Published in final edited form as:

J Mol Biol. 2017 July 21; 429(15): 2265–2277. doi:10.1016/j.jmb.2017.06.004.

Cell cycle control by PTEN

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Abstract

Continuous and error-free chromosome inheritance through the cell cycle is essential for genomic stability and tumor suppression. However, accumulation of aberrant genetic materials often causes the cell cycle to go awry, leading to malignant transformation. In response to genotoxic stress, cells employ diverse adaptive mechanisms to halt or exit the cell cycle temporarily or permanently. The intrinsic machinery of cycling, resting, and exiting shapes the cellular response to extrinsic stimuli whereas prevalent disruption of the cell cycle machinery in tumor cells often confers resistance to anti-cancer therapy. PTEN is a tumor suppressor and a guardian of the genome that is frequently mutated or deleted in human cancer. Moreover, it is increasingly evident that PTEN deficiency disrupts the fundamental processes of genetic transmission. Cells lacking PTEN exhibit cell cycle deregulation and cell fate reprogramming. Here we review the role of PTEN in regulating the key processes in and out of cell cycle to optimize genomic integrity.

Graphical abstract

Keywords

PTEN; Genetic transmission; Cell cycle; Quiescence; Senescence

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Introduction

Maintenance of genomic stability is critically important for tumor suppression. This principle is exemplified by tumorigenesis and cancer predisposition resulting from genetic alterations and chromosome aberrations, as manifested in both animal tumor models and human cancer patients. Phosphatase and tensin homolog (*PTEN*) is among the most frequently mutated genes in human cancer [1–3], and deletion or mutation of *Pten* causes various types of tumor in mice with high penetrance [4, 5]. As a bona fide tumor suppressor, PTEN has been recognized as a guardian of the genome since the revelation of its essential role in maintaining the structural integrity of chromosomes [6, 7]. PTEN deficiency not only results in disruption of the chromosomal structure but also causes whole chromosome abnormalities manifested by aneuploidy [8–10] and polyploidy [11]. These observations established that PTEN guards the genome against structural and numerical chromosome instability (CIN).

Faithful transmission of genetic information relies on orderly execution and timely integration of DNA replication and segregation with cell cycle progression, which is driven by the periodic activation and inactivation cyclin-dependent Ser/Thr kinases (CDKs). The activity of CDKs largely depends on their partnership with specific cyclins during distinct phases of the cell cycle. In mammalian cells, CDK1, CDK2, and CDK4/6 pair with cyclins A/B, cyclins A/E, and cyclin D1, respectively to coordinate the transition between distinct cell cycle phases [12, 13]. To ensure the unidirectional progression of the cell cycle, error-free completion of a prior phase is a prerequisite for initiation of the subsequent phase through ubiquitin-mediated protein degradation [14, 15]. A number of studies have shown that PTEN controls cell proliferation and survival by regulating every phase of the cell cycle, including the G1/S [16–20] and G2/M transitions [8, 21–25]. Moreover, PTEN is involved in the key processes of genetic transmission during the cell cycle to promote the fidelity of DNA replication [26–28] and chromosome segregation [9–11].

DNA replication and chromosome segregation are error-prone processes, during which erroneous DNA synthesis or incomplete chromosome disjunction normally activates checkpoint proteins to halt the cell cycle and trigger DNA repair. In addition to endogenous genotoxic stress, environmental stimuli as well as pharmacologic agents and radiotherapy can induce DNA damage and activate cell cycle checkpoints for repair of DNA lesions [29, 30]. The outcome of DNA repair depends on the checkpoint integrity, repair efficiency, and the severity of DNA damage. Defective cell cycle checkpoints and DNA repair promote CIN and may lead to uncontrolled cell proliferation and therapeutic resistance. PTEN is known to control DNA repair [6, 31] and increasing evidence suggests that PTEN also regulates multiple cell cycle checkpoints including the G1 [16], S [26], G2 [8], and M [21] checkpoints.

Errors generated during genetic transmission, when left unrepaired, may result in cell cycle exit. Subsequently, cells may either evolve adaptive mechanisms to survive severe genotoxic and environmental threats or undergo apoptosis if unfixable DNA lesions are detected. Cells can enter various functional states as they leave the cell cycle. Senescence and quiescence

are both non-proliferating conditions with distinct metabolic properties [32]. Quiescence is a reversible non-proliferating state resulting from a lack of nutrients and growth stimulation. In contrast, senescence represents a permanent cell cycle exit and an irreversible process initiated by serious DNA damage or saturated growth stimulation. As the prototype of regulated cell death, apoptosis evolves to ensure the demise of defective cells in response to disruption of the cell cycle and failure of continuous genetic transmission. Recent studies have also revealed the existence of regulated forms of necrosis [33].

Upon exit of the cell cycle, the ultimate decision between life and death may reflect the nature and severity of CIN. As a guardian of the genome, PTEN may inevitably participate in cell fate determination at this juncture. Indeed, PTEN has been shown to regulate senescence [34, 35], quiescence [36, 37], and cell death [38–41]. These multifaceted functions of PTEN all contribute to tumor suppression, which represents a compelling opportunity for translating new targets for cancer treatment. This review will summarize recent studies on PTEN regulation of cell cycle progression and cell fate determination in and out of the cell cycle. We will focus on the molecular mechanisms of cell cycle regulation to highlight the importance of PTEN and its role in genome maintenance.

Role of PTEN within the Cell Cycle: Guard Phase Transitions to Prevent CIN

A typical cell cycle consists of four distinct phases (G1, S, G2, and M) that occur in a successive order to complete the fundamental task of genetic transmission [42]. A large amount of data has shown that PTEN acts as a brake of cell cycle progression as an essential component of multiple checkpoints to prevent uncontrolled proliferation and CIN. For example, PTEN elicits an anti-proliferative effect in leukemic T cells by slowing progression through all phases of the cell cycle [43]. Although the PTEN-dependent control of the cell cycle may not occur in each phase of the cell cycle of all cell types, PTEN plays an important role in controlling the two major transitions during the cell cycle, from G1 to S and from G2 to M.

PTEN controls the G1-S transition

In G1 phase, newly daughter cells from the previous cell division accomplish most of their growth prior to entering S phase. G1 cells take up nutrients to synthesize necessary proteins and RNAs to prepare for DNA synthesis while integrating diverse environmental cues to make the decision of whether to continue cycling [44]. The G1 progression is mainly driven by activation of G1 CDKs (CDK4/6 and CDK2), following binding to their cognate cyclin partners (D and E types). The active kinase complex cyclin D-CDK4/6 phosphorylates the retinoblastoma protein (RB), which causes dissociation of both histone deacetylase 1 (HDAC1) and the transcription factor E2F-1 from RB. Release of HDAC1 and E2F-1 allows transcriptional activation of downstream genes including cyclin E that activates CDK2 to further phosphorylate RB and permit cell cycle progression.

Multiple mechanisms act to prevent premature entry into S phase and to restrict DNA replication to only once per cell cycle. For example, two families of small inhibitory proteins (INK4 and CIP/KIP) can suppress G1 CDKs by interfering with cyclin binding. The INK4 family proteins p16^{INK4a}, p15^{INK4b}, p18^{INK4c}, and p19^{INK4d} bind to CDK4/6 to block their

interaction with cyclin D. The CIP/KIP proteins (p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}) are potent inhibitors of cyclin E- and A-dependent CDK2 [45]. Moreover, an ubiquitin-mediated proteolysis mechanism plays an essential role in controlling the G1-S transition. SCF (Skp1/CUL1/F-box protein) is a well-characterized ubiquitin ligase complex that associates with Skp2, a F-box protein adaptor, to target multiple G1 regulators such as p21^{Cip1} and p27^{Kip1} for polyubiquitination-mediated proteolytic degradation [46, 47]. Interestingly, the activity of SCF^{Skp2} is inhibited in G1 by another E3 ligase complex APC/C (anaphase-promoting complex/cyclosome) through ubiquitination and degradation. CDH1 (Cdc20 homolog 1) functions as a substrate adaptor of APC/C from late M to the G1/S transition that recruits specific protein substrates such as SCF^{Skp2} for degradation. Therefore, the interplay between the two E3 ligase complexes SCF^{Skp2} and APC/C^{ADH1} controls G1 maintenance and progression.

The role of PTEN in regulating G1 progression and the G1-S transition has been well established. PTEN is a lipid phosphatase that antagonizes phosphoinositide 3-kinase (PI3K) by converting phosphatidylinositol-3, 4, 5-trisphosphate (PIP3) to phosphatidylinositol-4, 5-bisphosphate (PIP2) [48]. Loss of *Pten* in embryonic stem cells or neural stem/progenitor cells promotes cell cycle progression with acceleration of the G1-S transition [20, 49]. PTEN was found to suppress cell growth and survival in a phosphatase-dependent manner, likely through regulating the PI3K/AKT pathway [50, 51]. A study using a *PTEN*-null glioma cell model showed that ectopic expression of wild-type PTEN, but not a catalytic deficient PTEN mutant, induces G1 arrest [52]. This finding has been confirmed in other cancer models [16]. Mechanistic analysis demonstrated that PTEN induces the expression of p27^{Kip1} and reduces the activity of CDK2 to inhibit G1-S transition, leading to suppression of tumor development [53]. Further investigation demonstrated the requirement of p27^{Kip1} in PTEN-induced G1 arrest [17] and identified SCF^{Skp2} as the ubiquitin E3 ligase that mediates proteolytic degradation of p27^{Kip1} in the absence of PTEN [18] (Figure 1). The p27^{Kip1}-mediated G1 arrest is abrogated in mouse embryonic stem cells when *Pten* is deleted, resulting in an increased tolerance to serum deprivation [20]. In mice, concomitant deletion of *Pten* and *p27^{Kip1}* accelerates spontaneous development of prostate cancer [54], demonstrating the importance of Pten-p27^{Kip1}-mediated G1 regulation in suppressing tumorigenesis. These data suggest that PTEN is both required and sufficient for suppressing G1-S transition. In addition to the PTEN-p27^{Kip1} pathway, other important G1 regulators such as RB [55, 56], p18^{INK4C} [57], D- [58–61] and E-type cyclins [62, 63] are also involved in PTEN-mediated cell cycle control in the G1 phase.

As a lipid phosphatase that catalyzes the PIP3 to PIP2 conversion near the cytoplasmic membrane, PTEN was originally assumed to localize in the cytoplasm. PTEN was later found in the nucleus and interestingly, its nuclear localization was found to coincide with the G0–G1 phase of the cell cycle [64]. Several subsequent studies demonstrated that nuclear PTEN alone can elicit G1 arrest independent of AKT inhibition [65–67]. More specifically, the PTEN function in G1 regulation can be partitioned into cytoplasmic PTEN-mediated p27^{Kip1} upregulation and nuclear PTEN-mediated cyclin D1 downregulation [67]. In addition to reducing cyclin D1, nuclear PTEN can interact with p300 to promote p53 acetylation and the PTEN-p53 interaction in response to DNA damage, leading to G1 arrest [68]. Moreover, nuclear PTEN acts in a tumor suppressor network comprised of SPRY2

(Sprouty RTK Signaling Antagonist 2) and PP2A to mediate G1 arrest and suppress the development of prostate cancer [69]. Knockdown of *SPRY2* results in an elevated level of reactive oxygen species (ROS) followed by sequential activation of PP2A and GSK3B, which induces PTEN phosphorylation and nuclear accumulation. Nuclear PTEN subsequently elicits G1 arrest through a p53-dependent pathway (Figure 1). The enhanced understanding of PTEN function in controlling progression through the first gap phase (G1) of the cell cycle has implications for cancer treatment. For example, based on the prevailing upregulation of cyclin D1 following PTEN loss, a recent preclinical study demonstrated a significant therapeutic effect of cyclin D1-Cdk4/6 inhibition in a *Pten*-deficient endometrial tumor model [70]. In summary, PTEN inhibits the G1-S transition to prevent premature progression to DNA replication and resultant CIN.

DNA replication in S phase is a fundamental process of genetic transmission and recent studies revealed the essential role of PTEN in regulating DNA fork progression and restart. In response to replication stress, PTEN is enriched in the DNA replication fork to promote stalled fork recovery by recruiting multiple damage-response proteins such as RAD51 and CHK1 [26]. Additionally, PTEN physically interacts with RPA1, a single strand DNA binding protein, as well as MCM2, a DNA helicase, to stabilize the replication fork and prevent detrimental uncoupling between helicase and polymerase [27, 28]. Loss of PTEN thus results in failure of loading necessary components of the replisome and dysfunction of the DNA replication machinery, leading to accumulation of unreplicated DNA that may inevitably enhance genomic toxicity and impede subsequent phase transitions.

PTEN controls the G2-M transition and mitosis

In response to radiation-induced DNA damage, cells lacking PTEN exhibit an accelerated transition from G2/M to G1 whereas inhibition of PI3K/AKT signaling can elicit a G2 arrest in cells containing constitutively active AKT [71]. This earlier report suggests that PTEN and its lipid phosphatase pathway may be involved in cell cycle progression during the G2-M transition. Later, Parsons and colleagues characterized PTEN as an integral component of the DNA damage checkpoint by showing the premature exit from G2 in *Pten*^{-/-} embryonic stem cells following radiation treatment. Mechanistically, loss of PTEN induces AKT-mediated CHK1 phosphorylation, ubiquitination, and nuclear exclusion, leading to CIN and aneuploidy [8] (Figure 2).

Interestingly, CHK1 can also regulate PTEN to promote cell cycle reentry following stalled DNA replication via a phosphorylation-dependent mechanism. Specifically, stalled DNA forks recruits CHK1, which triggers a signaling cascade of casein kinase II stabilization and PTEN phosphorylation, allowing cells to reenter G2/M [72]. These results imply that PTEN phosphorylation may serve as a checkpoint bypass mechanism that promotes premature progression toward mitosis even prior to completion of DNA replication and repair of DNA damage (Figure 2). Consistent with this idea is the observation that dephosphorylation of PTEN can elicit a prometaphase arrest. This has been linked to inhibition of Notch signaling and consequent dephosphorylation of PTEN, which results in suppression of gastric tumors [23]. Moreover, PTEN forms a complex with cyclin B1 and CDK1, and inhibition of Notch induces nuclear translocation of the PTEN-cyclin B1-CDK1 complex to prevent cell cycle

progression [23]. Alternatively, PTEN can be dephosphorylated by TOPK (lymphokine-activated killer T-cell-originated protein kinase), a serine/threonine kinase downstream of CHFR (checkpoint protein with FHA and RING domains) that controls mitotic entry [21] (Figure 2).

In addition to the DNA damage checkpoint, cycling cells utilize another checkpoint in G2, referred to as the decatenation checkpoint, to ensure removal of entangled DNA prior to entering mitosis. This DNA decatenation checkpoint is primarily regulated by DNA topoisomerase II α (TOP2A), which plays an important role in genomic stability by preventing chromatin entanglement and chromosome bridges during segregation [73, 74]. PTEN has been shown to control the DNA decatenation checkpoint by physically interacting with TOP2A and promoting its stability [22]. Therefore, PTEN is involved in multiple checkpoints to control the G2-M transition and to protect the integrity of the genome by ensuring high fidelity DNA repair and decatenation (Figure 2).

When cells enter mitosis, PTEN is recruited to centrosomes and the mitotic spindle [10, 75]. Loss of PTEN impairs the mitotic spindle architecture and the integrity of mitotic centrosomes, leading to chromosome misalignment, missegregation, mitotic catastrophe, aneuploidy/polyploidy, and tumorigenesis [9–11, 75, 76]. These mitotic defects are partially mediated by enhanced expression and phosphorylation of polo-like kinase (PLK1), a mitotic kinase with oncogenic potential [11, 76]. PLK1 hyperactivation reportedly results in premature centrosome separation and lagging chromosomes, which causes tumorigenesis in *cyclin B* transgenic mice [77]. PTEN protects cell division and prevent polyploidy through controlling the phosphorylation and expression levels of PLK1 [11]. Besides serving as a mitotic target of PTEN, PLK1 has also been reported to control mitotic timing by phosphorylating PTEN and promoting its association with chromatin [78]. The mutual functional interaction between PTEN and PLK1 delineates a feedback loop to control the equilibrium of mitotic phosphorylation, particularly during mitotic exit. Mitotic CIN may result from concurrent PTEN deficiency and PLK1 activation, which serves as a potential therapeutic target.

Recent studies provide further mechanistic insights into the direct involvement of PTEN in the mitotic machinery [9, 10]. PTEN is recruited to pre-mitotic centrosomes in a PLK1-dependent manner, which creates a docking site for a PTEN-associated protein complex containing a microtubule plus end-directed kinesin, EG5 [9]. In addition, PTEN is also found to co-localize with EG5 on the mitotic spindle to regulate spindle size [10]. Loss of PTEN results in aberrant localization and modification of EG5, leading to impairment of mitotic spindle architecture and spindle pole motility [9, 10].

Similar to the mutual regulation between PTEN and PLK1, there is a reciprocal regulatory relationship between PTEN and APC/C^{CDH1}, an E3 ligase complex known to promote the transition from mitotic exit to the next cell cycle. For example, CDH1 interacts with PTEN in late mitosis and mediates its degradation to ensure timely mitotic exit [79]. On the other hand, PTEN interacts with APC/C^{CDH1} and promotes its activity in reducing the levels of Ets2 and p16^{INK4} to prevent cellular senescence [80]. While CDH1 mediates APC/C-induced PTEN ubiquitination, it can act in an APC/C-independent fashion to protect PTEN

stability. Specifically, CDH1 suppresses the activity of another E3 ligase, WWP2, to protect PTEN against ubiquitin-dependent proteasome degradation [81]. This APC/C-independent regulation of PTEN by CDH1, similar to the regulation of APC^{CDH1} by PTEN [80], may not have a direct link to the function of PTEN in mitotic control. Therefore, a PTEN-associated complex may exert diverse functions and the formation of such a functional complex serves in multiple cellular processes to guard the genome against CIN.

PTEN in cell size control

Cycling cells respond to DNA damage by undergoing G1 and G2 arrest, which is accompanied by a simultaneous cell size arrest, a control mechanism to ensure size stabilization. Besides regulating cell cycle progression, PTEN also controls cell size. The first evidence of PTEN controlling cell size was revealed in *Drosophila* during eye development, in which deficiency of *dPTEN* (the *Drosophila* homolog of mammalian PTEN) causes enlarged ommatidia [82]. The association of PTEN with cell size control has also been validated in various conditional *Pten* knockout mice showing oversized cells and organs [83–86]. For example, tissue-specific deletion of *Pten* in the mouse brain results in the formation of enlarged cells, leading to macrocephaly [83, 84]. Ectopic PTEN expression in Jurkat T cells results in reduced cell size and growth [87]. Using a gene-targeting approach, Waldman and colleagues proposed a concept of the cell size checkpoint. PTEN maintains the cell size checkpoint in a manner independent of the p53-mediated G1 or G2 checkpoint [88]. Further characterization revealed a PI3K/AKT-independent mechanism of actin remodelling that is responsible for PTEN function in controlling the DNA damage-related cell size checkpoint [89]. Given the distinct mechanisms employed by PTEN in regulating the DNA damage-induced cell size checkpoint, it is plausible that PTEN controls multiple G2 surveillance activities to ensure faithful genetic transmission from DNA replication to chromosome segregation.

Although there appears to be a disconnect between PTEN's function in cell size control and its role in cell cycle progression, these distinct activities converge at the interface of DNA damage responses and may reflect different types and degrees of CIN. In particular, the enlarged size of *PTEN*-deficient cells reminisces the morphology of senescent cells that permanently exit the cell cycle (as described below). It would be logical to explore whether the PTEN function in cell size control may contribute to cell fate determination at the entrance or exit of the cell cycle, or vice versa.

Role of PTEN in Cell Fate Determination upon Cell Cycle Exit

PTEN is well known to suppress both cell survival and proliferation. As described above, PTEN suppresses cancer cell growth mainly through inhibition of cell cycle progression, in particular, by controlling the G1-S and G2-M transitions [8, 52, 71]. Nevertheless, growth inhibition may result from cell death with and without cell cycle inhibition. For example, in Jurkat T cells, PTEN induces apoptotic cell death without affecting the cell cycle profile [87, 90]. In most scenarios, however, PTEN-mediated suppression of cell growth can be attributed to both reduced cell cycle progression and elevated cell death [91–97].

Apoptosis is a common path of exiting the cell cycle when endogenous and environmental threats exceed a threshold that is tolerable for survival. PTEN can function as a mediator of apoptosis, as often reported in terminally differentiated cells such as neurons [41, 98, 99]. For example, in primary hippocampal cultures, PTEN participates in the cellular intrinsic apoptotic machinery by interacting with mitochondrial Bax, a pro-apoptotic protein that translocates to mitochondria in response to apoptotic stimuli [41]. In cancer cells, however, PTEN is not a bona fide cell death inducer, despite its direct antagonism of the PI3K/AKT survival pathway. Unlike p53, overexpression of PTEN in cancer cells is usually unable to induce spontaneous cell death. Nevertheless, PTEN can sensitize cancer cells and augment apoptosis in response to various therapeutic agents [38–40]. PTEN acts as a protein phosphatase to suppress phosphorylation of CREB [100]. Loss of PTEN results in CREB activation, which subsequently suppresses the transcription of anti-apoptotic genes such as *Bcl-2*, leading to increase of cell death in prostate cancer cells treated with multiple chemotherapeutic drugs [40].

Quiescence and PTEN

As described above, *Pten*-null neural stem cells have a shortened cell cycle time due to the enhanced G1-S transition [49]. Further examination demonstrated that deficiency of *Pten* enhances neural stem/progenitor cell self-renewal by promoting exit from the quiescence state (G0) and entrance into the cell cycle [101]. Aberrant enhancement of self-renewal may disrupt the homeostatic control and promote extensive proliferation, leading to malignant transformation. Indeed, deletion of *Pten* in the hematopoietic system results in depletion of normal hematopoietic stem cells and generation of leukemic stem cells, which leads to development of myeloproliferative disorder and leukemic transformation [102, 103]. Therefore, PTEN loss plays an important role in tumor initiation by promoting cancer stemness.

Hair follicle stem cells (HFSCs) act as tumor-initiating cells for cutaneous squamous cell carcinoma and undergo defined cycles of quiescence and activation. Deletion of *Pten* in *K15-CrePR;Kras^{G12D}* mice promotes the transition from quiescence to activation and induces hyperplastic expansion of hair follicles, which allows Kras-mediated oncogenesis. These results suggest that PTEN suppresses tumorigenesis by maintaining the quiescent state [104].

In addition to quiescence-dependent tumor suppression, PTEN also regulates quiescence-related embryogenesis and tissue regeneration. In *Caenorhabditis elegans* primordial germ cells, DAF-18 (the worm homolog of mammalian PTEN) is required for the maintenance of a reversible cellular quiescence during embryogenesis. Depletion of DAF-18 results in bypass of this arrest and causes aberrant germline growth [36]. A recent study also demonstrated the important role of PTEN in stem cell-mediated tissue regeneration [37]. Myogenic stem cells are required for regeneration of adult skeletal muscles. Deletion of *Pten* in quiescent myogenic stem cells results in spontaneous activation and premature differentiation, leading to regeneration failure. Mechanistically, *Pten* deletion induces PI3K/AKT-mediated nuclear exclusion of Foxo1 and suppression of Notch signaling. These

findings suggest that PTEN maintains a pool of quiescent myogenic stem cells, which is required for proper regeneration of adult skeletal muscles.

Recent studies of tumor immunology revealed the role of PTEN in maintaining the lineage stability of regulatory T cells (Tregs) [105–108]. *Pten* interacts with Nrp1 (Neuropilin 1) in quiescent Tregs. This functional interaction thereby suppresses the PI3K/AKT pathway and maintains the nuclear localization of Foxo transcription factors for activation of Treg signature genes. Deletion of *Pten* or removal of the *Pten*-binding domain from Nrp1 results in Treg dysfunction, manifested by failure to elicit contact-dependent immune suppression [108]. These data highlight the role of PTEN in maintaining the functionality of Tregs by promoting their stability and quiescence. As a temporary departure from continuous cycling, quiescence represents a reserved mechanism for cell cycle revitalization and serves as a natural extension of the PTEN pathway of genome maintenance during the cell cycle.

Senescence and PTEN

Senescence limits the proliferation of damaged cells and may act as a natural barrier to cancer progression. This concept was illustrated in a mouse model of prostate cancer, in which combined deletion of both *Pten* and *p53* results in accelerated tumor onset and progression as compared to *Pten* deletion alone [34]. Specifically, *Pten* inactivation induces cellular senescence that is dependent on the presence of p53, whereas concomitant deletion of p53 lifts the senescence-mediated tumor suppressive effect and allows aggressive tumorigenesis. Besides concurrent deletion of p53, activation of an oncogene such as *Her2* can also overcome senescence in *Pten*^{-/-} mice to facilitate tumor development and progression [109]. A follow-up study further characterized the unique properties of PTEN-loss-induced cellular senescence (PICS). Unlike oncogene-induced senescence that is often preceded by hyperproliferation and a DNA damage checkpoint response, PICS occurs in the absence of cellular proliferation or DNA damage [35]. This special type of cellular senescence may provide a unique cell fate status for therapeutic intervention. Indeed, targeting the signaling mediators of PICS in *Pten*-null senescent tumors improves the efficacy of chemotherapy [110] and triggers antitumor immune response [111].

PTEN and p53 are among the most frequently mutated genes in human cancer and there are multiple forms of functional interaction between these two potent tumor suppressors. In response to DNA damage, p53 can bind to a site in the *PTEN* promoter to induce its transcription whereas PTEN is required for p53-mediated apoptosis [112]. Conversely, PTEN has also been shown to regulate p53 function through distinct mechanisms. For example, PTEN promotes p53 transactivity using its canonical lipid phosphatase activity likely by suppressing AKT-dependent phosphorylation and nuclear translocation of MDM2, a major negative regulator of p53 [113]. Alternatively, PTEN can increase p53 protein stability and its transcriptional activity, which is dependent on the physical association between PTEN and p53 but independent of the PI3K-AKT pathway [114]. Besides p53 itself, other members of the p53 family can also interact with PTEN to mediate DNA damage response. For example, PTEN forms a complex with p73 in the nucleus to promote p73 transactivity and induce apoptosis following doxorubicin-induced DNA damage [115]. Thus, PTEN can physically interact with both p53 and p73 in response to genotoxic stress.

Interestingly, the PTEN-p53 pathway controls cell cycle progression by activating p21 [113,114] while the PTEN-p73 complex induces PUMA-mediated apoptosis [115]. These findings suggest that the PTEN/p53/p73 axis plays an important role in directing cell fate commitment in response to genotoxic stress.

The PTEN status in human glioma cells has been shown to dictate the cell fate between senescence and apoptosis in response to radiation therapy [116]. In *PTEN*-deficient cells, radiation induces accumulation of ROS, which, together with the intrinsic activation of PI3K/AKT pathway, leads to cellular senescence. In contrast, *PTEN*-proficient cells undergo apoptosis following radiation treatment. Interestingly, ectopic PTEN expression or depletion of p53 prevents radiation-induced senescence in *PTEN*-null cells and redirects them to apoptosis.

Based on these studies, PTEN appears to function as a suppressor of cellular senescence. However, other findings suggest that PTEN may act to promote senescence. For example, PTEN has been shown to mediate replicative senescence in response to the aging-associated decline of CSIG (cellular senescence-inhibited gene) [117]. PTEN has also been shown to maintain the protein stability and acetylation of p53, a senescence inducer, by forming a complex with p300 [68]. In addition, a mouse model of *Pten* deletion specifically in pancreatic β -cells demonstrated that PTEN blocks cell cycle progression through induction of p16^{INK4a}, a well-known mediator of cellular senescence [118]. Although these results are contradictory to the findings with PICS, this disparity may reflect distinct cellular contexts as well as different levels of genotoxic stress. In one case, PTEN suppresses replicative senescence in response to sustained DNA damage, whereas in the other it mediates physiological senescence as a response to prolonged cellular hyperactivity.

Conclusions and Perspectives

Continuous and error-free progression of the cell cycle is an ideal approach for faithful transmission of genetic information. However, unavoidable errors associated with DNA replication generate cancer-driving mutations, as highlighted in a recent report by Vogelstein and colleagues [119]. When continuous mitotic cycling becomes impossible due to accumulation of unavoidable genetic errors, fail-safe mechanisms evolve to provide alternative paths for homeostatic restructuring and survival. PTEN controls both the primary and alternative pathways to guard the genome. During the cell cycle, PTEN not only acts as an integral component of the central genetic transmission machinery to ensure accurate genome duplication and segregation, but it also controls the consecutive phase transitions to warrant the cycling continuity. PTEN normally inhibits both the G1-S and G2-M transitions to prevent premature initiation of DNA replication and mitosis. PTEN also controls the in and out of the cell cycle by maintaining cellular quiescence and regulating the triggering of senescence (see graphic abstract). In response to genotoxic and environmental stress during the cell cycle and upon cell cycle exit, PTEN participates in the establishment of alternative fail-safe mechanisms through functional interaction with key regulators in and out of the cell cycle (Table 1). These include timely triggering of cell death programs to clear damaged cells and proper initiation of quiescence or senescence for autonomous and non-autonomous remodeling. Current knowledge of PTEN function in genome maintenance both within and

beyond the cell cycle provides a foundation for further elucidation of how PTEN controls the dynamic switch between cell cycle entry and exit.

During the cell cycle, accumulation of DNA damage and inactivation of cell cycle checkpoints occur simultaneously following PTEN inactivation and may represent a bilateral consequence of PTEN loss. It remains to be elucidated which may have the upper hand in causing CIN following *PTEN* depletion. Identification of the deregulated molecular events resulting from PTEN mutation that triggers the deleterious cascade of cell cycle defects has significant implications for efficient elimination of *PTEN*-deficient tumors. Upon cell cycle exit, cells undergoing quiescence or senescence are metabolically active despite their non-proliferative state. PTEN dysfunction reprograms the metabolome as tumor develops and grows [120–122]. Future research should be directed at elucidating our understanding of the genome-metabolome interaction and its effect on the continuum of cell cycle control and cell fate commitment. As a potent tumor suppressor, a key component of genome maintenance, and a regulator of metabolism in and out the cell cycle, PTEN is at the center of several potentially targetable processes that are relevant to cancer.

Acknowledgments

We thank Lorenzo Galluzzi for critical reading of the manuscript, thoughtful discussion, and editorial assistance. Research in the Shen laboratory is supported by National Institutes of Health (NIH) grant (R01GM100478) and the Irma T. Hirschl/Monique Weill-Caulier Trust.

Abbreviations

PTEN	phosphatase and tensin homolog
PI3K	phosphoinositide 3-kinase
CIN	chromosome instability
CDK	cyclin-dependent kinase
RB	retinoblastoma protein
HDAC	histone deacetylase
APC/C	anaphase-promoting complex/cyclosome
CDH1	cdc20 homolog
SCF	Skp1/CUL1/F-box protein
PI3K	phosphoinoside 3-kinase
SPRY2	Sprouty RTK Signaling Antagonist 2
ROS	reactive oxygen species
PLK1	polo-like kinase
TOPK	(lymphokine-activated killer T-cell-originated protein kinase)

CHFR	checkpoint protein with FHA and RING domains
TOP2A	topoisomerase IIa
Tregs	regulatory T cells
Nrp1	Neuropilin 1
PICS	PTEN-loss-induced cellular senescence
CSIG	cellular senescence-inhibited gene

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Research Highlights

- Continuous and error-free progression of the cell cycle is key to genomic stability
- PTEN is a tumor suppressor and a guardian of the genome
- Cells lacking PTEN exhibit cell cycle deregulation and aberrant genetic transmission
- PTEN controls cell cycle progression and cell fate determination upon cell cycle exit
- Attentive and continuous cycling with PTEN is required to ensure genome integrity

Figure 1.

PTEN induces G1 arrest to prevent uncontrolled proliferation and chromosome instability (CIN). Multiple mechanisms are involved in PTEN-mediated inhibition of the G1-S transition. PTEN can increase the expression of p27^{Kip2} or block its proteolytic degradation. Loss of PTEN induces SCF^{Skp2}-dependent ubiquitination of p27^{Kip2}, which results in the reduction of p27^{Kip2} and activation of CDK2, leading to acceleration of the G1-S transition (shown with a green background to indicate aberrant cell cycle regulation). While the induction of p27^{Kip2} is attributed to the function of cytoplasmic PTEN (shown with a yellow background), PTEN has a nuclear function (pink background) of downregulating cyclin D. In addition to reducing cyclin D, nuclear PTEN can interact with p300 to promote acetylation and stability of p53, resulting in G1 arrest. Moreover, PTEN can translocate into the nucleus to elicit a p53-dependent G1 arrest by serving as a target of phosphorylation signaling cascade comprised of PP2A and GSK3B, in response to an increased ROS level following SPRY2 depletion.

J Mol Biol. Author manuscript; available in PMC 2018 July 21.

Table 1

PTEN interactors involved in the control of cell cycle progression and exit

	Molecule	Functional relationship & phenotype	Reference
G1-S transition	p27 ^{Kip1}	upregulation by PTEN/cooperation with PTEN in tumor suppression	17,20,53,54,58,67
	CDK2	inhibition by PTEN	17,18,20,53,63
	SCF ^{Skp2}	downregulation by PTEN	18
	RB	upregulation/dephosphorylation/activation by PTEN	17,55,56,57
	p18 ^{INK4C}	cooperation with PTEN in tumor suppression	57
	Cyclin D	downregulation by PTEN	17,58,59,60,61,62,67
	Cyclin E	downregulation by PTEN	18,53,58,62,63
	Cyclin A	downregulation by PTEN	17,53
	p21 ^{Cip1}	downregulation by PTEN	63
	p300	association with PTEN	68
	p53	acetylation by PTEN-associated p300/stabilization/interaction with PTEN	68,69
	SPRY2	suppression of PTEN nuclear accumulation	69
	GSK3B	induction of PTEN phosphorylation and nuclear accumulation	69
	PP2A	increase of PTEN phosphorylation	69
S (DNA replication)	RAD51	association with PTEN on replication forks to promote fork restart	26
	CHK1	association with PTEN on replication forks	26
	RPA1	association with PTEN and induction of accumulation on replication forks by PTEN	28
	MCM2	association with PTEN and dephosphorylation by PTEN to restrict replication fork progression	27
G2-M2 transition	CHK1 (downstream of PTEN)	suppression of PI3K/AKT-mediated phosphorylation, ubiquitination, nuclear exclusion by PTEN	8
	CHK1 (upstream of PTEN)	induction of PTEN phosphorylation, allowing entry into G2/M	72
	Notch	dephosphorylation, nuclear translocation and activation of PTEN, leading to G2/M arrest	23
	Cyclin B1	association with PTEN	23
	CDK1	association with PTEN	23
	TOPK	phosphorylation and inactivation of PTEN, leading to premature mitotic entry	21
	CHFR	prevention of TOPK-mediated PTEN phosphorylation and inactivation	21
	TOP2A	association with PTEN and stabilization by PTEN for activation of the DNA decatenation checkpoint	22
M (Mitosis)	γ -tubulin PLK1	downregulation by PTEN	75
	(downstream of PTEN) PLK1	downregulation and dephosphorylation by PTEN	11,75
	(upstream of PTEN)	recruitment of PTEN to pre-mitotic centrosomes, promotion of PTEN phosphorylation and chromatin enrichment	76
	DLG1	association with PTEN	9

	Molecule	Functional relationship & phenotype	Reference
	EG5	association with PTEN for spindle pole motility and maintenance of spindle architecture	9,10
Quiescence	FOXO1	nuclear retention by PTEN for the maintenance of quiescent myogenic stem cell for muscle regeneration	37
	NRP1	association with PTEN in quiescent Treg cells for the maintenance of Treg lineage stability	108
Senescence	p53	suppression or stabilization by PTEN	34,68
	HER2/3	overcome of senescence caused by PTEN loss	109
	MEK	overcome of senescence caused by PTEN loss	109
	CSIG	suppression of PTEN to prevent replicative senescence	113
	p16 ^{INK4a}	induction by PTEN	114