Review Article

Portrait of *PTEN*: Messages from mutant mice

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***PTEN* is a tumor suppressor gene mutated in many human sporadic cancers and in hereditary cancer syndromes such as Cowden disease. The major substrate of PTEN is phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P3), a second messenger molecule produced following PI3K activation induced by a variety of stimuli. PI(3,4,5)P3 activates the serine-threonine kinase Akt, which is involved in antiapoptosis, proliferation and oncogenesis. In mice, heterozygosity for a null mutation of *Pten* (*Pten*$^{+/-}$ mice) frequently leads to the development of a variety of cancers and autoimmune disease. Homozygosity for the null mutation (*Pten*$^{-/-}$ mice) results in early embryonic lethality, precluding the functional analysis of *Pten* in adult tissues and organs. To investigate the physiological functions of *Pten* in viable mice, we and other groups have used the *Cre-loxP* system to generate various tissue-specific *Pten* mutations. The present review will summarize results obtained from the study of conditional mutant mice lacking *Pten* in specific tissues, and discuss the possible biological and molecular explanations for why *Pten* deficiency leads to tumorigenesis. (*Cancer Sci* 2008; 99: 209–213)**

The phosphoinositide-3-kinases (PI3K) are evolutionarily conserved lipid kinases[1] that are activated in response to the engagement of receptors for growth factors, cytokines, hormones such as insulin, and antigens. Activated PI3K generate phosphatidylinositol-3,4,5-trisphosphate (PIP3), which activates the downstream molecule Akt that plays an important role in antiapoptosis, proliferation and oncogenesis (Fig. 1).

PTEN is a multifunctional phosphatase, the lipid phosphatase activity of which is associated with tumor suppression.[2,3] The major substrate of the lipid phosphatase activity of PTEN is PIP3.[4] By dephosphorylating the D3 position of PIP3, PTEN negatively regulates the PI3K pathway and Akt activation and thus suppresses tumorigenesis (Fig. 1). The protein phosphatase activity of PTEN inactivates FAK, Shc, PDGFR and PTEN itself.[5–7] Mice deficient for *Pten* spontaneously develop various cancers. Other phosphatases, such as SHIP, SHIP2 and SKIP, also dephosphorylate PIP3, but do so at the D5 position. Mice lacking SHIP or SHIP2 do not show elevated cellular PIP3 or Akt activity at basal level and do not develop malignancies.[8,9]

PTEN is the second most frequently mutated tumor suppressor gene in human sporadic cancers,[10] and reduced PTEN protein expression occurs in approximately half of all tumors. Germline mutations of PTEN lead to hereditary disorders such as Cowden disease, Bannayan–Zonana syndrome, Lhermitte–Ducros disease (LDD) and Proteus syndrome. These syndromes are characterized by multiple hamartomas and increased risk of cancer.[11] We and others have been analyzing the functions of PTEN *in vivo* by using the *Cre-loxP* system to generate *Pten* null or conditional Pten-deficient (*Pten*^{flox}) mice. Here we review the phenotypes of these animals and discuss how they have helped us to molecularly investigate why *Pten* deficiency leads to tumorigenesis.

Phenotypes of *Pten*-deficient mice that develop spontaneous cancers

Mice with a complete null mutation of *Pten*. Null mutation of *Pten* in mice results in early embryonic lethality (~E9.5).[12–15] In addition, half of *Pten*$^{+/-}$ mice die within 1 year of birth. Survivors develop a broad range of tumors, including mammary, thyroid, endometrial and prostate cancers, as well as T-cell lymphomas. This spectrum of neoplasias closely resembles that in humans with PTEN mutations. *Pten*$^{+/-}$ mice also show signs of autoimmune disease.[16]

Mice with Pten-deficient T cells. We have generated mice in which *Pten* is disrupted in T cells (*tPten*$^{flox/-}$ mice) by crossing Lck-Cre transgenic mice to *Pten*$^{flox/-}$ mice.[17] *tPten*$^{flox/-}$ mice die of T-cell lymphomas within 20 weeks of birth. Moreover, *tPten*$^{flox/-}$ mice harboring the HY-TCR transgene show defective thymic-negative selection and symptoms of autoimmune disease. Peripheral tolerance to SEB is also impaired. Pten-deficient T cells hyperproliferate, are autoreactive, secrete increased levels of Th1/Th2 cytokines, resist apoptosis, and show increased phosphorylation of Akt and Erk and elevated expression of

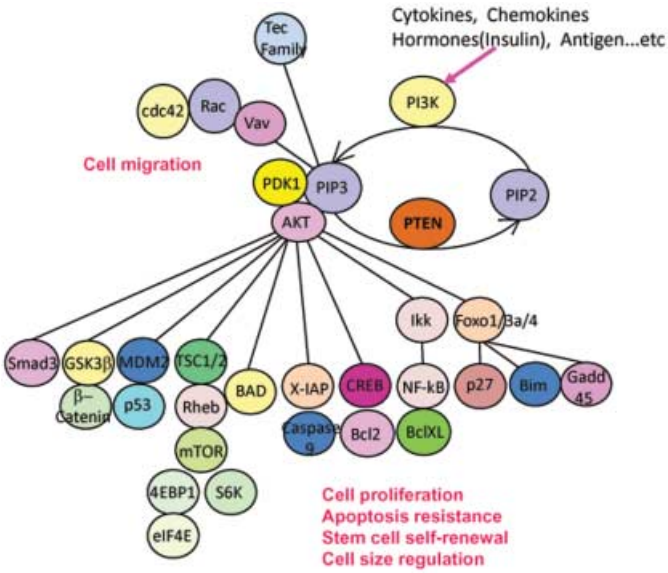

Fig. 1. Schematic representation of the PI3K/PTEN signaling network and cellular outcomes.

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Bcl-X_L. *Pten* is thus important for preserving self-tolerance and inhibiting T-cell malignancy.

Our group has also generated mice deficient for the *p110γ* catalytic subunit of PI3Kγ.[18] *p110γ* deficiency and *Pten* deficiency appear to have opposing effects in T cells. A *p65*^{PI3K} transgenic mouse[19] that expresses a constitutively active truncated form of p85α in T cells exhibits phenotypes very similar to those of *tPten*$^{flox/-}$ mice. Thus, the phenotypes of *tPten*$^{flox/-}$ mice are PI3Kγ-dependent.

Mice with *Pten*-deficient keratinocytes. Keratinocyte-specific Pten-deficient mice (*kPten*$^{flox/flox}$ mice) have been created by crossing *Pten*^{flox} mice to Keratin5-Cre transgenic mice.[20] *kPten*$^{flox/flox}$ mice exhibit epidermal hyperplasia and hyperkeratosis, and shaggy hair. Most *kPten*$^{flox/flox}$ mice are significantly smaller than their wild-type littermates, and 90% of the mutants die of malnutrition within 3 weeks of birth, possibly due to esophageal hyperkeratosis. *kPten*$^{flox/flox}$ mice that survive past 2 months of age have a normal lifespan. Significantly, 23% of *k5Pten*$^{flox/+}$ and 100% of surviving *k5Pten*$^{flox/flox}$ mice develop spontaneous cancers within 9 months of birth. 7,12-Dimethylbenz[a]anthracene (DMBA) plus 12-0-tetradecanoylphorbor-13 acetate (TPA) treatment accelerates the onset of these tumors. Most of the spontaneous tumors are squamous papillomas, but squamous cell carcinomas, sebaceous carcinomas and adenocarcinomas of the sweat gland are also observed. *In vitro*, Pten-deficient keratinocytes are hyperproliferative, resistant to apoptosis, and show increased activation of Akt and Erk. Zhao *et al*. have demonstrated that *Pten* deficiency enhances electric field-induced PI3K signaling in keratinocytes and accelerates the directed movements of skin epithelial cells in response to endogenous electric signals generated by wounding.[21]

Mice with *Pten*-deficient hepatocytes. The mating of *Pten*^{flox} mice to *Albumin-Cre* transgenic mice results in *hPten*$^{flox/flox}$ mice that have a hepatocyte-specific null mutation of *Pten*.[22] *hPten*$^{flox/flox}$ mice show massive hepatomegaly and steatohepatitis and an accumulation of triglycerides similar to that in human non-alcoholic steatohepatitis (NASH). Gas chromatographic analysis of the total fatty acid composition of the *hPten*$^{flox/flox}$ liver showed that C16:1 and C18:1 fatty acids are significantly increased. Adipocyte-specific genes (adipsin, adiponectin, and aP2) are induced in the mutant hepatocytes, implying adipogenic-like transformation of these cells. Genes involved in lipogenesis (*fatty acid synthetase* [*FAS*], *acetyl CoA carboxylase* [*ACC*], and *stearoyl-CoA desaturase* [*SCD1*]) and PPAR-α-regulated peroxisomal fatty acid β-oxidation genes (*acyl-CoA oxidase* [*AOX*], *peroxisomal enoyl-CoA thiolase* [*L-PBE*], and *peroxisomal 3-ketoacyl-CoA thiolase* [*PTL*]), are also induced in *hPten*$^{flox/flox}$ hepatocytes, possibly because these cells show elevations of the transactivating factors SREBP1c and PPAR-γ that act downstream of Akt/Foxo1. By 78 weeks of age, all *hPten*$^{flox/flox}$ mice exhibit liver adenomas and 66% show hepatocellular carcinomas (HCC). *hPten*$^{flox/flox}$ mice also show decreased serum glucose levels due to insulin hypersensitivity, and reduced serum insulin. *hPten*$^{flox/flox}$ hepatocytes are hyperproliferative and display increased hyperoxidation with abnormal activation of Akt and Erk. We speculate that NASH patients may often suffer from reduced PTEN functions, particularly NASH patients who develop HCC.

Mice with *Pten*-deficient urothelial epithelial cells. Approximately 53% of primary bladder cancer patients exhibit decreased or absent PTEN protein in the cytoplasm or nucleus of their tumor cells.[23] This PTEN deficiency may augment the activation of Akt and ERK pathways triggered by various growth factors (such as EGF) present in urine. Indeed, in mice, the overexpression of epidermal growth factor receptor (EGFR), H-Ras, or fibroblast growth factor receptor (FGFR)-3, all of which activate Akt and Erk, causes urothelial hyperplasia,[24–26] that can progress to superficial papillary carcinomas. We have generated a urothelium-specific null mutation of *Pten* in mice (*uPten*$^{flox/flox}$ mice) by mating Fabp-Cre transgenic mice to *Pten*^{flox} mice.[23] All *uPten*$^{flox/flox}$ mice exhibit urothelial hyperplasia in which the cells display enlarged nuclei and increased cell size. Pedicellate papillary transitional cell carcinomas (TCC) occur spontaneously in 10% of *uPten*$^{flox/flox}$ mice, and in both *uPten*$^{flox/+}$ and *uPten*$^{flox/flox}$ mice treated with the chemical carcinogen BBN. *In vitro*, *uPten*$^{flox/flox}$ urothelial cells are hyperproliferative and showed increased Akt and Erk activation. This hyperproliferation may increase the chance of additional genetic alterations that could tip the balance towards the development of superficial papillary TCC and then more advanced cancers.

Mice with *Pten*-deficient lung epithelial cells. To examine PTEN function in the lung, we mated $(tetO)_7$-Cre transgenic mice to *SP-C-rtTA* and *Pten*$^{flox/flox}$ mice and generated *lPten*$^{flox/flox}$ mice with a bronchioalveolar epithelium-specific mutation of *Pten* that is under the control of doxycycline.[27] Ninety per cent of *lPten*$^{flox/flox}$ mice that receive doxycycline *in utero* (E10–16) die of hypoxia within 2 h of birth. Most of the surviving *lPten*$^{flox/flox}$ (E10–16) mice, as well as mice that received doxycycline postnatally (P21–27), develop spontaneous lung adenocarcinomas. Urethane treatment accelerates the number and size of lung tumors in both *lPten*$^{flox/flox}$ (E10–16) and *lPten*$^{flox/flox}$ (P21–27) mice. Histological and biochemical examination of *lPten*$^{flox/flox}$ (E10–16) lungs reveals hyperplasia of bronchioalveolar epithelial cells and myofibroblast precursors, enlarged undifferentiated alveolar epithelial cells, and impaired production of surfactant proteins. In addition, there are increased numbers of bronchioalveolar stem cells (BASC), which are putative initiators of lung adenocarcinomas. Lungs of *lPten*$^{flox/flox}$ (E10–16) mice show increased expression of Akt, c-Myc, Bcl-2 and Shh as well as Spry2, which inhibits the maturation of alveolar epithelial cells. Furthermore, *K-ras* is frequently mutated in adenocarcinomas in *lPten*$^{flox/flox}$ lungs. Thus, the expansion of BASC in *lPten*$^{flox/flox}$ mice might increase the risk of additional oncogenic mutations in these cells, including alterations of *K-ras*. The combined effects of *Pten* deficiency, *K-ras* mutation and perhaps other tumorigenic events might further increase the proliferation of BASC such that they eventually initiate lung adenocarcinomas.

Phenotypes of *Pten*-deficient mice that develop paraneoplastic effects

For a tumor to progress, proper tumor angiogenesis must occur and the malignancy must escape eradication by immune sentinels such as natural killer (NK)T cells, NK cells and cytotoxic T cells. In the absence of angiogenesis, tumors become necrotic or apoptotic and do not grow beyond 2–3 mm^3 in size.[28] In the absence of Vα14iNKT cells, tumor rejection is impaired.[29] We have examined the roles of *Pten* in both angiogenesis and NKT cell function.

Mice with *Pten*-deficient endothelial cells. We have generated mice with an endothelial cell-specific mutation of *Pten* (*ePten*$^{flox/flox}$) by mating Tie2-Cre transgenic mice to *Pten*^{flox} mice.[30] *ePten*$^{flox/+}$ mice display enhanced tumorigenesis due to increased angiogenesis driven by vascular growth factors. *In vitro*, *ePten*$^{flox/+}$ endothelial cells show enhanced proliferation/migration. e*Pten*$^{flox/flox}$ mice die during embryogenesis due to bleeding and cardiac failure caused by impaired recruitment of pericytes and vascular smooth muscle cells to blood vessels, and of cardiomyocytes to the endocardium. These effects partially depend on the PI3K subunits p85α and p110γ, and are associated with decreased expression of Ang-1, vascular cell adhesion molecule (VCAM)-1, connexin-40 and ephrin-B2, but increased expression of Ang-2, vascular endothelial growth factor (VEGF)-A, Flt-1 and Flk-1. Thus, *Pten* is required for normal blood vessel remodeling and suppresses tumor angiogenesis.

Mice with *Pten*-deficient NKT cells. *LckCrePten*$^{flox/flox}$ (*tPten*$^{flox/flox}$) mice show deletion of *Pten* not only in T-lineage cells but also

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in Vα14iNKT cells. In response to *in vivo* administration of αGalCer, a known activator of NKT cells, both *Pten*$^{+/-}$ and *tPten*$^{flox/flox}$ mice display reduced serum γ-interferon (IFN-γ). *Pten*-deficient Vα14iNKT cells do not mature and show reduced proliferation and cytokine secretion in response to αGalCer stimulation *in vitro*.[31] These effects require the functions of PI3K p110γ and p110δ. In addition, when *Pten*-deficient Vα14iNKT cells interact with αGalCer-loaded dendritic cells (DC), PI3K becomes hyperactivated. Lastly, neither *Pten*$^{+/-}$ nor *Pten*-deficient Vα14iNKT cells can protect mice against metastasis of melanoma cells to the lung. The *Pten*–PI3K pathway is therefore indispensable for antitumor surveillance by Vα14iNKT cells.

Lessons learned from *Pten*-deficient mice

In addition to the above, conditional *Pten*-deficient mice have been generated that develop teratomas,[32] prostatic cancers,[33,34] pancreatic cancers,[35] breast cancers,[36] thyroid cancers,[37] cholangiocellular carcinomas,[38] or leiomyosarcomas[39] (Fig. 2). PTEN is thus a crucial tumor suppressor in a variety of organs. Cowden disease is a hereditary syndrome of cancer susceptibility caused by heterozygous germline mutations of PTEN. Our results have provided fresh insight into this syndrome and suggest that an individual who inherits a mutated PTEN allele is not only at risk for the onset of cancers, but may also experience accelerated growth of any incipient tumors due to enhanced angiogenesis and impaired NKT-mediated antitumor surveillance (Fig. 3). Moreover, *Pten* haplo-insufficiency in both mice and humans can clearly promote tumor development despite the retention of a wild-type allele.[40] Thus, even a partial loss of *Pten* may give a tumor cell a selective advantage.

In mice, Pten deficiency causes increases in cell proliferation, apoptotic resistance, stem-cell renewal/maintenance, centromeric instability, and DNA double-strand breaks.[27,32,41–44] All of these defects enhance an animal's susceptibility to carcinogens and the occurrence of secondary genetic or epigenetic alteration(s) that can lead to cancer development. Furthermore, *Pten* deficiency contributes to autoimmune disease,[16,17] heart failure,[45] NASH,[22] insulin hypersensitivity,[22,46] macroencephaly,[47] defects in immunoglobulin class switch recombination,[48] and increased bone mineral density.[49] Finally, *Pten* is essential for normal embryogenesis[12,14] (Fig. 4).

In every Pten-deficient organ or tissue examined, the component cells always demonstrate inappropriate activation of Akt. Transgenic mice in which Akt is constitutively activated in T cells develop T-cell lymphomas,[50] and Akt1 deficiency in *Pten*$^{+/-}$ mice largely protects these animals from developing tumors.[51]

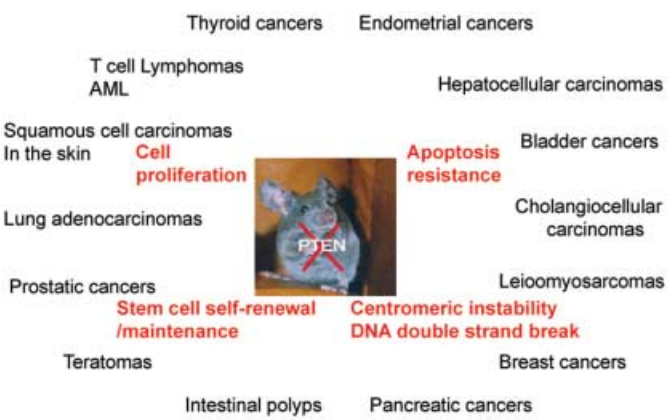

Fig. 2. Tumor spectrum observed in tissue-specific Pten-deficient mice.

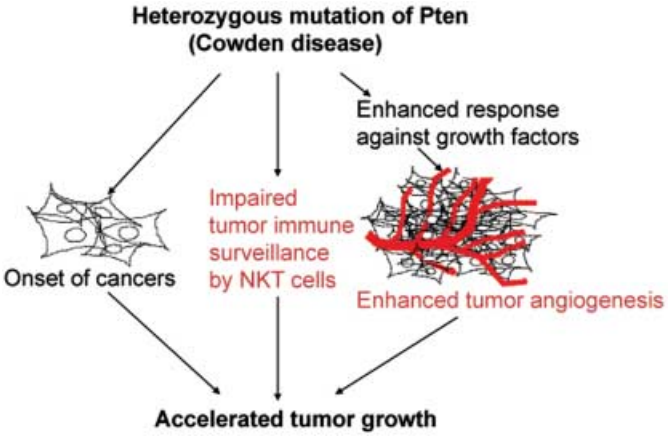

Fig. 3. Clinical significance of paraneoplastic effects in humans with heterozygous mutations of PTEN. NKT cells, natural killer T cells.

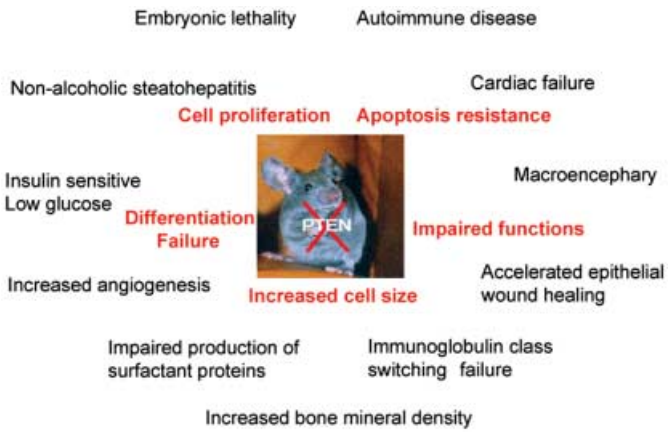

Fig. 4. Non-cancerous phenotypes observed in tissue-specific Pten-deficient mice.

These observations point to an important role for Akt in oncogenesis. However, mice transgenic for Akt do not develop mammary tumors,[52] suggesting that Akt is necessary but not sufficient to drive the tumorigenesis triggered by *Pten* deficiency. Another important enzyme that is usually, not always, activated by *Pten* deficiency is Erk. The onset of skin tumor formation in mice requires SOS/Ras/Erk signaling in addition to Akt signaling.[53] Indeed, the Erk pathway acts in synergy with the PI3K pathway to stimulate CycD1 transcription in NIH3T3 cells.[54] Thus, the onset of tumors in Pten-deficient mice is most likely due to Akt hyperactivation, with a contribution by deregulated Erk activation. PTEN possesses protein phosphatase activity that can dephosphorylate integrin-signaling mediators such as FAK and Shc.[5,6] When phosphorylated, these mediators signal through Erk to drive cell adhesion, migration and growth. Because the Erk activation observed in some Pten-deficient melanocytes cannot be inhibited by the PI3K inhibitor wortmannin (Inoue-Narita *et al.*, 2007, unpub. data), the protein phosphatase activity of *Pten* may be more important than its lipid phosphatase activity for Erk activation in at least some cell types.

The role of PTEN at the plasma membrane is well-defined; however, PTEN is also strongly expressed in the nucleus, particularly in differentiated and resting cells.[55,56] Nuclear PTEN suppresses cell growth not by inducing apoptosis but rather by a mechanism that is independent of Akt.[57–59] In normal cells, PTEN binds to molecules such as p53, MSP58, CENP-C and E2F-1, all of which are expressed in the nucleus and involved in

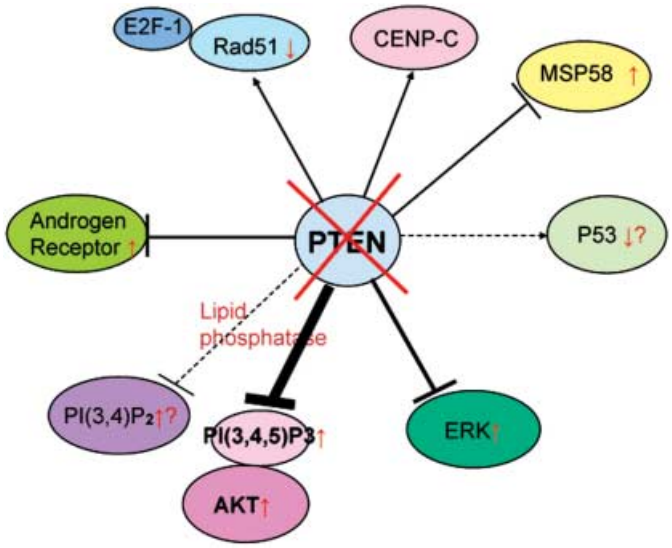

Fig. 5. Interaction with PTEN of molecules known to be involved in oncogenesis.

tumorigenesis. Indeed, decreased levels of nuclear PTEN correlate with increased tumorigenicity.[60] *In vitro*, PTEN directly associates with p53 in the nucleus to form a complex that can inhibit p53 degradation and increase p53 transcriptional activity in both phosphatase-dependent and -independent ways.[61] However, such mechanisms have seldom been observed *in vivo* because Pten-deficient cells do not generally show a concomitant decrease in p53 expression. In prostate cells and endothelial cells, *Pten* deficiency or Akt activation can induce p53 upregulation that triggers cell senescence.[62,63] In normal resting cells, nuclear PTEN binds to MSP58 and inhibits MSP58-induced cell transformation in a phosphatase-independent manner.[64] Nuclear PTEN also binds to CENP-C, an integral component of the kinetochore, and helps to prevent centromeric instability and chromosomal translocations. Lastly, PTEN binds to the Rad51 promoter and enhances E2F-1-mediated transcription of Rad51, reducing the incidence of spontaneous DNA double-strand breaks.[44]

A striking effect associated with *Pten* deficiency in mice is sex bias. Tumors caused by loss of *Pten* in hepatocytes or bladder epithelial cells are much more frequent in male mice than in female, whereas T-cell lymphomas and autoimmune disease are much more frequent in female mice. PTEN suppresses the activity of the androgen receptor (AR) via Akt/Foxo1-dependent and -independent pathways.[65–67] In addition, PTEN can interact directly with AR such that the nuclear translocation of AR is impaired. This inhibition promotes degradation of the AR protein and blocks AR transactivation and inhibition of apoptosis.[68] Accordingly, mice with prostate-specific *Pten* deficiency develop prostatic cancers that are refractory to androgen ablation therapy.[34,69] In contrast, loss of *Pten* activates Akt-mediated phosphorylation and activation of the estrogen receptor (ER)-α even in the absence of estrogen, and this activation drives endometrial neoplastic transformation in *Pten*$^{+/-}$ mice.[70,71] Breast cancer cells showing activation of Akt are also less sensitive to hormone withdrawal, consistent with the resistance of these types of breast cancers to antihormonal therapy.[70]

An issue in the PTEN field that requires urgent investigation is the potential effect on tumorigenesis of PI(3,4)P2 accumulation. In addition to dephosphorylating PI(3,4,5)P3, PTEN also dephosphorylates PI(3,4)P2, at least *in vitro*.[72] It is therefore possible that PTEN deficiency leads to an abnormal build-up of PI(3,4)P2 *in vivo*. The normal function of PI(3,4)P2 has yet to be clarified, and its role in cancer development, if any, is unknown.

In conclusion, work with conditional Pten-deficient mice has definitively shown that PTEN functions as a highly effective tumor suppressor in a wide variety of tissues, and that PTEN is essential for the normal development and/or homeostasis of numerous organ systems. The onset of tumors induced by PTEN deficiency is most likely due to cooperation between Akt and other downstream PTEN targets that promote hyperproliferation, resistance to apoptosis, increased migration, enhanced stem-cell self-renewal/maintenance, and genetic instability (Fig. 5). Targeted inhibition of the PI3K–PIP3–Akt pathway may therefore be an attractive approach for treating a wide variety of malignancies and non-cancerous diseases.

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