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Review

PTENless means more

Bangyan Stiles, Matthias Groszer, Shunyou Wang, Jing Jiao, Hong Wu*

Department of Molecular and Medical Pharmacology, UCLA David Geffen School of Medicine, CHS23-234, Los Angeles, CA 90095, USA

Abstract

Recent studies indicate that certain key molecules that are vital for various developmental processes, such as Wnt, Shh, and Notch, cause cancer when dysregulated. PTEN, a tumor suppressor that antagonizes the PI3 kinase pathway, is the newest one on the list. The biological function of PTEN is evolutionarily conserved from *C. elegans* to humans, and the PTEN-controlled signaling pathway regulates cellular processes crucial for normal development, including cell proliferation, soma growth, cell death, and cell migration. In this review, we will focus on the function of PTEN in murine development and its role in regulating stem cell self-renewal and proliferation. We will summarize the organomegaly phenotypes associated with *Pten* tissue-specific deletion and discuss how PTEN controls organ size, a fundamental aspect of development. Last, we will review the role of PTEN in hormone-dependent, adult-onset mammary and prostate gland development.
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Introduction

Lloyd and Dennis (1962) published the first case report on a "rare, familial, developmental disease" called "Cowden's disease", after the family name of the proposita. Patients with Cowden's disease suffer from hamartomas (benign growths) in multiple organs with a tendency of malignant transformation. In 1997, three research laboratories independently cloned a tumor suppressor gene located on human chromosome 10q23 (Li and Sun, 1997; Li et al., 1997; Steck et al., 1997). Sequence analysis indicated that this gene encodes a phosphatase and shares a large region of homology to tensin and auxilin. This tumor suppressor gene was therefore named *PTEN* for phosphatase and tensin homolog deleted on chromosome 10 (or *MMAC1/TEP1*). In the same year, the link between Cowden's disease and *PTEN* mutations was formally established (Liaw et al., 1997; Nelen et al., 1997). Many studies using either primary tumor tissues or established tumor cell lines demonstrated high frequencies of *PTEN* mutation/deletion in various

human cancers, including brain, bladder, breast, prostate, and endometrial cancers (Ali et al., 1999; Aveyard et al., 1999; Dahia, 2000; Dreher et al., 2004; Li et al., 1997; Rasheed et al., 1997), marking *PTEN* the second most frequently mutated human tumor suppressor gene (Stokoe, 2001). *PTEN* mutation was also found to be responsible for two other autosomal dominant tumor predisposition syndromes, Bannayan–Zonana syndrome (BZS) and Lhermitte–Duclos disease (LDD) (Arch et al., 1997; Iida et al., 1998; Koch et al., 1999; Marsh et al., 1999).

Deletion of *Pten* in mouse models revealed that PTEN is critical for animal development. *Pten* null embryos die early during embryogenesis (Di Cristofano et al., 1998; Podsypanina et al., 1999; Stambolic et al., 1998; Suzuki et al., 1998) and thus much of our current knowledge regarding the functions of PTEN in development is acquired from animals with tissue-specific *Pten* deletion using the Cre-loxP system. Because the physiological functions of PTEN in individual mouse tissues have been reviewed recently by Kishimoto et al. (2003), we will emphasize how PTEN-controlled signaling pathways regulate key biological events related to normal development, including stem cell self-renewal and proliferation, cell differentiation and migration,

* Corresponding author. Fax: +1 310 267 0242.
E-mail address: hwu@mednet.ucla.edu (H. Wu).

organ size control, and the hormone-regulated organogenesis. Although this review will focus on the role of PTEN in murine development, it is worth noting that PTEN- and PTEN-controlled signaling pathways are evolutionary conserved and play fundamental roles in *Drosophila* (Gao et al., 2000; Huang et al., 1999) and *C. elegans* (Gil et al., 1999; Mihaylova et al., 1999; Rouault et al., 1999) development and chemotaxis response of *Dictystelium* (Funamoto et al., 2002; Iijima and Devreotes, 2002).

PTEN-controlled signaling pathways and development

Studies on the molecular mechanism of PTEN function revealed that the signaling pathways controlled by the PTEN tumor suppressor are vital for both cell growth and animal development (Vivanco and Sawyers, 2002). PTEN functions as a phosphatase with both lipid and protein phosphatase activities in vitro (Li and Sun, 1997; Maehama and Dixon, 1998; Myers et al., 1998). While its in vivo protein phosphatase activity remains to be further investigated, PTEN's lipid phosphatase activity has been demonstrated both in vitro and in vivo. As a lipid phosphatase, PTEN dephosphorylates phosphotidylinosital-3,4,5-triphosphate (PIP_3), a product of phosphotidylinosital-3-kinase (PI3Kinase) (Maehama and Dixon, 1998; Stambolic et al., 1998; Sun et al., 1999). By dephopshorylating PIP_3, PTEN inhibits the growth factor signals transduced through PI3Kinase, thus has a broad impact on cell growth, cell migration, cell death, and cell differentiation, processes involved in normal development (Fig. 1). PTEN deficiency leads to accumulation of PIP_3 that in turn activates several signaling molecules including the phosphotidylinositol-dependent kinases (PDKs), the serine/threonine kinases AKT/PKB, S6 kinase, and mTOR, as well as small GTPases Rac1 and Cdc42 (Anderson et al., 1998; Liliental et al., 2000; Pene et al., 2002; Stiles et al., 2002; Sun et al., 1999; Wu et al., 1998). Among these downstream signaling molecules, AKT is the best characterized one (Anderson et al., 1998; Sun et al., 1999; Wu et al., 1998). A number of substrates have been identified for AKT kinase, including proapoptotic factor BAD (Datta et al., 1997; del Peso et al., 1997), caspase 3 and 9 (Cardone et al., 1998), metabolic enzyme glycogen synthase kinase (Cross et al., 1995), key cell cycle modulators MDM2 (Ashcroft et al., 2002; Zhou et al., 2001b), p21 and p27 (Collado et al., 2000; Zhou et al., 2001a), members of the forkhead transcription factor family (FOXOs) (Biggs et al., 1999; Brunet et al., 1999; Guo et al., 1999; Kops et al., 1999; Nakae et al., 1999; Takaishi et al., 1999; Tang et al., 1999), and nuclear receptors AR (Lin et al., 2001) and ER (Campbell et al., 2001; Mita et al., 2003). Phosphorylation of these molecules leads to changes in their subcellular localization, activities, or half lives, which in turn controls cell metabolism, cell death, cell cycle progression, and cell differentiation (for review, see Vivanco and Sawyers, 2002).

The PTEN/PI3 kinase/AKT signaling pathway also interacts with other signaling pathways known to be essential for normal development, including the TGF-β/Smad pathway and the Wnt/β-catenin pathway. TGF-β regulates many cellular processes that are crucial in normal development. PTEN, also called TEP1 for TGF-β-regulated and epithelial cell-enriched phosphatase (Li and Sun, 1997), is rapidly downregulated by TGF-β in keratinocytes and pancreas (Ebert et al., 2002; Li and Sun, 1997). Conversely, PI3 kinase/AKT phosphorylates SMAD3, a receptor-regulated SMAD, and inhibits SMAD3-dependent TGF-β signaling (Song et al., 2003). Similar to the TGF-β pathway, the Wnt signaling pathway is also conserved in various organisms from worms to mammals, and plays important roles in development, cellular proliferation, and differentiation. In mammals, the Wnt signal transduction pathway is involved in many differentiation events during embryonic development (for review, see Polakis, 2000). Activation of the Wnt downstream molecule β-catenin leads to tumor formation of various origins (Polakis, 2000). The cytosolic pool of β-catenin has a short half-life in the absence of Wnt signaling. Its turnover is controlled by the action of a multi-protein complex (for a review, see (Kikuchi, 2000), including at least Axin, APC, PP2A, GBP, and GSK-3β. GSK-3β kinase phosphorylates β-catenin on specific serine and threonine residues at its N-terminus, targeting it to ubiquitin-mediated degradation (Munemitsu et al., 1996; Yost et al., 1996). AKT kinase phosphorylates and inhibits GSK-3β, leading to β-catenin nuclear translocation and activation (Pap and Cooper, 1998; Yost et al., 1996). In addition to the above signaling pathways, recent studies have pointed out the role of PTEN in regulating the expression of homeobox genes, such as NKX3.1 (Wang et al., 2003) and hepatic nuclear factors (Wolfrum et al., 2003). Taken together, PTEN can modulate animal development by multiple mechanisms.

PTEN expression and function in early development

Pten expression can be detected as early as embryonic stem cell stage, which is derived from embryonic day 3.5 blastocysts (Sun et al., 1999). In situ hybridization and immunohistochemistry analyses indicate that *Pten* is expressed in both extraembryonic and embryonic tissues (Luukko et al., 1999; Podsypanina et al., 1999). Within the embryo proper, *Pten* is expressed ubiquitously during the early stage of embryonic development (E7–11) but becomes more restricted in the later stage (E15–19) in tissues and organs (Podsypanina et al., 1999). *Pten* is highly expressed in the central nervous system, liver, heart, skin, and gastrointestinal tract, similar to the expression
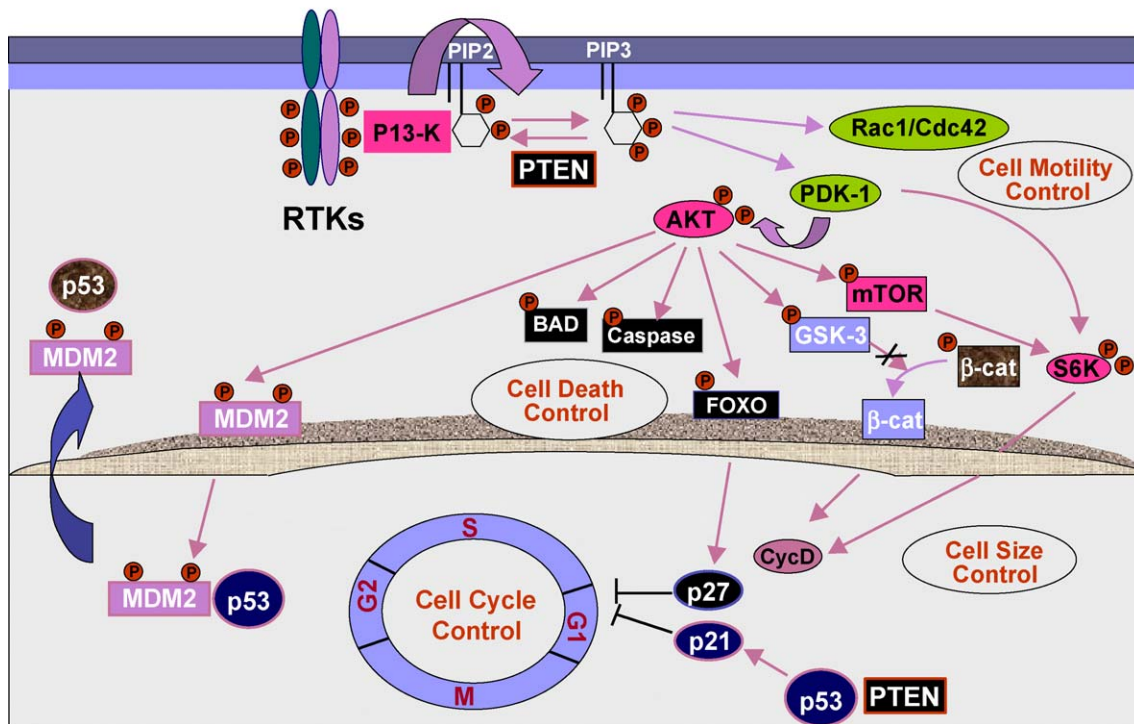
Signaling Pathways Controlled by PTEN

Fig. 1. Signaling pathways controlled by PTEN. PTEN is a phosphatase that inhibits the growth factor/PI3 kinase signaling. One of PTEN's enzymatic activities is to dephosphorylate PIP3, the product of PI3 kinase and a major cellular second messenger. By dephosphorylating PIP3, PTEN inhibits the activity of several downstream molecules, of which, the most important one is AKT. Activation of AKT by growth factors leads to increased cell cycle progression, suppressed cell death as well as increased translation.

patterns observed during human development (Gimm et al., 2000). At the cellular level, PTEN is present in both cytosol and nucleus (Freeman et al., 2003; Gimm et al., 2000; Li and Sun, 1997). Our recent study indicates that the subcellular localization of PTEN may be developmentally regulated and differential nuclear and cytoplasmic expression of PTEN may reflect its role during different stages of development and cellular functions (Wang and Wu, unpublished observations).

To study the biological functions of PTEN, independent groups have generated *Pten* mutant animals by deleting different regions of the *Pten* locus (Di Cristofano et al., 1998; Podsypanina et al., 1999; Suzuki et al., 1998). Animals heterozygous for *Pten* developed normally but homozygotes died early during embryogenesis, bearing different onsets and phenotypes. While all groups agreed that PTEN are essential for normal embryonic development, they reached different conclusions on the exact embryonic function of PTEN. Di Cristofano et al. (1998) could not recover null embryos post E7.5 and observed differentiation defects in *Pten*$^{-/-}$ ES cell-derived embryoid bodies. *Pten* null ES cells also failed to contribute to the formation of the chimeric organism. Thus, they concluded that PTEN is required for the differentiation and organization of three germ layers (Di

Cristofano et al., 1998); Suzuki et al. (1998) obtained gastrulated null embryos at E7.5 with severely expanded and abnormally patterned cephalic and caudal regions at E8.5. Knockout embryos generated in our laboratory have similar defects as reported by Suzuki et al. (Freeman and Wu, unpublished observations). In addition, we found that *Pten* null ES cells were able to contribute efficiently to chimeric animals. These discrepancies could be due to several mechanisms, including the nature of deletions made by individual groups, the genetic background of the mutant animals, as well as the specific assays conducted (Bradley and Luo, 1998). Nevertheless, in light of the ubiquitous expression nature of *Pten* gene during early embryogenesis, one should not be surprised if *Pten* null embryos are dead due to abnormal development of multiple organs and tissues.

PTEN regulates stem cell function

Development fulfills two major functions: it generates cellular diversity and order within an individual, and it assures the continuity of life from one generation to the next. Stem cells play essential roles in both of the aforementioned functions. Stem cells are generally defined

as clonogenic cells capable of both self-renewal and multi-lineage differentiation. Embryonic stem cells (ES) or primordial germ cells (PGC or EG) derived from early embryos are totipotent, which can give rise to every single cell type in our body, including germ cells that are vital for propagating genetic materials from one generation to the next one. Somatic stem cells present in individual organs or tissues, on the other hand, are generally regarded as having more limited differentiation potentials than ES cells and are responsible for normal organogenesis and tissue repair throughout the life of an individual (Weissman et al., 2001). Among many key molecules that are crucial for normal development and tumorigenesis, Wnt, Shh, Notch, as well as their controlled signaling pathways are known to have important roles in regulating stem cell self-renewal, proliferation, and differentiation (Reya et al., 2001). Because *Pten* is highly expressed in ES cells, the functions of PTEN in ES cells have been studied independently by different groups. Di Cristofano et al. showed that *Pten* null ES cells have enhanced anchorage-independent growth property. However, under regular adherent conditions *Pten* null ES cells behaved similar to WT controls in their growth rate and cell cycle distribution (Di Cristofano et al., 1998). Our studies, on the other hand, indicated that $Pten^{-/-}$ ES cells exhibited an increased growth rate under normal growth condition and could proliferate and survive even in the absence of serum. ES cells lacking PTEN function also displayed advanced entry into S phase (Sun et al., 1999). Importantly, deletion of *Akt-1*, the major *Akt* family member in *Pten* null ES cells, completely reversed the growth advantage phenotype seen in $Pten^{-/-}$ cells (Stiles et al., 2002), further supporting the essential role of AKT in PTEN-controlled ES cell proliferation and survival.

ES cells are a rather special type of stem cells because they do not exhibit contact inhibition and will not enter G_0 cell cycle arrest. In contrast, most somatic stem cells are situated in a quiescent stage. The mechanisms controlling stem cell G_0–G1 transition, self-renewal, and proliferation are poorly understood. By specifically deleting *Pten* in the brain during mid-embryonic development, we generated mutant mice in which the brain size as well as weight is doubled, similar to macrocephaly found in humans with inherited *PTEN* deletions/mutations (Groszer et al., 2001). Further studies indicated that the enlarged brain results from increased cell proliferation, decreased cell death, and enlarged cell size. The histoarchitecture of the mutant brain also appears abnormal. However, cell fate commitments of the progenitors were largely undisturbed. Because all neural cell types are derived from a common stem cell, the neural stem cell, the abnormal phenotypes observed in the mutant brain prompt us to study the neural stem cell population. Our in vitro analysis indicates that there are more stem cells in the mutant brain, and these stem cells are undergoing more self-renewal divisions. Moreover, PTEN-deficient neural stem/progenitor cells

have a greater proliferation capacity, which is due, at least in part, to a shortened cell cycle time (Groszer et al., 2001).

The role of PTEN in regulating stem cell self-renewal and proliferation is further supported by the generation of *Pten* null primordial germ cells (PGCs) (Kimura et al., 2003; Moe-Behrens et al., 2003). To study the intracellular signaling pathways that control PGC proliferation and differentiation, Kimura et al. have generated mice with PGC-specific deletion of the *Pten* gene. PGC-specific *Pten* deletion resulted in impaired mitotic arrest and outgrowth of cells with immature characters accompanied by bilateral testicular teratoma formation. Interesting, *Pten* null PGCs in culture have greater proliferative capacity, similar to what we have observed in *Pten* null ES cells. They also demonstrated enhanced pluripotent embryonic germ cell colony formation from *Pten* null PGCs (Kimura et al., 2003), providing additional proof for the role of PTEN in regulating stem cell self-renewal.

PTEN and cell migration, cell fate determination

Even though PTEN plays important roles in stem cell self-renewal and proliferation, *Pten* deletion does not change the overall cell differentiation program. Humans with *PTEN* germline mutations develop hamartomas in tissues derived from all three germ layers (Liaw et al., 1997; Marsh et al., 1998; Nelen et al., 1997). These focal hyperplastic or dysplastic lesions are formed by overgrowth of tissue elements normally present at these sites, indicating that *PTEN*-deficient cells in a variety of tissues remain responsive to exogenous and endogenous differentiation cues. Concordantly, it has been shown that PTEN is dispensable for fate determination of forebrain cortical (Groszer et al., 2001) and cerebellar (Marino et al., 2002) progenitor cells in vivo and in vitro. Similarly, over-expression of *Akt* or deletion of *dPTEN* in *Drosophila* did not affect cell-fate determination either (Gao et al., 2000; Huang et al., 1999; Verdu et al., 1999).

PTEN, on the other hand, does control cell migration. It has been suggested that PTEN negatively regulates cell migration by directly dephosphorylating p125[FAK] and changing MAP kinase activity (Gu et al., 1999; Tamura et al., 1998). However, we failed to detect hyperphosphorylation of p125[FAK] or hyperactivation of MAP kinase in *Pten* null tissue or cells. Instead, we observed that PTEN negatively regulates MEF cell mobility by downregulation of Rac-1 and Cdc-42, two small GTPase activities in vitro (Liliental et al., 2000). In *Dictyostelium*, PTEN coordinates G protein coupled signaling pathways during chemotaxis. PTEN is localized opposite to the migration leading edge, and deletion of PTEN leads to dramatically prolonged and broadened PH domain relocation and actin polymerization responses (Funamoto et al., 2002; Iijima and Devreotes, 2002). However, in tissues with multiple cell types, PTEN's

effect on cell migration is more complicated. Even though *Pten*$^{+/-}$ SVZ progenitor cells seem to migrate faster (Li et al., 2002b), abnormal layering and morphological alterations have been observed in *Pten* conditional deleted brain, suggesting possible defects in cellular migration (Backman et al., 2001; Marino et al., 2002). In *En2-Cre*$^{+/-}$; *Pten*$^{lox/lox}$ mice older than 3 weeks, Purkinje cells are clustered above the forth ventricle without recognizable orientation and do not migrate toward the cerebellar surface (Marino et al., 2002). Moreover, clusters of cerebellar granule cells, which failed to migrate inward, were found in the molecular layer (Marino et al., 2002). Postmitotic granule cells that express mature granule cell markers showed a similar phenotype in *GFAP-Cre*$^{+/-}$; *Pten*$^{lox/lox}$ mice (Backman et al., 2001; Kwon et al., 2001). Therefore, the authors concluded that these might indicate a defect in granule cell migration rather than a persistence of the EGL (Kwon et al., 2001). Whether the observed defects are due to changes in cell intrinsic properties of granule cells or caused by alterations in environmental cues requires further investigation.

PTEN negatively regulates cell and organ size

Size regulation is fundamental for developmental biology. The size of a particular organ can be influenced by both extrinsic and intrinsic factors. Recent evidence suggests that insulin and IGF-1 may play critical roles in organ size controls in both mammals and other multi-cellular organisms (Beck et al., 1995; Bohni et al., 1999; Brogiolo et al., 2001; McMullen et al., 2004; Petrik et al., 1999; Powell-Braxton et al., 1993; Xu et al., 2002). Studies in *Drosophila* suggest that the pathway responsible to insulin/IGF-1 signaling is the determination factor for organ size control (Bohni et al., 1999; Brogiolo et al., 2001; Goberdhan and Wilson, 2002). Loss of *dPten*, the negative regulator of the insulin and IGF-1 signaling pathway, causes increased cell size, organ size, as well as animal size (Bohni et al., 1999). Overexpression of *dPten*, on the other hand, results in completely opposite effects (Gao et al., 2000; Huang et al., 1999). Similarly, in *Pten* conditional knockout mice, deletion of *Pten* bypasses the organ size control and leads to organomegaly in liver, brain, heart, prostate, and skin (Backman et al., 2001; Crackower et al., 2002; Groszer et al., 2001; Kwon et al., 2001; Li et al., 2002a; Stiles et al., 2002; Suzuki et al., 2003; Wang et al., 2003).

Organ size control involves two processes: cell size control and cell number regulation. Under normal conditions, these two processes are reciprocally controlled to determine organ size. This relationship is best illustrated in experiments with salamanders. Tetraploid salamanders are composed only half the number of cells that are seen in the diploid ones. The tetraploid salamanders developed giant cells to compensate for this lack of cell number (Frankhauser, 1945). Apparently, PTEN controls both cell size and cell number checkpoints:

PTEN regulates cell number by inhibiting cell cycle progression and inducing apoptosis (Sun et al., 1999); PTEN also controls translation mechanisms through its regulation of mTOR/S6K signaling, thus influencing cell size (Groszer et al., 2001; Kwon et al., 2003; Neshat et al., 2001).

PTEN may control cell number and cell size in a cell-type- and cell differentiation status-dependent manner. Even though *Pten* null ES cell, MEF cells, and thymocytes do not show any apparent size control defect, *Pten*-deleted neurons and prostatic epithelial cells are clearly larger than their WT controls (Backman et al., 2001; Groszer et al., 2001; Kwon et al., 2001, 2003; Wang et al., 2003). Increased cell proliferation is evidenced in undifferentiated *Pten* null ES cells and neural stem cells (Groszer et al., 2001; Sun et al., 1999), but not in differentiated neurons and MEFs (Backman et al., 2001; Sun et al., 1999). Similarly, activated AKT may differentially modulate cell size and cell number checkpoints. Activation of AKT in the *Drosophila* imaginal disc and mouse pancreatic β-cells leads to increased cell size and compartment size, independent of cell proliferation (Tuttle et al., 2001; Verdu et al., 1999). Overexpression of Akt in the prostate epithelium, on the other hand, increases both cell number and cell size (Majumder et al., 2003).

PTEN in regulation of hormone responses

The development of sexual organs involves additional regulation by hormones, such as androgen and estrogen. At the onset of sexual maturity, these hormones stimulate the proliferation, differentiation, and maturation of mammary, endometric and prostate epithelium, resulting in significant changes in their morphological appearances and functions.

Female patients with germline *PTEN* deletion suffer from bilateral hypertrophy of the virginal breasts and early malignant transformation (Lloyd and Dennis, 1962). However, the exact mechanisms of PTEN in controlling mammary gland development are unclear. Conditional deletion of *Pten* in the virgin mammary gland leads to hypersensitivity to hormone stimulation, resulting in dramatically increased ductal branching and BrdU incorporation (Li et al., 2002a). The virgin mutant gland also contains many lobulo-alveolar buds and expresses milk-specific proteins, which are normally associated with hormonal stimulation during pregnancy. Conversely, overexpression of *Pten* inhibits differentiation of mammary tissues and resulted in dysfunctional lactation (Dupont et al., 2002). The *Pten* null mammary gland is also less sensitive to hormone withdrawal during weaning as evidenced by decreased cell apoptosis and impaired involution process. Recent studies suggest that PI3Kinase/AKT can active estrogen receptor in the absence of estrogen (Campbell et al., 2001). These results suggest that PTEN is one of the important factors controlling the hormone responsiveness of mammary epithelial cells during normal mammary gland development and mammary gland cycling, and provide a mechanistic explanation for the bilateral

virginal mammary gland hypertrophy seen in Cowden's disease patients.

Similarly, deletion of *Pten* in prostate resulted in enlarged prostate glands as early as postnatal 4 weeks, which is partially due to increased cell proliferation and cell size of *Pten* null prostate epithelium (Wang et al., 2003). *Pten* null prostate cancer cells are also capable of proliferation in the absence of androgen. Surprisingly, *Pten* null prostatic epithelial cells are sensitive to androgen withdrawal and die rapidly after castration even though AKT is hyper-activated in these cells. Several recent studies suggest the

presence of cross talks between the PI3Kinase/AKT and AR signaling pathways. Unfortunately, the effects in different cell lines and from different laboratories often seem contra-dictory, leading to confusion in the field as to whether PI3Kinase/AKT exerts a positive or negative effect on AR (Lin et al., 2002; Manin et al., 2002). For example, Manin et al. (2002) have claimed that the PI3kinase/AKT pathway is required for basal and dihydrotestosterone-induced AR expression in prostate cell lines; whereas Lin et al. (2002) have shown that AKT and Mdm2 form a complex with AR and promote phosphorylation-dependent AR ubiquitination,
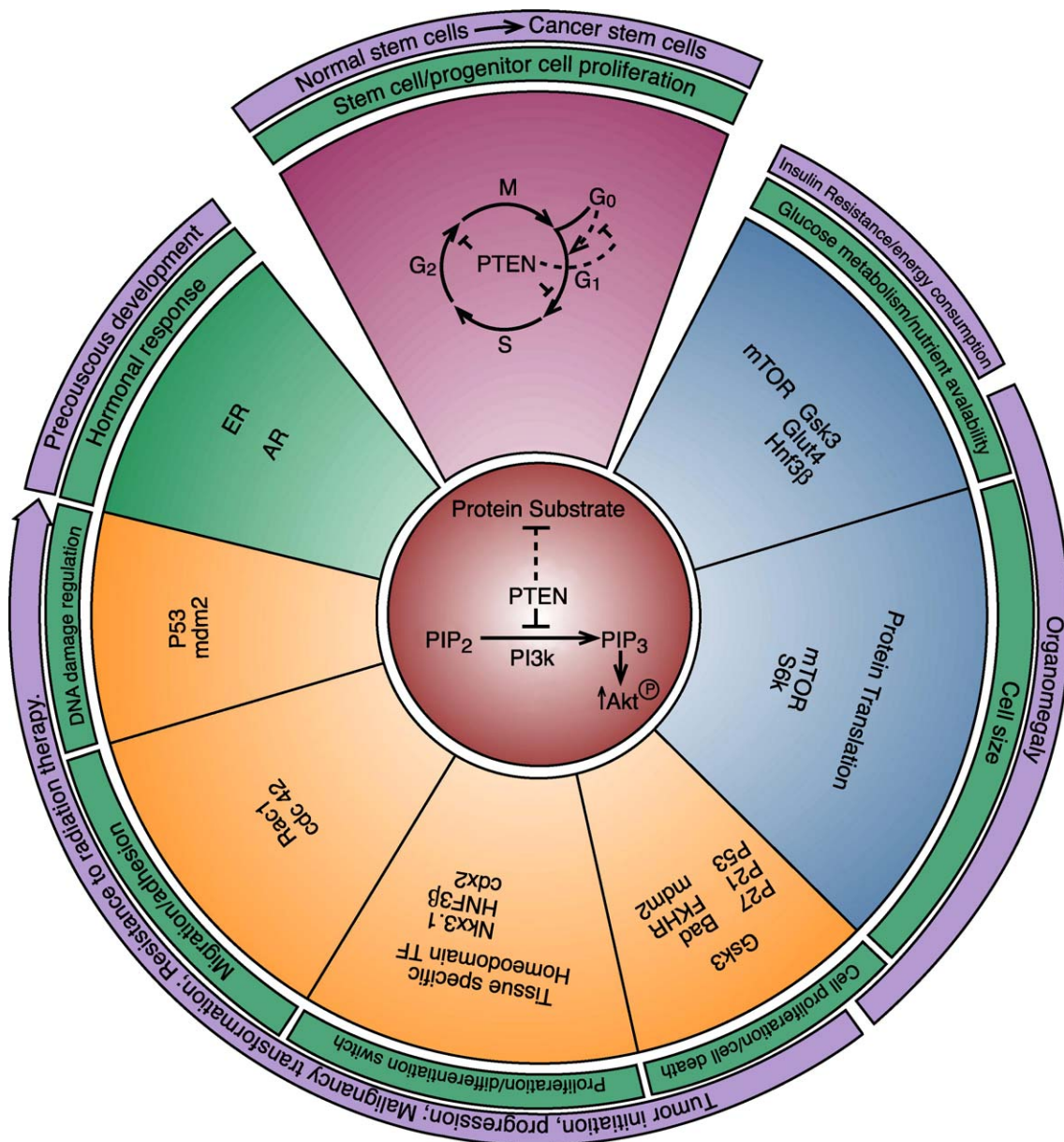

Fig. 2. The biological function of PTEN and its role in disease progression. In stem/progenitor cells, PTEN acts to regulate not only G1/S and G2/M switch, but also determines whether cells in G0 resting stage re-enter the cell cycle. This role of PTEN in regulating cell cycle re-entry maybe an important switch controlling whether normal stem cells is to become cancer stem cells. In postmitotic cells, PTEN plays a wide range of function regulating from metabolism to cell proliferation and differentiation. Through regulating these processes, PTEN plays a role in insulin resistance, organ size control, tumor development as well as normal organ development.

resulting in AR degradation by the proteasome. Further studies, by employing in vivo systems, may help clarify this controversial observation.

Perspectives

Studies from the last few years clearly established the vital role of PTEN in regulating normal development and physiology, and illustrated the detrimental effects of PTEN dysregulation in tumorigenesis (Fig. 2). PTEN functions in multiple tissues and organs, from early embryogenesis to sexual organ development in adulthood. The role of PTEN is best illustrated by its broad expression pattern and abnormal phenotypes associated with animal models with *Pten* tissue-specific deletion. PTEN regulates stem cell self-renewal and proliferation without perturbing the fate of cell differentiation. PTEN also controls organ size by modulating the checkpoints of cell proliferation and soma growth.

Several important issues raised by current studies are worth further investigation. First, self-renewal is the hallmark property of stem cells, but how PTEN controls stem cell self-renewal remains unknown. Majority of somatic stem cells are arrested at G_0 until proper stimulations trigger them to proliferate to generate more stem cells and to repopulate the injured tissues. We have observed hyperproliferation and enhanced self-renewal in *Pten* null neuronal stem/progenitor cells, suggesting that PTEN may modulate G_0–G_1 transition (Groszer et al., 2001). Nuclear expression of PTEN varies throughout the cell cycle with highest expression at G_0–G_1 phase and lower levels in S phase (Ginn-Pease and Eng, 2003). Together, these studies suggest nuclear PTEN may play a more direct role in regulating cell cycle reentry. Further study is necessary to elucidate the signaling pathways involved. Second, whether PTEN has protein phosphatase activity in vivo and the importance of such activity remain to be determined. Sequence analysis suggests that PTEN contains a sequence motif that is highly conserved among members of the protein tyrosine phosphatase family (Li and Sun, 1997; Li et al., 1997). Structurally, the PTEN phosphatase domain is similar to protein phosphatase, but its active site is large enough to accommodate lipid substrates, for example, PIP3 (Lee et al., 1999). In vitro analysis suggests that PTEN possesses phosphatase activity on phosphotyrosyl-, phosphothreonyl-, and phosphoseryl-containing substrates (Li and Sun, 1997; Myers et al., 1998). Furthermore, FAK has been proposed as a possible protein substrate of PTEN (Gu et al., 1999; Tamura et al., 1998), although FAK phosphorylation status does not change in *Pten*-deficient embryonic stem cells or fibroblasts (Liliental et al., 2000). It has also been shown that PTEN-null mutation in *Drosophila* does more than just affect the PI3kinase pathway (Gao et al., 2000; Huang et al., 1999). Another interesting observation is that PTEN can dephosphorylate itself and PTEN's protein phosphatase activity is required for self-regulation (Raftopoulou et al., 2004). So an attractive hypothesis is that PTEN may regulate its own activity and the activity of various protein substrates by controlling their phosphorylation and localization. Third, maintaining proportional organ size is fundamental to development. However, the mechanism underlying organ size regulation remains poorly understood. The enlarged organ and cell size phenotypes observed in *Pten* tissue-specific-deleted animals may provide a unique opportunity to address this fundamental question.

Development and tumorigenesis are tightly linked, and tumor formation may be viewed as abnormal development. Signaling pathways critical for development processes could cause cancer when dysregulated. Thus, studying how PTEN regulates normal development may shed light on our understanding of the molecular mechanisms that underlie PTEN-controlled tumorigenesis and vice versa.

Acknowledgments

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