-damaged responses can either result in cell-cycle stalling, finalized to

give time to activate DNA repair mechanisms, or in the removal of cells that are irreparably injured via apoptosis, MC, or necrosis. At the same time, drugs that influence microtubule stability, influencing mitotic spindle formation or chromosomes segregation, end up in the elimination of cells incapable to complete a correct mitosis. The same can be said about deficiency in cell-cycle checkpoints (especially the ones related to DNA structure analysis and to mitotic spindle): when these checkpoints are compromised, cells might prematurely enter into mitosis (M phase), as when DNA has still not been repaired, thus going toward MC.[9,12] MC-related alternations are associated with morphological changes, as the acquisition of big cellular dimensions and the formation of abnormal nuclei, mainly due to micronucleation (chromosomes or chromosome fragments not evenly segregated into the two daughter nuclei) and multinucleation (missegregated chromosome fragments enveloped by one or multiple nuclear membranes of different dimension). The latter two cases generally represent the final step of MC.[9] By this description, it appears evident that MC is a type of mitosis-related cell death that occurs during or shortly after a defective or failed mitosis.[3,13] This concept is further corroborated by the evidences that MC is also associated with incomplete DNA duplication and premature chromosomes condensation (PCC).[14–16] Nevertheless, chromatin condensation is a key feature of another well-defined type of cell death, apoptosis, which, anyway, can be distinguished from MC by the TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling) test, which individuates nuclear fragmentation, and by cell shrinkage. The morphological dissimilarities let, initially, hypothesize that MC was a direct cause of death, not connected with apoptosis, a hypothesis further corroborated by the evidence that inhibition of caspases did not influence the formation of giant cells.[10] Nevertheless, some papers suggest that MC could also be associated with caspase-2 and 3 activation, mitochondrial release of pro-apoptotic factors, and DNA fragmentation, all apoptotic features.[13,17,18] This initially created debate about MC: is it a distinct type of cell death, or is MC only a "pre-stage" of apoptosis? Additional questions arise when it was demonstrated that heated HeLa cells underwent PCC and premature reconstitution of nuclear envelope around asymmetric cluster, but did not ended up in nuclear fragmentation and apoptosis, but rather in the formation of multiple micronuclei that lead to a necrotic-like death.[14] Moreover, MC has also been characterized as delayed form of reproductive death, described as the inability to form a viable progeny able to undergo new mitotic divisions, since giant cells exhibiting multiple nuclei can be temporally viable.[19–21] This underlines that a big debate is still going on about MC, which, by some, is seen as distinct type of cell death, while others believe that it is a process that ultimately leads to apoptosis or necrosis. Agreement has been found on the concept that MC is a mitotic-related cell death and that, without entering the M phase, a cell cannot undergo MC.[10] Since MC is induced by DNA damage, as well as by alterations in cell-cycle checkpoints, it might be possible that MC is a defense mechanism to avoid aneuploidization, which might bring to neoplastic transformation. This is likely to be stimulated not only by DNA alterations, but also by deficiencies in various proteins involved in cell-cycle regulation, as cell-cycle-specific kinases Cdk1 (cyclin B1-dependent kinases) or Aurora kinases, cell-cycle checkpoint proteins and mitotic spindle assembly as Chk1 (checkpoint kinase 1) and Chk2 (checkpoint kinase 2), or cell death inducers as p53, p21 and caspases.[10] When the DNA damage is the inducer of MC, which can be induced by various chemotherapeutic agents, cells generally die during interphase, before entering the M phase. If they survive to interphase,

DNA-altered cells will stop in G1 or G2 to restore the damage and, eventually, reenter the cycle. Nevertheless, DNA alterations are not always repaired, leading to the activation of the pathways of ATM (ataxia-telangiectasia-mutated) kinases and of ATR (ataxia-telangiectasia and Rad3-related) kinases which, through an evolutionary conserved kinases cascade, activate the checkpoint-regulated Chk1 and Chk2.[22,23] Chk1 and Chk2 kinases are especially essential for cell-cycle arrest in G1, S, and G2 phases. Chk1 is phosphorylated, and activated, in response to DNA alterations, and seems to play a role at every point of the cell cycle.[24,25] Indeed, the conditional knockout of Chk1 in transgenic mice induces a vast cell death in proliferating somatic cells, which show clearly visible morphological alterations in the nucleus typical of cell that are undergoing MC.[26] To further demonstrate that an intact DNA structure checkpoint is an essential prerequisite to prevent MC, we can look at the substrate targets of the Chk2 kinases, such as p53,[27,28] the cell-cycle-regulating phosphatases Cdc25A and Cdc25C[29,30] and Mdm2,[28] which are not only required for MC, but appear to have an apoptosis-sensitizing effect,[13] remarking the interconnection between MC and apoptosis. Anyway, some authors point on the differences between MC and apoptosis and sustain that the two pathways are distinct,[31] since some manipulations able to block the apoptotic cascade (as overexpression of Bcl-2) increase the frequency of MC.[32] To further sustain the hypothesis that MC is unrelated to apoptosis, Nabha *et al*. demonstrated that caspase inhibitors such as Z-VAD.fmk are unable to halt the appearance of giant multinucleated cells, induced by treatment with spindle poisons.[33] However, the latter demonstration does not account for the possibility that apoptosis might not be mediated by caspases, which has been proved by different authors.[34–36] Moreover, the pharmacological inhibition or genetic deletion of genes related to the "DNA structure checkpoint" such as ATM, ATR, Chk1 and Chk2, lead to DNA damage-induced MC.[31,37–39] Thus, inhibition of this checkpoint limits the time available for DNA repair, constraining the cell to prematurely advance through G1, S and G2 phase and leading to MC,[40,41] a process which is markedly different from apoptosis, yet at macroscopic level, due to large cells formation characterized by multiple micronuclei clustered around individual or group of chromosomes.[31,42–45] Mitotic phase entry, however, relies essentially on cyclin B/Cdk1 kinase (or cdc2) action, which must be timely activated and form a complex that gain functional activity.[46–48] Cyclin B transcriptional levels and activity begin during late S phase and peak in M phase, while its active form translocates to the nucleus in early mitosis.[46,49] On the other side, Cdk1 is kept inactivated by the phosphorylation operated by Myt1 and Wee1 until early mitosis, when the phosphate groups are removed by a member of the Cdc25 family phosphatases and CAK kinases phosphorylate Cdk1, ensuring its maximal activity to enter in early mitosis.[47] The interactions between these two elements give rise to the mitosis-promoting factor (MPF), which ensures the G2 to M phase transition. The Cdk1/cyclin B1 heterodimer induces mitosis by activating phosphorylation of enzymes that control chromatin condensation, nuclear membrane disaggregation, and microtubule restructuration, leading to cellular roundup.[50] The perfect coordination of spatiotemporal patterns of Cdk1/cyclin B1 activity is crucial to ensure a regular cell cycle and is controlled at multiple steps. Thus, the so-called DNA structure checkpoints, by activating Wee1 and Myt1, as well as the checkpoint kinases Chk1 and Chk2, avoid Cdk1 action and entry into M phase. While the "DNA structure checkpoint" stops cells before entering the M phase in case of DNA alteration or not complete duplication, another checkpoint, the so-called spindle assembly

checkpoint, prevents anaphase entry until all chromosomes are bound on both sides with the mitotic spindle. Indeed, this will ensure a balanced chromosome segregation between daughter cells.[47,51,52] To do this, the "spindle assembly checkpoint" promotes the formation of the anaphase-promoting complex (APC), which ensures the rapid destruction of cyclin B1 thanks to its E3 ubiquitin ligase activity.[50,53,54] Moreover, APC-mediated ubiquitination of securin allows the separase to bind securin itself, event that ends in the breakdown of the cohesins that maintain the linkage between sister chromatids. By this, sister chromatid segregation and anaphase entry are granted. When alteration in the mitotic spindle dynamics activates this checkpoint, the cell stops M phase before anaphase onset. This may lead to prolonged inhibition of APC (extended Cdk1 temporal activity), which is known to induce MC generally characterized by centrosome overduplication. Analogously, defects in checkpoint signaling are able to lead to abortive centrosome duplication,[55] multipolar mitosis, and premature segregation of unaligned chromosomes with uneven partition of genetic material into the offspring.[56,57] This causes MC. In this way, the "spindle assembly checkpoint" prevents aneuploidy by allowing unbounded kinetochores on chromosomes that would be missegregated to delay the transition from metaphase to anaphase until they become appropriately attached. This is why MC can be induced by drugs that, by acting on mitotic spindle formation and cell division, affect progression through mitosis. Indeed, spindle poisons are mitotic inhibitors that have various impacts on microtubule dynamics, since by binding with tubulin they can prevent either microtubules assembly or their disassembly. Thus, altered assemblage of mitotic spindle deficits in components of "spindle assembly checkpoint" and regulators of cell cycle, as DNA damage can be associated with MC. Moreover, it has to be remembered that MC induced during experimental procedures greatly depends on the genetic background, energy, and metabolism state of the cell.[9] An important concept when talking about MC is the one related to checkpoint adaptation and mitotic slippage. Checkpoint adaptation is the capacity of a cell to enter the M phase even after a prolonged checkpoint-imposed cell-cycle arrest in the presence, for instance, of DNA alterations.[58,59] These cells are committed to become arrested before entering anaphase, which is a way that conduce to MC as just described,[55] thus inducing to hypothesize that checkpoint adaptation might represent a different way to remove cells with unrecoverable damage.[60] Similar to adaptation, mitotic slippage promotes the inhibition of checkpoint activity by prolonging temporal extension of mitotic arrest. This leads to the development of tetraploidy in cells that can complete mitosis thanks to the constitutive activation and/or permanence of cyclin B1, which should be, instead, degraded by APC to allow progression to anaphase.[61–63] However, when the arrest is prolonged, cyclin B1 cannot escape from the slow but continuous proteasomal degradation, thus forcing cells to exit from M phase, with subsequent nuclear envelope reconstitution around cluster of misaggregated and altered chromosomes to form multiple nuclei.[62] These tetraploid cells will subsequently experience a definitive arrest in G1 state due to checkpoint activation. Mitotic slippage is one of the important characteristics of cell undergoing MC: they increase in size up to 200 times and become giant cells with anomalous nuclei.[20,64] The presence of multinucleated, MC-derived cells can be easily distinguished from apoptotic death by carefully observing the morphology of their nuclei. Apoptotic cells are, indeed, characterized by nuclear fragmentation, condensed chromatin, and cellular shrinkage; on the contrary, MC is identified by the formation of clusters of nuclear envelopes

that contains misaggregated chromosomes with various and different grade of chromatin condensation.[9,14,41] But how do MC-related giant cells die? Notwithstanding the morphological differences between apoptosis and MC, they do share biochemical hallmarks, as further demonstration that MC represents a pre-stage of apoptosis. Indeed, Chk2 inhibition is able to induce MC in HeLa cells, a process that was associated with caspase-2 and caspase-3 activation, permeabilization of mitochondria that release apoptotic-involved factors (as cytochrome c) and DNA fragmentation.[14,16, 65] Moreover, caspases appear to be essential in the final stages of MC, thus supporting the hypothesis that morphological alterations associated with MC are just the initial steps that will lead to apoptosis.[12,66] To further sustain this concept, it has been proved that mitotic slippage and mitotic arrest address to apoptotic death.[67–69] Nevertheless, MC-mediated death do not always require caspases activation and apoptosis, as demonstrated by the capacity of some giant cell to undergo slow death in a necrosis-like fashion, with loss of nuclear and plasma membrane integrities ending in cell lysis.[13] Indeed, some evidences demonstrated that MC can happen in a caspase-independent fashion, since MC can induce several rounds of abnormal mitosis, associated with polyploidy plus multi-nucleation, which direct the cells to necrotic death.[70] However, a central point when talking about MC is p53. Several studies proved that spindle-damaged cells that are able to cross the M phase will be halted, in a tetraploid state, during G1 phase thanks to the activation of G1 checkpoint by p53.[71,72] This, in its turns, irreversibly activates p21, thus blocking cell-cycle progression of tetraploid cells in order to avoid altered-cell propagation. This will all end into cell death.[7,71] Moreover, p53 is the key factor that activates apoptosis after endomitosis and endoreplication. p53 and apoptosis, anyway, are not always required for MC-related cell death. Several papers evidenced how MC and necrosis can be morphologically distinguished especially when referring to loss of nuclear membrane and plasma membrane integrities. [42,73] While more detailed characteristics of MC still remain elusive and need to be investigated to elucidate all the different involved pathways, great consensus can be found on the determinants that will push MC-associated cell to choose between apoptosis or even necrosis: the genetic status of the cell and the profile of proteins involved in cell-cycle regulation.[13]
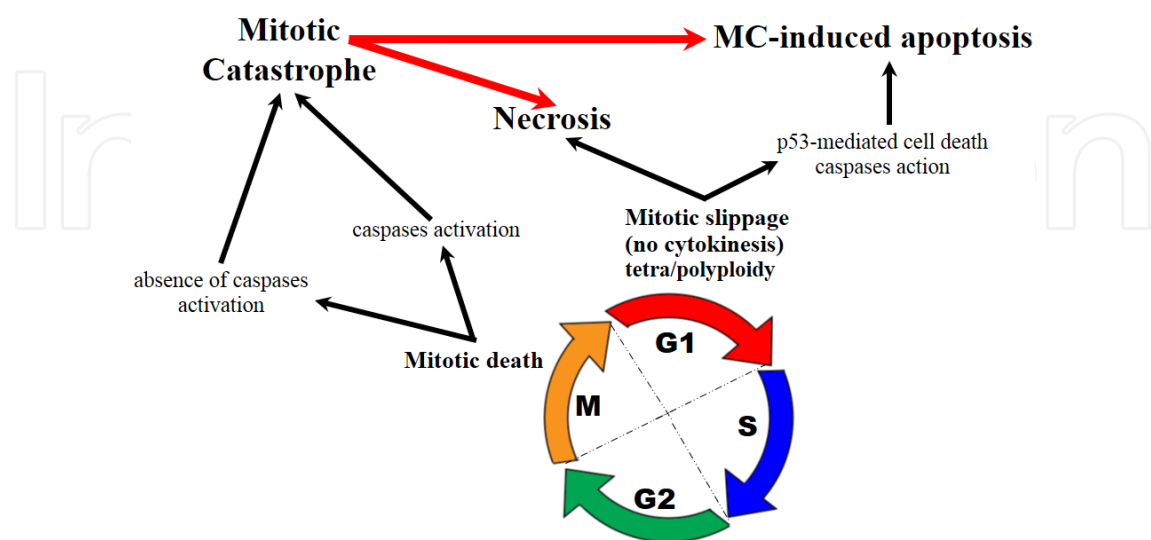

Figure 2. Schematic representation of main cell death path related to MC.

4. Cell-cycle alterations in post-mitotic neurons

Mature neurons of central nervous system are typically described as permanently post-mitotic cells that have been recently revealed to be in a continuously activated but arrested cell-cycle status. Thus, neurons must constantly keep their cell cycle in check, avoiding its re-initiation, since vigilance relaxation would mean death. During the development, neurogenesis takes place mainly in a tightly packed layer of nuclei lining the lumen of the neural tube (the ventricular zone, VZ) and later in the closely apposed region known as the subventricular zone (SVZ). After birth, the VZ has been depleted of all mitotic cells, but cells with stem cell precursors properties are present throughout the adult brain particularly in the SVZ (to a greater or lesser extent in different vertebrates) and can give rise to neurons in the adult in case of injury. Following the last cell division, neurons mature and the still unclarified mechanisms that will ensure a permanent mitotic arrest begin. What would happen if a neuron lost control of its cell cycle and reentered cell division? Evidence suggests that the neuron would die. One of the first descriptions of this phenomenon was given in 1992 analyzing the effects of expression of SV40 T antigen in Purkinje cells or in photoreceptor. While the expression of this viral oncogene typically promotes tumorigenesis in mammalian cells, its expression in post-mitotic neurons induced the appearance of mitotic figures, and entry in S phase not followed by proliferation but by cell death.[74,75] RB protein has an important role in the maintenance of neuron cell-cycle control and mice deficient in Rb show in the nervous system, ectopic mitoses, and massive neuronal death.[76] Freeman *et al.* (1994)[77] demonstrated that the death of cultured sympathetic neurons following NGF deprivation led to the upregulation of cyclin D1. These experimental data support the hypothesis of an association between ectopic cell division and cell death in the nervous system. All these data converge on the idea that during development, once a neuron leaves the VZ, its cell cycle must be actively held in check and cell-cycle re-initiation leads to death. In the adult life, cell-cycle re-activation has been claimed as the cause of neuronal death in many neurologic disorders such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ASL), ataxia telangiectasia, Parkinson's disease (PD), and even stroke.[78] In the early nineties of the last century, it became increasingly clear that mitogenic pathways in neurons are aberrantly activated early during AD. In the neurons of patients who had died with AD have been reported the presence of phosphorylated tau protein, usually found only in dividing cells[76] and of various cell-cycle proteins, such as cyclin A, B, D and E,[79,80] PCNA and CKIs of both the Ink and Cip/Kip families.[81] Evidence of cell-cycle activation in post-mitotic neurons has been also observed in murine models of AD, such as increased expression of the cell proliferation marker PCNA, and the CDK inhibitor Cdkn2a, as detected by immunohistochemistry in cortical neurons of the APP/PS1 mice.[82] Bonda *et al.* demonstrated that phosphorylated MCM2 protein, a component of the DNA replication machinery, is localized to the cytoplasm, rather than nucleus, in neurons of AD patients, while it was completely absent in control cases.[83] These data support the notion that neurons in AD reenter the cell cycle and progress through the S phase, but the aberrant cellular distribution of MCM2 may induce cell-cycle stasis and consequent neuronal degeneration. Entry into the S phase not followed by progression through the M phase in dying neurons in AD patients has been reported also by others.[84] Similarly, Ogawa *et al.*, using immunohistochemistry,

reported an increase in phosphorylated histone H3 in hippocampal neurons in AD patients. However, phosphorylated histone H3 is localized within the nucleus in active dividing cells, while in neurons of AD patients the proteins were aberrantly localized in the cell cytoplasm, indicating that cells underwent MC that leads to neuronal dysfunction and neurodegeneration. [85] In animals, AD-like neurodegeneration can be experimentally induced by the administration of okadaic acid (OA), a potent phosphatase inhibitor[86,87] that increases the degree of phosphorylation of various proteins, such as the microtubule-associated protein tau. *In vitro* exposure of neuroblastoma cells to OA induces the expression of G2/M phase marker cyclin B1 and cyclin D1,[88] causing neurons to aberrantly reenter cell cycle, but the cells fail to complete the mitotic cycle, resulting in MC. Indeed, Chen *et al.* demonstrated that disturbance of the protein kinase–phosphatase system caused by OA is sufficient to induce neuronal cyclin B1 expression, force neurons into the mitotic phase of cell cycle, and cause MC.[89] Although the presence of binucleated neurons has been reported,[90] no cytokinesis has ever been described, consistent with the idea that susceptible neurons may be arrested at the G2/M transition before they die. In mitotic-competent cells, G2 phase is associated with the activation of CDK1 and phosphorylation of target proteins, that is, microtubule-associated protein tau, that allow cell-cycle progression.[91,92] However, in neuron the presence of a highly specialized and stabilized cytoskeleton probably hinders the possibility to disassemble cytoskeleton structures to commit to mitotic spindle formation and cytokinesis. Interestingly, in AD patients the presence of abnormally increased level of phosphorylated tau has been reported and this could be explained as an unsuccessful effort to modulate G2 neuronal architecture and prepare it for mitosis.[93] In addition, the pro-apoptotic BAD protein is another protein phosphorylated and activated by CDK1 in G2 phase, linking the cell cycle to the programmed cell death mechanisms.[94] However, the apoptotic nature of neuron death in AD remains controversial. Indeed, although extensive DNA fragmentation has been reported, only a small number of cells show signs of caspase-mediated apoptosis. Interestingly, activation of caspases in human neurons does not lead to a rapid process of cell death but provokes a prolonged form of apoptosis, thus explaining the long-term survival of neurons that have progressed in late phases of the cell cycle and the protracted nature of AD. Indeed, quantification of neuron-expressing cell-cycle proteins revealed that 5-10% of neurons are dying at any moment. This implies that death by cell cycle in adult neurons must be a very slow process requiring in the order of 6–12 months; otherwise, during the average course of AD (10 years), 95% of neurons should be dead in less than a month. Thus, it is possible that in fully mature neurons, neuronal death by cell-cycle re-initiation requires an additional stimulus to make the transition from cycle to death.

The involvement of cell-cycle molecules was found also in ALS. Nuclear accumulation of phosphorylated RB protein, with concurrent increase in cytoplasmic levels of cyclin D, and redistribution of E2F-1 into the cytoplasm occur in motor neurons and glia during ALS, suggesting overcoming of G1/S checkpoint during ALS as mechanisms regulating motor neuron death.[95] More recently, Ranganathan *et al.* linked elevated levels of p53 in ALS spinal cord motor neurons and activation of G1 to S phase cell-cycle regulators to their cell death.[96] Using microarray analysis in single motor neuron in the SOD1 transgenic murine model of familial ALS, researchers found a significant increase in the expression of cyclins D2, E2, and

I.[97] In ataxia-telangiectasia (A-T), the extent of Purkinje cell loss correlates with the severity of the clinical phenotype. Re-expression of cell-cycle proteins in the cerebellum of patients has been reported in particular in Purkinje cells and striatal neurons in both human and mouse A-T.[98] This linkage of the neurodegeneration with events of the cell cycle has important implications in understanding the etiology of A-T. The involvement of cell-cycle-associated mechanisms to neurodegeneration in PD is suggested by a series of experimental evidence. In human, the expression of phosphorylated Rb protein, E2F1, as well as DNA synthesis, has been reported in postmortem samples of PD patients.[99,100] Administration of 6-hydroxydopamine (6-OHDA) induced a rat model of PD in which overexpression of Cdc2 was found in dopaminergic neurons of the substantia nigra.[101] *In vitro* treatment of PC12 cells with 6-OHDA lead to oxidative stress and was associated with upregulated expression of several markers of cell-cycle reactivation.[102] The role of cell-cycle reentry in neuronal dysfunction and death observed in many neurodegenerative diseases has been clarified by Lee *et al.*.[103] These researchers developed a new transgenic mouse model in which forebrain neurons were induced to reenter the cell cycle by overexpression of the proto-oncogene Myc under the control of the CAMKII promoter. In this animal model, the induction of ectopic cell-cycle reentry (determined by the expression of PCNA, Ki-67 and cyclin D1, and BrdU incorporation) results in neuronal cell death, gliosis, and cognitive deficit, thus conclusively demonstrating that dysregulation of cell-cycle reentry is a key determinant of neurodegeneration *in vivo*. Activation of the cell cycle mediated by the loss of CDKI function is also reported as a cause of ischemia-induced delayed neuronal death *in vivo* and *in vitro*.[104] Similarly, the phosphorylation of the RB protein is altered by transient brain ischemia.[105] However, the activation of cell cycle and the rapid expression of cell-cycle inhibitors, such as p53 and p21cip1, may trigger neuronal death in acute hypoxic stress,[106] while preventing cell death in the presence of sublethal stimulus.[107] All these data suggest that cell cycle is aborted mostly at G1/S checkpoint in ischemic death. However, in some systems, authors have shown that neurons start to replicate DNA, thus entering the S phase before they die, as demonstrated by induction of PCNA and incorporation of BrDU in several models of focal and global ischemia.[108–110] However, there is no *in vivo* evidence of G2 entry in ischemic neurons. In spite of the accumulating *in vivo* experimental studies demonstrating aberrant deregulation of cell cycle in stroke, there is little known about how/whether these processes occur in humans. Studies in human biopsy material indicate that the neuronal death in temporal lobe epilepsy is also associated with the expression of cell-cycle-related proteins.[111] Finally, in adult central nervous system, some DNA-damaging agents, such as UV radiation and cytotoxic drugs, have deleterious effects because they induce neuronal death via the activation of the cell division cycle[112] and the cell death follows a MC that can be prevented by cyclin-dependent kinase inhibitors.[112,113] The evidence to date is compatible with the requirement for a second "hit" for a neuron to progress cell-cycle initiation and DNA replication to death. This finding offers several important insights. The most important is that any intervention of blocking "second" processes might prevent or slow the neuronal cell death in the process of disease. Finally, which type of cell death follows the reported cell-cycle alterations in post-mitotic neurons is not completely understood, but MC is one possibility.

5. Cell-cycle alterations in podocytes

Podocytes are specialized renal epithelial cells that, through cytoplasmic extensions called foot processes, interdigitate with neighboring podocytes and cover the surface of the glomerular capillary loops, thus forming the glomerular filtration barrier (GBM). Podocytes, like neurons, are terminally differentiated post-mitotic cells, with a sophisticated actin cytoskeleton, whose disruption due to genetic, mechanic, immunologic, or toxic injury leads to the detachment of cells from glomerular basement membrane, and finally to podocyte loss. Decrease in the number of podocytes in the glomerular capillary tuft is associated with the development of glomerular sclerosis in several human and experimental diseases. Two cellular strategies that could act to compensate for cell stress or relative cytopenia (e.g., during organogenesis or injury) are hypertrophy, which is an increase in cell size, and hyperplasia, which is an increase in cell number. Both these processes require that quiescent cells reenter the G1 phase to increase the amount of cell organelles and proteins. Podocytes can only undergo hypertrophy, producing additional foot processes to compensate for podocytopenia. Indeed, several protective mechanisms prevent podocyte progression to mitosis and arrest their cell cycle at the restriction points of G1 and G2 phases. The ability of podocytes to arrest at the restriction point is well documented. *In vitro* exposure of quiescent mesangial cells and podocytes to antibody and a complement source induces sublytic injury and entry into G1 phase.[114,115] Mesangial cells progress through the cell cycle, synthesized DNA, and divide, with resultant proliferation and increased cell number. In contrast, podocytes undergo limited DNA synthesis, but do not proceed beyond the G2/M phase of the cycle and do not proliferate.[116] This arrest is associated with an increase in protein levels for p53, the CDKI p21, growth-arrest DNA damage-45 (GADD45), and the checkpoint kinases 1 and 2.[116] Sublytic C5b-9 injury causes DNA damage in podocytes, but not in mesangial cells,[117] and DNA damage prevents proliferation by arresting cells at G2/M phase. Re-expression of cell-cycle proteins has been reported during glomerular disorders, and in recent years, cell-cycle regulatory proteins have become an area of intense research in order to understand the changes that occur in various renal diseases. The roles of these proteins in renal diseases have been addressed in several reviews:[118–120] cyclin A and Ki-67 staining was observed in podocytes of children collapsing glomerulopathy[121,122] and focal segmental glomerulosclerosis (FSGS);[123] cyclin D was observed in cellular lesion of FSGS.[123] Moreover, p27 and p21 but not p57 was decreased in CGN (crescentic glomerulonephritis), as in FSGS when compared to normal.[122] A uniform decrease in p27 and p57 immunostaining in FSGS and collapsing glomerulopathy was reported also by Shankland *et al.*.[124] However, these authors reported de novo expression of p21. Downregulation in the expression of cyclin kinase inhibitors such as p21 and p27 has been observed in podocytes in children with idiopathic nephrotic syndrome. This downregulation is not followed by upregulation of cyclin D and cyclin A that are needed to overcome the G1/S transition and move the cell forward in the cell-cycle process. Thus, the podocytes remain trapped in the G1 arrest phase. Similarly, an altered expression of 27, p21, and cyclin A was reported by Srivastava and colleagues also in patients with minimal change disease,[122] while Shankland *et al.* did not reported change in expression of p21, p27 and 57 in podocytes of patients affected by minimal change disease and membranous glomerulopathy.[124] Using animal models of glomerular disease such as the passive Heymann nephritis (PHN) and anti-

glomerular antibody models, Hiromura *et al.* reported a marked decrease in p57 expression that was diffuse in PHN, whereas in the murine model, loss of expression of p57 occurred predominantly in podocytes expressing PCNA.[125] Marked podocyte expression of the CKIs, such as p21 and p27, was reported during Heymann nephritis in diabetic ZDF-fa/fa rats,[126, 127] and in glomerular tufts affected by crescentic glomerulonephritis,[128] suggesting upregulation of CKIs as a generalized response of podocytes to stress or injury. Interestingly, mechanical stretch of cultured podocytes reduced cell-cycle progression and induced hypertrophy in both wild-type and p27−/− podocytes.[129] Moreover, this event required the presence of p21 and was prevented by specifically blocking extracellular signal-regulated kinase 1/2 (Erk1/2) or Akt.[129] These data suggest that upregulation of CKIs in podocytes is an attempt to maintain cell-cycle quiescence and preserve normal physiological function. However, it is possible to overcome the resistance and force podocytes entering the cell cycle, under the pressure of sufficiently strong stimuli. We recently provided evidence that the dramatic consequences of this forced entry in mitosis are the trigger for a catastrophic mitosis. Indeed, as already reported for neurons, podocytes are programmed to maintain their complex cytoskeleton and cannot assemble an efficient mitotic spindle due to poor expression of Aurora kinase B, which is essential for cytokinesis[130] (Figure 3). Thus, podocytes cannot survive mitosis and, if forced to override these cell-cycle restriction points, detach and are lost in the urine. This is confirmed by the presence of many binucleate podocytes in the urine in patients affected by FSGS[131] and lupus nephritis,[132] suggesting that podocytes carrying nuclear abnormalities generated during an abnormal cytokinesis are more susceptible to detachment and loss. Moreover, in mitotic podocytes actin is required for the formation of the mitotic spindle and is no more compatible with maintaining the cytoskeletal structure of secondary foot processes. Indeed, in adherent cells the actin network is rapidly dismantled and rearranged to allow the cell to form the mitotic spindle and to enter mitosis. Therefore, mitotic cells acquire a rather rounded shape and easily detached and are lost in urine. Thus, acquisition of functional specialization in a cell type, such as podocytes, neurons, and cardiomyocytes, is coupled with the permanent exit from the cell cycle[133] and the arrest in a "post-mitotic" state. Therefore, when expression of cell-cycle markers (such as Ki-67) in podocytes is observed in certain diseases this is probably a sign that they undergo hypertrophy, but they are unlikely to undergo mitosis. Podocytes with mitotic figures are only occasionally observed because they are susceptible to detachment and/or death. Nagata *et al.* reported one mitotic figure in a single podocyte in a case of FSGS among 164 renal biopsy specimens with glomerular disease. [134] Mitotic podocytes associated with proteinuria may be a desperate but aborted attempt to regenerate epithelial injury.

Podocyte multinucleation on the other hand is a recognized feature of aberrant mitosis, also described by Nagata *et al.* in 1998.[135] More recently, Mulay *et al.* in lieu of the fact that aberrant mitosis and podocyte binucleation are synonymous to MC, retrospectively reviewed the consecutive renal biopsy specimens and found twice as many (*n*=12) multinucleated podocytes.[136] All cases had significant proteinuria, and diagnoses included minimal change, FSGS, IgA nephropathy, membranous nephropathy, collapsing glomerulopathy, and membranoproliferative glomerulonephritis. Foot process effacement was invariably present in association with binucleated podocytes. These binucleated, and sometimes trinucleated, podocytes enlarge, with edematous nuclei, with one or more
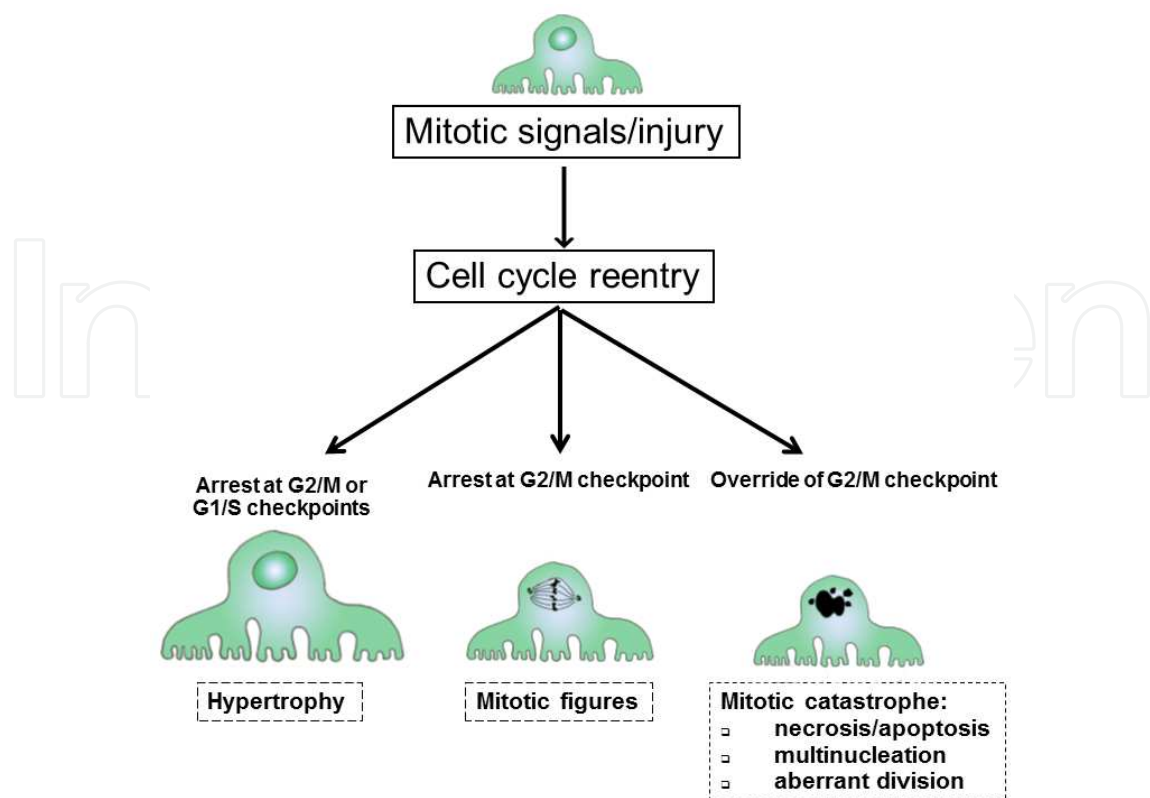
Figure 3. Podocytes are terminally differentiated post-mitotic cells. Pathological activation such as exposure to stress induces a compensatory response. The most important response to stress is podocyte hypertrophy, which results from cell-cycle arrest at the G1 or G2/M checkpoint. Persistence of mitogenic stimuli forces the podocyte to complete mitosis. This could lead to the formation of mitotic spindles and aberrant divisions with the formation of aneuploid cells or to the execution of a mitotic death program.

nucleoli. The presence of micronuclei is also reported and it is indicative of genomic instability and characteristic of MC. Moreover, these podocytes exhibit other alteration typical of MC, such as increased number of organelles, mitochondria and lipid droplets or cytoplasmic vacuoles.[137] The classic example of mature podocytes reentering the cell cycle is HIV-associated nephropathy (HIVAN). In HIVAN, the presence of tightly packed multinucleated podocyte is frequently observed.[138,139] These multinucleated podocytes to large extent remain attached to the GBM, and there is no evidence of overt foot process effacement and of apoptotic nuclear condensation. The cytoplasm appears fragile with cytoplasmic dense (osmiophilic) bodies (lysosomes). Eventually, these multinucleated podocytes detach from the GBM, disrupt the cytoplasm, and release the nucleus and cytoplasmic contents into the Bowman space.[137] Podocyte mitoses can be seen.[137] However, the connection with MC and aberrant cell death is still vague and little studied experimentally or clinically. Interestingly, in HIVAN p27, p57, and cyclin D are absent in podocytes, and p21, cyclin A, and Ki-67 are induced.[121,124,140] This implies a dysregulated podocyte phenotype characterized by bypassing cell-cycle restriction points and podocyte loss via MC. Recently, we demonstrated that Notch activation may represent an important driver of MC in podocytes during glomerular disorders.[130] Indeed, Notch activation in podocytes *in vitro* induced the downregulation of the p21 and p27, which

pushed progression toward mitosis of a cell that cannot assemble an efficient mitotic spindle, leading to the formation of bi- or micro-nucleated cells with disrupted cytoskeleton. [130] Accordingly, Notch protein expression was not detected in glomeruli of healthy adult kidneys, while several studies demonstrated strong Notch upregulation in podocytes of patients affected by several types of glomerular disorders characterized by podocyte death. [130,141,142]

6. Cell-cycle alterations in renal tubular epithelial cells

6.1. G1 arrest

Acute kidney injury (AKI) is a potentially devastating, increasingly common syndrome characterized by rapid impairment of kidney function as a result of a toxic or ischemic insult. In the first 24 h following injury, tubular cells undergo apoptotic and necrotic cell death, and 70% of the surviving, normally quiescent proximal tubule epithelial cells enter the S phase of the cell cycle.[143,144] This is documented by an increase in PCNA,[145–147] incorporation of 3H-thymidine and 5-bromo-2-deoxyuridine into nuclear DNA, and induction of mRNA for "immediate-early" genes, c-fos, c-jun, and egr-1.[148,149] A rapid induction of p21, in several models of AKI, has also been reported.[149] This cell-cycle reentry after injury has traditionally been viewed as an appropriate repair response to the loss of adjacent cells after an initial insult. However, this is in contrast to the observation that cell-cycle inhibition is protective against several form of AKI. The strategic role of p21 in AKI is demonstrated by a series of experiments. Administration of an adenovirus vector directing the expression of p21 protects mouse proximal tubule cells in culture from cisplatin toxicity.[150] Similar results can be obtained by treating cells with several cell-cycle inhibitors, such as roscovitine and olomoucine.[150] *In vivo*, Megyesi *et al*.[151] demonstrated the upregulation of p21 mRNA in ARF (Acute Renal Failure) induced by ischemia, cisplatin, or ureteral obstruction. In addition, p21 knockout mice were shown to have increased kidney cell-cycle activity, increased cisplatin nephrotoxicity, and higher mortality than wild-type animals.[145] In p21 (−/−) mice, 5-bromo-2-deoxyuridine incorporation into nuclear DNA and increases in PCNA content were much higher compared with p21(+/+) mice. Following either cisplatin administration or ischemia–reperfusion injury (IRI), compared with their p21(+/+) littermates, p21(−/−) mice developed more severe morphological damage, displayed a more rapid onset of the physiological signs of AKI, and had a higher mortality.[152] These findings suggested that the induction of p21 plays a protective role in kidney cells by preventing DNA-damaged cells from progressing in the cell cycle without repair, which eventually would result in death. Miyaji *et al*.[153] speculated that p21 induction may play a protective role to prevent the development of cisplatin-induced AKI, stopping the cell cycle in the G1 phase and providing enough time for DNA repair. Similarly, Nath and co-workers[154] reported that LLC-PK1 kidney cells overexpressing heme oxygenase-1 were resistant to several apoptotic stimuli and that this resistance was mediated by p21 upregulation. Similarly, Golzalez-Michaca *et al*.[155] demonstrated in a rat proximal tubule cell culture that blocking the apoptotic effects of hemin was correlated with upregulation of p21 levels.

The hypothesis that cell-cycle inhibition post-insult protects against AKI is supported by several experimental findings. As reported above, the cell-cycle-inhibitory drug roscovitine is effective in protecting kidney cells *in vitro* from cisplatin-induced apoptosis.[150] Similarly, the broad spectrum small-molecule CDKI, purvalanol, can actually protect against cisplatin-induced cell death[156] and expression of an inactive Cdk2 mutant in mouse kidney cells protected from cisplatin nephropathy.[157] Importantly, small-molecule inhibition of CDK2 leads to a G2/M block or an intra-S phase arrest, and G2/M arrest would likely be an undesirable long-term effect as it has been recently reported that this induces progressive interstitial fibrosis in the kidney and increased cell apoptosis (see following paragraph). Interestingly, early G1 and late S phases are reported to be more resistant to genotoxic damage.[158] Thus, delaying this cell-cycle progression after injury should also ensure more time for DNA damage to be repaired before mitosis. DiRocco *et al.* recently demonstrated that a small-molecule inhibitor of CDK4/6 effectively promotes transient G0/G1 arrest in renal epithelial cells, protects these cells from DNA damage and apoptosis as a result of exposure to cytotoxic chemotherapeutic agents *in vitro*, and ameliorates kidney damage following AKI *in vivo*.[159] Their results suggest the possibility that epithelial cells may die through MC after IRI.

Thus, following injury, tubular cells enter the cell cycle, but rapidly arrest in the G1 phase. This G1 cell-cycle arrest prevents cells from dividing when the DNA may be damaged and arrests the process of cell division until the damage can be repaired lest resulting in the cell's demise or senescence. Two inducers of G1 cell-cycle arrest, insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2), have been recently identified and their urinary levels serve as sensitive and specific biomarkers to early prediction of AKI and of renal recovery.[160,161] IGFBP7 directly increases the expression of p53 and p21 and TIMP-2 stimulates p27 expression. The upregulated p proteins in turn block cell-cycle promotion acting on the cyclin-dependent protein kinase complexes (CyclD-CDK4 and CyclE-CDK2), thereby inducing G1 cell cycle presumably to avoid cells with possible damage from dividing. Markers of cell-cycle arrest such as TIMP-2 and IGFBP7 may signal that the renal epithelium has been stressed and has shut down various function but may still be able to recover without permanent injury to the organ.[161]

6.2. G2/M arrest

For several decades, AKI was usually assumed to be transient with usual expected recovery of renal function if the individual survived the acute illness. Observational clinical studies and animal models however link AKI to chronic kidney disease (CKD) progression. When kidney injury is of mild entity with normal baseline function, the repair process can be adaptive with few long-term consequences. On the contrary, if injury is more severe, repeated, or to a kidney with underlying disease, the repair can be maladaptive. Maladaptive repair leads to CKD, a process characterized by persistent parenchymal inflammation, with increased numbers of myofibroblasts and accumulation of extracellular matrix (Figure 4). The mechanism that triggers the fibrogenic response after injury is not well understood but a G2/M arrest of tubular cells has been demonstrated being an important driver of maladaptive repair and progressive CKD after AKI.[162–164] Indeed, characterization of the cell-cycle profile of tubular epithelial cells *in vivo* at various times after an acute insult in multiple experimental models of murine kidney injury, including severe bilateral IRI, unilateral IRI, aristolochic acid-induced nephr-

opathy, and unilateral ureteral obstruction (UUO), identified the accumulation of cells in G2/ M growth arrest as the common feature predicting progressive fibrotic kidney disease.[162] In the UUO model, authors demonstrated that fibrotic injury induced TGF-β1 secretion by tubular epithelial cells. TGF-β1 then induced G2/M cell-cycle arrest and profibrotic phenotype through the upregulation of p21 and activation of the JNK pathway, respectively. TGF-β1 and PDGF subsequently stimulated pericyte–myofibroblast transition through differentiation and proliferation, respectively.[165] In another study, authors demonstrated how severe IRI induces a sustained epidermal growth factor receptor (EGFR) activation, which is essential for the tubular cell regenerative response at the early stage of reperfusion, but eventually leads to the activation/proliferation of renal interstitial myofibroblasts and development of renal fibrosis.[164] The correlation between G2/M-arrest and fibrosis is supported by several studies. Pharmacological inhibition of G2/M-arrested cells reduced fibrosis, whereas increases in the proportion of G2/M-arrested cells in the cell-cycle exacerbated fibrosis, thus confirming that G2/M arrest in tubular cells contribute to progressive CKD after AKI.[164–166] The link between inadequate DNA repair and renal fibrosis and CKD is underlined in study on humans with the FAN1 mutation (a DNA damage response signaling pathway devoted to repair of DNA interstrand crosslink damage). These patients develop karyomegalic interstitial nephritis, with evidence of increased levels of DNA damage and cell-cycle arrest in the late G2 phase. [167] In conclusion, all these recent findings on the pathophysiology of AKI underline the important role of tubular cell-cycle arrest in the process of maladaptive repair. Moreover, these findings open new therapeutic perspectives to prevent, slow down, or arrest chronic fibrosis progression and progressive CKD, as suggested by experiments in rodents performed using agents such as histone deacetylase inhibitors[166] or p53 inhibitors[162,168], by blocking the initiation of the G2/M checkpoint, or by stimulating transit through G2/M to complete mitosis.
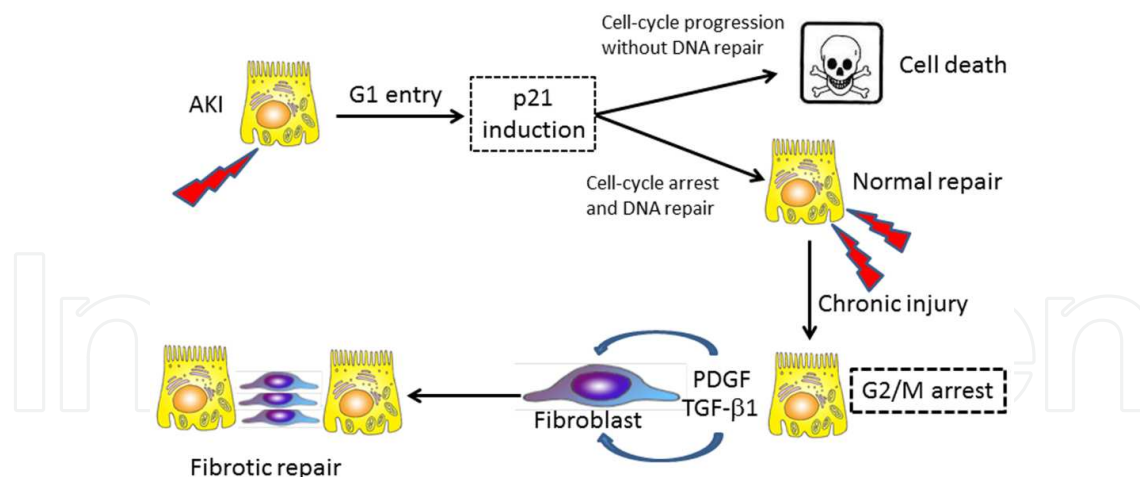

Figure 4. After AKI, normally quiescent proximal tubule epithelial cells enter the cell cycle, event traditionally viewed as an appropriate repair response to the loss of adjacent cells after an initial insult. However, rapid induction of p21 and arrest of cell-cycle progression play a protective role in kidney cells by preventing DNA-damaged cells from progressing in the cell cycle without repair, which eventually would result in death, probably by MC. In cases where the kidney injury is persistent or repeated, increasing numbers of epithelial cells stall between the G2 and M phases of the cell cycle, which stimulates the production of considerable amounts of TGF-β1 and PDGF that induce a fibrotic response. Pharmacological inhibition of G2/M-arrested cells reduced fibrosis, whereas increases in the proportion of G2/M-arrested cells in the cell-cycle exacerbated fibrosis, thus confirming that G2/M arrest in tubular cells contribute to progressive CKD after AKI.

However, application of these therapeutic strategies in humans needs careful assessment of the safety of the drugs and pathways under investigation because the G2/M checkpoint is extremely important in preventing the perpetuation of dangerous DNA mutations.

A great comprehension of the mechanisms involved in cell-cycle arrest could thus help not only in the discovery of novel therapeutic strategies to prevent podocyte loss, glomerulosclerosis, proteinuria and progressive kidney disease, but also in the selection and utilization of new specific and sensitive biomarkers for AKI.

7. Conclusions

Terminal differentiation invariably involves two closely linked phenomena: permanent withdrawal from the cell cycle and cell type-specific differentiation characterized by the upregulation of a panel of tissue-specific genes. Typically, post-mitotic cells do not reenter the cell cycle when exposed to growth signals, and in some cases further increases in tissue mass are achieved through an increase in cell size or hypertrophy. One long-standing theory to explain the lack of cytokinesis in post-mitotic cells, such as neurons, podocytes, and adult cardiac myocytes, is the presence of highly organized mature myofibrils which physically prevent cell division. Because cells must disassemble their cytoskeletal filaments before entering cell division, disassembly of the cytoarchitecture in these cell types would presumably negatively impact their function. However, in these cells expression of a wide range of cell-cycle proteins has been described, although no cases of cell division have ever been reported. This, together with the finding that the expression of cell-cycle proteins is necessary to execute cell death in response to certain stress signals, has led to the proposition that in post-mitotic cells, cell cycle is part of a well-regulated response to stress signals. The mechanisms by which cell-cycle reentry causes cell death are not completely known, but exploring the trigger(s) that induce normally post-mitotic cells to re-express cell-cycle proteins late in life as well as the molecular mechanism by which the induction of these proteins leads to cell death may produce great advances in the treatment and prevention of several neuro- and renal degenerative diseases.

8. Abbreviations

2N double chromosome quantity

3N triple to on chromosome quantity

MC mitotic catastrophe

MPF mitosis-promoting factor

M phase mitosis

PCC premature chromosomes condensation

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