Faithfull Modeling of *PTEN* Loss Driven Diseases in the Mouse

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Abstract A decade of work has indisputably defined *PTEN* as a pivotal player in human health and disease. Above all, *PTEN* has been identified as one of the most commonly lost or mutated tumor suppressor genes in human cancers. For this reason, the generation of a multitude of mouse models has been an invaluable

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strategy to dissect the function and consequences-of-loss of this essential, evolutionary conserved lipid phosphatase in tumor initiation and progression.

In this chapter, we will summarize the mouse models that have allowed us to faithfully recapitulate features of human cancers and to highlight the network of connections between the *PTEN* signaling cascade and other oncogenic or tumor suppressive pathways.

Notably, *PTEN* represents one of the most extensively modeled genes involved in human cancer and exemplifies the strength of genetic mouse modeling as an approach to gain information aimed to improve our understanding of and ability to alleviate human disease.

1 Introduction

In 1997, *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) was identified as the frequently lost tumor suppressor gene in a region of human chromosome 10 (10q23) that was known to be highly susceptible to deletion in malignant tumors of the prostate and the brain (Li et al. 1997; Steck et al. 1997).

Soon after its discovery, the work of Maehama and Dixon (1998) unveiled the biochemical function of PTEN as a plasma-membrane lipid phosphatase that hydrolyzes the 3-phosphate on the second-messenger molecule phosphatidylinositol-3,4,5-triphosphate (PIP_3) to generate phosphatidylinositol-4,5-bisphosphate (PIP_2). In the following years, several groups (Di Cristofano et al. 1998; Podsypanina et al. 1999; Stambolic et al. 1998) showed that PTEN exerts its function as a tumor suppressor at least in part through negative regulation of the crucial cell survival serine/threonine kinase AKT (PKB).

Since then, PTEN has been shown to affect pleiotropic cellular processes such as cell cycle progression, cell proliferation, senescence, chemotaxis, apoptosis, aging, muscle contractility, DNA damage response, angiogenesis, and cell polarity. In line with its role in multiple crucial cellular processes, PTEN has a role in the pathogenesis of numerous diseases such as diabetes, autism, and cancer.

Indeed, *PTEN* is one of the most frequently mutated, deleted, and silenced tumor suppressor genes in human cancer. The importance of *PTEN* as a tumor suppressor is supported by the observations that germline *PTEN* mutations in humans can result in autosomal dominant syndromes collectively referred to as the *PTEN* hamartomas tumor syndromes (PHTS), characterized by developmental defects, neurological deficits, multiple hamartomas in various tissues including skin, breast, intestine and brain, and an increased risk of breast, thyroid and endometrial cancers (Liaw et al. 1997; Marsh et al. 1997; Zhou et al. 2000).

The identification of *PTEN* as an important tumor suppressor gene led to a rapid outburst of several mouse models aimed at understanding the consequences of *Pten* loss. During this time, these mouse models have been further refined to study specific organs and specific cell lineages. This has allowed us to faithfully recapitulate some features of human cancers and to reconstruct the intricate connections

between the PTEN signaling cascade and other oncogenic or tumor suppressive pathways.

Overall, this chapter will focus on the role of PTEN as a critical player in human diseases and, specifically, on the faithful mouse models generated to dissect the roles of this phosphatase in tumor initiation and progression in different organs. Additionally, a particular relevance will be given to the work carried out *in vivo* in the mice to identify the network of signaling pathways enabling PTEN to exert its tumor suppressive function. Finally, emphasis will be given to the differential outcomes observed in different contexts as a consequence of loss of *Pten*.

2 Spectrum of Human Diseases Associated with Loss of PTEN

Over the last decade a multitude of important studies have identified *PTEN* gene mutations in a wide range of sporadic malignancies and at a high frequency in cancer-susceptibility syndromes.

Sequencing of the PTEN gene has revealed that this non-redundant, evolutionary conserved phosphatase is one of the most commonly mutated tumor suppressors in human malignancies (Cairns et al. 1998; Dahia et al. 1997; Duerr et al. 1998; Rasheed et al. 1997; Shao et al. 1998; Tashiro et al. 1997; Wang et al. 1997).

Genetic alterations of the PTEN gene include various types of abnormalities ranging from point mutations (encoding mostly unstable and/or catalytically inactive proteins) to large chromosomal deletions (Georgescu et al. 1999, 2000; Li et al. 1997; Steck et al. 1997). PTEN mutations can affect both alleles in various cancers with the following frequencies: endometrial (∼50%), glioblastoma (∼30%), melanoma (∼12%), prostate (∼10%), and breast (∼5%) (Ali et al. 1999; Birck et al. 2000; Cairns et al. 1997; Celebi et al. 2000; Chiariello et al. 1998; Duerr et al. 1998; Haluska et al. 2006; Lin et al. 1998; Saal et al. 2005; Shao et al. 1998; Steck et al. 1997; Tashiro et al. 1997; Wang et al. 1997; Zhou et al. 2002). Loss of one PTEN allele is frequently observed in the following malignancies: glioma (∼75%), breast (∼40%), colon (∼20%), lung (∼37%), prostate (∼42%) (Bose et al. 1998; Feilotter et al. 1998; Lin et al. 1998; Rubin et al. 2000; Teng et al. 1997).

Importantly, PTEN expression is regulated not only genetically but also at the transcriptional/translational level. DNA methylation, transcriptional repression, and microRNA-directed mRNA degradation and translational abrogation have been reported to be important mechanisms in reducing PTEN expression in several cancers(Wiencke et al. 2007; Yang et al. 2008; Poliseno et al. 2010).

Overall, these findings imply that loss of function of PTEN is a common event in cancer, which is accomplished though several layers of control and mechanisms.

Germline deletion/mutation of PTEN is associated with several autosomal dominant tumor predisposition syndromes including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Lhermitte-Duclos disease, Proteus syndrome, and Proteus-like syndrome (Liaw et al. 1997; Marsh et al. 1997; Zhou et al. 2000, 2001). These patients often suffer from hamartomas in multiple organs with the risk of

progression to malignant cancer transformation. In addition to hamartoma development, patients affected by Cowden syndrome and Bannayan–Riley–Ruvalcaba also develop macrocephaly (Buxbaum et al. 2007; Herman et al. 2007). This observation led to an association of PTEN mutation with autism, which is characterized by patients with macrocephaly (Butler et al. 2005). This observation expanded the role of PTEN to suppress human diseases of non-neoplastic nature. Recently, PTEN loss has also been associated with neurological diseases such as Parkinson's (Gasser 2007) and metabolic syndromes such as diabetes. The latter implication is supported by studies in animal that have demonstrated that Pten deletion causes an insulin sensitivity phenotype (Stiles et al. 2004, 2006). This finding supports the notion that patients affected by PHTS characterized by Pten mutations also present increased insulin sensitivity (Iida et al. 2000).

Together these data point to a role for PTEN as a key regulator of several cellular processes and that deregulation of its function can causes a wide spectrum of human diseases.

3 Modeling *PTEN* Loss in Specific Murine Organs

Extensive mouse modeling has been performed to elucidate the importance of PTEN and the consequences of its loss in human health and disease.

Homozygous deletion of *Pten* in the mouse embryo is lethal and is characterized by developmental defects in the mesoderm, endoderm and ectoderm (Di Cristofano et al. 1998). Heterozygous *Pten* mice develop multiple neoplasias in a wide spectrum of tissues including prostate, thyroid, colon, lymphatic system, mammary gland, and endometrium (Di Cristofano et al. 1998; Podsypanina et al. 1999; Stambolic et al. 2000; Suzuki et al. 1998). These mouse models also recapitulate some of the features of the PTEN-associated hamartoma syndromes in humans.

To further analyze the consequences of *Pten* loss in other organs, several tissue specific models of *Pten* deletion have been developed using a conditional gene-targeting approach (Table 1). In this chapter, we will examine several mouse models of *Pten* conditional inactivation as examples of the human tissues where loss of *Pten* is observed, such as brain, prostate, and breast.

3.1 Brain

The first three mouse models of tissue-specific inactivation of *Pten* were generated in 2001 (Backman et al. 2001; Groszer et al. 2001; Kwon et al. 2001) with the brain chosen as a target organ. The choice was most likely dictated by the fact that: (1) *PTEN* is very highly frequently mutated in glioblastoma (30%), the most aggressive primary brain tumor in humans (Knobbe et al. 2002) and (2) syndromes associated with germline mutation of PTEN are characterized by neurological abnormalities.

Table 1 Tissue-specific mouse models of *Pten* deletion

Promoter	Tissue	References	Phenotype
Adipose tissue			
aP2Cre	Adipocytes	Kurlawalla-Martinez et al. (2005)	No alterations in adiposity or plasma fatty acids. Increased systemic glucose tolerance and insulin sensitivity
Bone and cartilage			
Col2a1Cre	Osteo-chondro progenitors	Ford-Hutchinson et al. (2007)	Alterations in skeletal size and bone architecture. Metastatic osteosarcomas at very low penetrance
OcCre	Osteoblast	Liu et al. (2007)	Increase in bone mineral density throughout life. In vitro osteoblasts lacking Pten show more differentiation and reduced apoptosis
	Bladder		
FabpCre	Urothelium of the bladder, kidney and ureter	Tsuruta et al. (2006), Yoo et al. (2006)	Urothelial hyperplasia in which component cells show enlarged nuclei and increased cell size. With time, 10% of mutant mice spontaneously develop pedicellate papillary transitional cell carcinomas (TCC) These mice develop also adenocarcinoma of prostate, seminal vescicles, and urethra; vaginal squamous cell carcinoma; adenocarcinoma of colon
Breast			
MMTVCre	Breast epithelium. Also prostate and skin are targeted	Backman et al. (2004), Li et al. (2002)	Breast development abnormalities. Breast tumors at 9–10 months. High-grade PIN that progresses to prostrate carcinoma. Mild epidermal hyperplasia

(*continued*)

Table 1 (continued)

Promoter	Tissue	References	Phenotype
Central nervous system			
En2Cre	Vermis	Marino et al. (2002)	No differences in cell differentiation. Mild defect in cell migration and decreased proliferation
GfapCre	Granule cells of cerebellum, dentate gyrus, cortical neurons, in a fraction of Bergmann-glia as well as astrocytes and oligodentrocytes	Backman et al. (2001), Kwon et al. (2001), Yue et al. (2005)	Premature death. Defects in neuronal migration and specialized subcellular structure. Increased cell size. Abnormalities phenocpying the ones observed in patients with Lhermitte–Duclos disease
L7Cre	Purkinje cells	Marino et al. (2002)	Subtle irregularities of Purkinje cell lining but no major architectural disturbances. Noticeable increase in cell size, including thickening of dendrites and descending axons
NesCre	Neural stem/progenitor cells	Groszer et al. (2001, 2006)	Enlarged, histoarchitecturally abnormal brains, which resulted from increased cell proliferation, decreased cell death, and enlarged cell size. Enhanced self-renewal capacity of neural stem cells
NesCre	Layers II–V of cerebral cortex, granular and polymorphic layers of dentate gyrus	Kwon et al. (2006)	Abnormal social interaction and exaggerated responses to sensory stimuli. Macrocephaly and neuronal hypertrophy, including hypertrophic and ectopic dendrites and axonal tracts with increased synapses. Abnormal behavior resembling autism spectrum disorder
PomcCre	Hypotalamic proopiomelanortin neurons	Plum et al. (2006)	Hyperphagia and sexually dimorphic diet-sensitive obesity. Neuronal membrane hyperpolarization and reduction in basal

			firing rate due to increased ATP-sensitive potassium (KATP) channel activity
Endothelium			
Tie2Cre	Endothelial and endocardial cells	Hamada et al. (2005), Suzuki et al. (2007)	Heterozygous mice display enhanced tumorigenesis due to an increase in angiogenesis driven by vascular growth factors. Homozygous mice die before embryonic day 11.5 (E11.5) 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
Immune system			
CD19Cre	B cells	Anzelon et al. (2003), Suzuki et al. (2003)	Increased serum autoantibodies and elevated numbers of B1a cells. Defects in immunoglobulin class switch recombination associated with impaired induction of activation-induced cytidine deaminase
LckCre	T cells	Suzuki et al. (2001), Hagenbeek et al. (2004), Hagenbeek and Spits (2008)	T-cell lymphomas leading to a premature death. Increased proliferation and decreased apoptosis
LysMCre	Myeloid lineage; granulytes	Zhu et al. (2006), Subramanian et al. (2007)	Augmented chemoattractant-induced transwell migration and superoxide production. Enhanced recruitment of neutrophils to the inflamed peritoneal cavity
LysMCre	Myeloid lineage; macrophages	Kuroda et al. (2008)	Enhanced susceptibility to Leishmania infection
Mx1Cre	HSCs	Yilmaz et al. (2006), Zhang et al. (2006)	Myeloproliferative disease within days and transplantable leukaemias within weeks.

(*continued*)

Table 1 (continued)

Promoter	Tissue	References	Phenotype
			Enhanced hematopoietic stem cell (HSC) proliferation leading to HSC depletion via a cell-autonomous mechanism
Liver			
AlbCre	Hepatocytes	Horie et al. (2004), Stiles et al. (2004)	Massive hepatomegaly and steatohepatitis with triglyceride accumulation. Insulin hypersensitivity. 100% incidence of hepatoadenomas and 66% of hepatocellular carcinomas
Lung			
SpcCre (doxycyclin-inducible)	Lung epithelium	Yanagi et al. (2007)	Death caused by hypoxia soon after birth. Surviving mice develop spontaneous lung adenocarcinomas. Mice with postnatal deletion of *Pten* show lung tumors with high penetrance
Pancreas			
RipCre	Pancreatic β-cells and hypotalamus	Nguyen et al. (2006), Stiles et al. (2006)	Significant whole-body growth restriction and increased insulin sensitivity Increased islet mass without compromise of beta-cell function. Protection from developing streptozotocin-induced diabetes
Pdx1Cre	All pancreatic cell lineages	Stanger et al. (2005)	A fraction of mice develop ductal malignancy. Ductal metaplasia results from the expansion of centroacinar cells
Prostate			
PbCre	Prostatic epithelium	Trotman et al. (2003)	High-grade PIN that progresses to invasive prostate carcinoma
PbCre4	Prostatic epithelium	Wang et al. (2003), Trotman et al. (2003)	

			High grade PIN with progression to invasive adenocarcinoma and, only in Wang et al. model, lung metastatis
PsaCre	Prostatic luminal epithelial cells	Ma et al. (2005c)	Increased size of the luminal epithelial cells, large areas of hyperplasia, focal PIN that progresses tofocal microinvasion and invasive prostate carcinoma
PbCreER(T2)	Prostatic epithelium	Luchman et al. (2008)	Low-grade PINthat progresses to overtly malignant lesions, characterized by high-grade PIN and microinvasive carcinoma
PsaCreER(T2)	Prostatic epithelium	Ratnacaram et al. (2008)	PIN lesions that progresses to adenocarcinoma
Reproductive system			
Gdf9Cre	Oocytes	Reddy et al. (2008)	Activation of the entire primordial follicle pool resulting in premature ovarian failure
TnapCre	Primordial germ cells	Kimura et al. (2003)	Bilateral testicular teratoma, characters. Primordial germ cells with greater proliferative capacity and enhanced pluripotent embryonic germ cell colony formation
Skeletal muscle and heart			
MckCre	Skeletal muscle cells and cardiac myocytes	Crackower et al. (2002), Wijesekara et al. (2005)	Hypertrophy, and decrease in cardiac contractility. Protection from insulin resistance and diabetes caused by high-fat feeding
Skin			
K5Cre	Keratinocytes	Suzuki et al. (2003)	Wrinkled skin because of epidermal hyperplasia and hyperkeratosis. Spontaneous tumors and acceleration in the onset of chemical-induced tumors
DctCre	Pigment producing cells: melanocytes, melanocyte stem	Inoue-Narita et al. (2008)	Half of the mice die shortly after birth with enlargements of the cerebral cortex and

(*continued*)

Table 1 (continued)

Promoter	Tissue	References	Phenotype
	cells, retinal pigment epithelial cells, cells in brain (dentate gyrus of the hippocampus and the cortex)		hippocampus. Resistance to hair graying and susceptibility to carcinogen-induced melanomagenesis
Smooth muscle			
TaglnCre	Smooth muscle cells	Hernando et al. (2007)	Widespread smooth muscle cell hyperplasia and abdominal leiomyosarcomas, with a very rapid onset and elevated incidence (approximately 80%)
Thyroid gland /			
TpoCre	Thyroind epithelium	Yeager et al. (2007)	Diffuse goiter characterized by extremely enlarged follicles. Increase in the thyrocyte proliferative index. Over two thirds of the mutant females develop follicular adenomas

For instance, patients with Lhermitte–Duclos disease (LDD) develop dysplastic gangliocytoma, which is described clinically as a benign overgrowth of neurons in the cerebellum that causes increased intracranial pressure, ataxia and seizure (Zhou et al. 2003). Although patients affected by LDD have inherited only one normal copy of PTEN, the dysplastic cells have either completely lost PTEN expression or express only the mutant allele due to loss of heterozygosity (LOH); both events are characterized by an increase in the phosphorylation of AKT (Abel et al. 2005; Iida et al. 1998; Zhou et al. 2003). Two of these mouse models generated faithfully recapitulated the features of LDD. In these models, $Pten^{flox/flox}$ mice were crossed with transgenic mice in which Cre recombinase expression is under the control of the glial fibrillary acidic protein (Gfap) promoter. In these mice, *Pten* is deleted late in the development of granule neurons of the cerebellum and results in a cell-autonomous loss of size regulation (Backman et al. 2001; Kwon et al. 2001). As a consequence, the size of Pten-deficient granule neurons progressively increases without evidence of abnormal proliferation. This observation is reminiscent of the focal lesions in LDD that rarely contain proliferative cells. Furthermore, LDD is characterized by dysplastic neurons ectopically placed in the molecular layer, which is similar to the ectopically positioned granule neurons resulting from a neuronal migration defect in mouse models (Abel et al. 2005; Backman et al. 2001; Kwon et al. 2001). Indeed, several studies have established that deletion of Pten in different neuronal types during development results in marked defects in migration and patterning in brain (Backman et al. 2001; Kwon et al. 2001; Marino et al. 2002; Yue et al. 2005). Another similarity between humans and the mouse models is that abnormalities in synaptic structure have been identified in LDD patients as well as in Pten conditional knockout mice (Fraser et al. 2008; Kwon et al. 2006). Overall, these mouse models suggest that the abnormalities observed in LDD can be attributed to key roles of PTEN in neuronal migration, size regulation, and specialized subcellular structure. Notably, this nonproliferative disease resulting from PTEN inactivation, although not malignant, is often associated with premature morbidity.

The third mouse model developed in 2001 utilized the neural stem cell specific nestin promoter (NesCre) to deliver Cre (Groszer et al. 2001) in neural stem cells, thereby resulting in *Pten* deletion throughout the entire brain. These mice succumb to an early postnatal death, presumably due to a continuous increase in brain size with individual cells being larger than those from wild-type mice brains. In contrast to the other two mouse models previously described, these mutant mice showed increased cell proliferation and decreased cell death. Using this model, Groszer et al. concluded that *Pten* most likely negatively regulates neural stem/progenitor cells self-renewal capability by modulating G_0–G_1 cell cycle entry (Groszer et al. 2006).

Other neurological abnormalities observed in patients with germline mutations of *PTEN* were also modeled in mice. One manifestation of inherited *PTEN* mutation includes macrocephaly; several studies have identified autism or autistic behaviors in macrocephalic PHTS patients (Butler et al. 2005; Goffin et al. 2001). Moreover, it is interesting to note that neurological phenotypes associated with

PHTS are very variable. As outlined above, the development of LDD is characterized by a second hit that inactivates the wild-type allele of PTEN in the lesions of the cerebellum. It is possible that other neurological deficits observed in PHTS patients, such as macrocephaly, mental retardation, and autism, are also associated with second hits that occur stochastically during development. In such a scenario, the timing and specific cell populations in which *PTEN* function is lost during development would determine the specific neurological outcome observed. For instance, a mouse model where *Pten* was deleted in subsets of differentiated neurons in the cerebral cortex and hippocampus showed anxiety-like behavior and decreased learning, that may recapitulate the autistic features of some PHTS patients (Kwon et al. 2006).

Although PTEN is frequently inactivated in malignant human brain tumors, PHTS is not associated with an increased incidence of brain tumors, and mice with heterozygous loss of *Pten* fail to develop brain tumors. Brain tumors are also not observed in conditional knockouts targeting *Pten* deletion in the brain, indicating that cooperating mutations in other genes are required for the neoplastic process (Backman et al. 2001; Fraser et al. 2004; Groszer et al. 2001; Kwon et al. 2001; Marino et al. 2002) (see following paragraphs).

3.2 Prostate

Prostate cancer and glioblastoma cell lines were the first cellular models where deletion of the chromosomal region containing *PTEN* was reported. These findings led to the identification of *PTEN* as a tumor suppressor gene (Li et al. 1997; Steck et al. 1997). It is reported that the majority of primary prostate cancers show loss of only one allele of *PTEN*, whereas homozygous inactivation of *PTEN* is generally associated with advanced cancer and metastasis (Gray et al. 1998).

Although human and mouse prostates are structurally dissimilar, prostate cancer progression in mice and humans is strikingly similar. In both species, epithelial hyperplasia is followed by low-grade prostatic intraepithelial neoplasia (PIN), which can progress to high-grade PIN. As the lesion becomes more neoplastic and aggressive, the prostate epithelium invades through the basement membrane into the surrounding stroma, thus establishing a localized yet invasive adenocarcinoma (De Marzo et al. 2003; Marandola et al. 2004).

In an effort to define the role of *PTEN* loss in prostate tumorigenesis, a series of *Pten* loss mouse models, the so called "hypomorphic *Pten* allelic series" (*Pten* heterozygous, *Pten* hypomorphic, and *Pten* conditional knock-out), have been generated (Trotman et al. 2003). Specific deletion of *Pten* in the prostate was achieved by crossing $Pten^{loxP/loxP}$ mice with *Probasin-Cre* (*PB-Cre*) transgenic mice. *PB-Cre* transgenic mice express *Cre* recombinase under the control of the ARR_2 *Probasin* promoter specifically in the prostate epithelium post-puberty (Wu

et al. 2001). The generation of the "hypomorphic *Pten* allelic series" has revealed that the prostatic epithelium is exquisitely vulnerable to subtle variations of PTEN expression levels. For instance, loss of one allele of *Pten* is associated with the development of high-grade PIN with incomplete penetrance after a long latency (9 months), whereas when the level of *Pten* is reduced to ~30% (hypomorphic mouse model), mice developed invasive prostatic adenocarcinoma albeit with incomplete penetrance (Trotman et al. 2003). Furthermore, complete loss of *Pten* results in the development of high-grade PIN (HG-PIN) as early as 8 weeks of age, together with the concomitant activation of cellular senescence response (see below) (Chen et al. 2005). HG-PIN lesions progress to invasive prostate cancer with complete penetrance at 6 months of age, once the senescence response has been evaded (Chen et al. 2005; Trotman et al. 2003). These analyses imply that (1) loss of PTEN is critical for prostate cancer initiation and that (2) the level of PTEN expression is inversely associated with prostate tumorigenesis.

Wang et al. also used *PB-Cre* transgenic mice to generate mice with conditional inactivation of *Pten* in the prostate, which also results in invasive prostate cancer (Wang et al. 2003). Additionally, these mice developed metastatic prostate cancer of the lymph nodes and lung, which is not observed in other mouse models of *Pten* conditional inactivation in the prostate (Abate-Shen et al. 2003; Chen et al. 2005; Wang et al. 2003). This may be due to the different genetic background strain of the mice, which is known to influence cancer susceptibility.

In a later report, by crossing $Pten^{loxP/loxP}$ mice with *MMTV-Cre* transgenic mice, Backman et al. inactivated *Pten* in the prostate during development (Backman et al. 2004). Deletion of *Pten* in the prostate before puberty resulted in the onset of neoplastic lesions at a very early time point, with mice displaying high-grade PIN by the age of 2 weeks at complete penetrance that frequently progressed to invasive adenocarcinomas by 7–14 weeks (Backman et al. 2004). These data show that, if *Pten* has already been deleted in the prostate during development, the incidence, penetrance, and progression of neoplasia are much greater than if *Pten* is lost during or after puberty.

In 2005, yet another model of complete *Pten* inactivation in the prostate was generated using $Pten^{loxP/loxP}$ mice crossed with prostate-specific antigen (PSA)-*Cre* transgenic mice (Ma et al. 2005c). The onset of prostatic neoplastic lesions is significantly delayed in these mice which show focal PIN at the age of 4–5 months. By 7–9 months, focal microinvasion was observed which progressed to frank invasive adenocarcinoma at 10–14 months (Ma et al. 2005c).

Recently, two groups have generated two mouse models where *Pten* deletion is temporally controlled through the use of $Pten^{loxP/loxP}$ mice crossed with tamoxifen-inducible Cre recombinase transgenic mice (Luchman et al. 2008; Ratnacaram et al. 2008). Like other models before, deletion of *Pten* results in the development of PIN lesions that later progress to invasive adenocarcinoma.

Together, models of conditional *Pten* inactivation in the prostate clearly demonstrate the sensitivity of the prostatic epithelium to alterations of *Pten* and they recapitulate the sequential stages of the human disease from PIN to invasive

prostate cancer where time to progression is dictated solely by the developmental time of *Pten* excision and the remaining dose of functional Pten.

3.3 Breast

A characteristic feature of Cowden disease is the development of benign breast hamartomas that are accompanied by a higher risk of breast cancer. Although somatic *PTEN* mutations are detected only in a smaller fraction of breast cancer cases (Dahia 2000), LOH at the *PTEN* locus (10q23) is frequently found (40%) (Bose et al. 1998; Garcia et al. 1999). Furthermore, immunohistochemical studies suggest that loss of PTEN protein expression is a common event in breast cancer (33–48%), with strong correlation with lymph node metastasis, loss of estrogen receptor staining, and disease related death (Depowski et al. 2001; Perren et al. 1999). Thus, epigenetic mechanisms are hypothesized to be responsible for a number of cases in which PTEN levels are downregulated or even totally ablated in the absence of a detectable mutation.

The relevance of *Pten* in breast tumorigenesis was initially highlighted in the mouse model of *Pten* germline heterozygous loss generated by Stambolic et al. (2000). Female $Pten^{+/-}$ developed mammary tumors at incomplete penetrance, with most of them having features of well-differentiated adenocarcinoma. Similar to CS patients (Schrager et al. 1998), breast lesions in $Pten^{+/-}$ mice displayed marked proliferation of the stroma. The authors observed an increased penetrance of the breast tumors with age in $Pten^{+/-}$ mice thereby suggesting a requirement for additional hits for tumor progression in this tissue.

After that, to fully understand the role of *Pten* in breast tumorigenesis, in 2002 Li et al. crossed $Pten^{loxp/loxp}$ mice with transgenic mice expressing MMTV-Cre transgenes in order to achieve *Pten* deletion in the mammary epithelium (Li et al. 2002). The deletion of *Pten* in mammary epithelium triggered increased cell proliferation, hyper-branched ductal structure, precocious development, delayed involution and severely impaired apoptosis. *Pten*-deficient mammary epithelium also displayed remarkable neoplastic changes. Females with mammary-specific *Pten* deletion develop tumors as early as 2 months. Histological features of the tumors varied from benign fibroadenomas to pleiomorphic adenocarcinomas. Furthermore, immunohistochemistry analysis revealed up-regulation of cytokeratins 5 and 6 in these mice (Li et al. 2002). Interestingly, this finding nicely correlates with over-expression of these two cytokeratins in human breast tumors of the basal subtype (Sorlie et al. 2001). The basal subtype often occurs in patients with germline BRCA1 mutations and is associated with a poor prognosis. Importantly, it has been recently shown that heterozygous inactivation of *Pten* leads to the formation of basal-like mammary tumors in mice, and that loss of *PTEN* expression is significantly associated with this subtype of breast cancer in human sporadic and BRCA1-associated hereditary breast cancers (Saal et al. 2008). In addition, Saal and colleagues have identified frequent gross *PTEN* mutations, involving

intragenic chromosome breaks, inversions, deletions and micro copy number aberrations, specifically in BRCA1-deficient tumors (Saal et al. 2008).

It has recently been shown that even a subtle reduction in *Pten* dose determines breast cancer susceptibility (Alimonti et al. 2010). Indeed, *Pten* hypomorphic mice, expressing 80% normal levels of *Pten*, develop a spectrum of tumors, with breast occurring at the highest penetrance (Alimonti et al 2010). Overall, all these observations underscore the essential role of *PTEN* during normal mammary gland development and in suppressing breast cancer formation.

4 In Vivo Deconstruction of the PI3K-AKT-mTOR Axis

The numerous mouse models generated to study the PI3K-AKT-mTOR pathway have been valuable tools to shed light on the role of various components of the PI3K signaling cascade in disease and tumorigenesis. Overall, these mouse models have defined the mTOR pathway as a crucial converging node downstream PI3K-AKT signals required for oncogenic transformation driven by loss of *PTEN*.

4.1 PI3K-PDK-AKT

The PI3K (phosphatidylinositol-3-kinase) pathway starts at the plasma membrane where the binding of ligands to the growth factor receptor tyrosine kinases activate PI3K, which phosphorylates PIP2 to produce PIP3, thereby directly antagonizing PTEN (Klinghoffer et al. 1996).

Class I PI3K contains four p110 isoforms, α, β, γ, and δ. The association between p110α and tumorigenesis is well established and has been corroborated by the occurrence of gain of function p110α mutations in human cancer (Samuels and Velculescu 2004). Recently, the p110β isoform has also been connected to oncogenesis (Ciraolo et al. 2008; Jia et al. 2008). With regards to *PTEN*-loss driven cancer, conditional inactivation of PIK3CB, the gene encoding p110β, blocked prostate tumorigenesis mediated by loss of *PTEN* whereas prostate-specific knockout of the α-isoform did not alter tumor formation. The observations of Jia and coworkers identify a previously unknown role for p110β in cancer, specifically in *PTEN* mutated tumors. These studies collectively suggest that p110β may represent a potential "druggable" target, specially in *PTEN* null cancers.

PI3K signaling induces a series of growth-promoting events through the activation of the protein kinases PDK1 and AKT, which directly bind to and are activated by PIP3 (Alessi et al. 1997; Currie et al. 1999). Upon PIP3 binding, PDK1 induces AKT kinase activity 30-fold by phosphorylating it on residue T308 in addition to the phosphorylation of numerous other target proteins within the T loop (such as Serum and Glucocorticoid-regulated kinases, SGK) enabling their activation

(Alessi et al. 1997). AKT, in turn, phosphorylates multiple targets to activate the cell cycle, prevent apoptosis and trigger cellular growth (Manning and Cantley 2007). *In vivo* studies in the mice have genetically highlighted the important epistasis of *Pten* and *Pdk1* and *Akt*. Compound mutant mice have provided clear genetic evidence for the roles of *Akt* and *Pdk1* as mediators of cancer phenotypes identified upon heterozygous Pten-loss in mice. Specifically, Akt1 deficiency suppresses tumor development in $Pten^{+/-}$ mice (Chen et al. 2006). Similarly, the hypomorphic expression of Pdk1 (levels that are 80–90% reduced compared with normal) inhibits tumor formation in $Pten^{+/-}$ mice (Bayascas et al. 2005). Therefore, these mouse models have validated AKT1 and PDK1 as critical players in mediating tumorigenesis upon PTEN-loss.

4.2 TSC1/2-Rheb-mTOR

Among the many downstream targets of AKT, the mammalian target of rapamycin (mTOR) has been demonstrated to be an essential effector in promoting cell proliferation and susceptibility to oncogenic transformation. mTOR is a serine/threonine kinase that regulates protein synthesis, cell growth, and proliferation in response to pleiotropic inputs including growth factors, nutrients, energy, and stress (Wullschleger et al. 2006). mTOR differentially regulates PI3K/AKT signaling by acting as a key component of two multiprotein complexes: mTOR complex 1 (mTORC1) which is activated downstream of AKT, and mTOR complex 2 (mTORC2) which has been demonstrated to phosphorylate AKT on Ser 473 (Sarbassov et al. 2005). This modification, in conjunction with the phosphorylation on Thr308 by PDK1 triggers full activation of AKT in response to mitogenic stimuli (Sarbassov et al. 2005). Moreover, in many cell types, mTORC1 has been reported to elicit a negative feedback regulation on the PI3K pathway through the ability of its downstream target ribosomal S6 kinase 1 (S6K1) to inhibit IRS-1 (reviewed in Guertin and Sabatini 2007). The elusive cross-talk between the AKT and mTOR pathways was uncovered by the finding that tuberous sclerosis complex 1 (TSC1) and 2 (TSC2) negatively regulates mTORC1 (Gao et al. 2002; Tapon et al. 2001). These studies demonstrated that AKT phosphorylates and inactivates the TSC1/TSC2 complex, and as a consequence results in mTORC1 activation (Jaeschke et al. 2002; Tee et al. 2002). Specifically, Ras homologue enriched in brain (Rheb), a small guanosine triphosphate (GTP)-binding protein, was discovered as a novel substrate for TSC2, which could also lead to the activation of mTOR (Garami et al. 2003; Inoki et al. 2003; Zhang et al. 2003). TSC2 was shown to display a GTPase activating protein (GAP) activity towards the Rheb GTPase; this event stimulates the intrinsic GTP-hydrolysis activity of Rheb to promote its transition from an active GTP-bound to an inactive guanosine diphosphate (GDP)-bound form (Garami et al. 2003; Zhang et al. 2003). Conversely, inactivation of the TSC1/TSC2 complex by AKT phosphorylation, results in GTP loading and activation of Rheb, which ultimately promotes the activation of mTORC1. AKT also promotes mTORC1 activity through phosphorylation of PRAS40, which prevents its inhibitory function

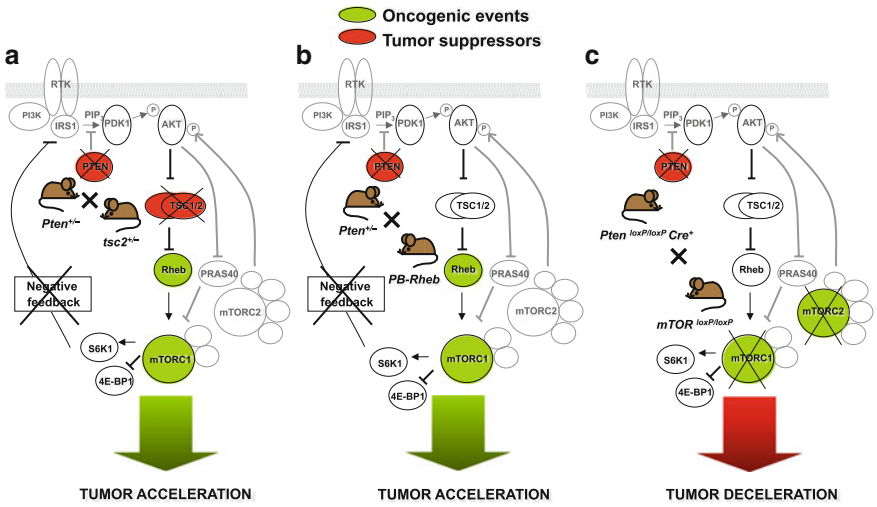

Fig. 1 Analysis of the contribution of the PI3K pathway to *Pten* loss-driven disease, through the combination of genetic events in the mouse. *Pten*-loss driven disease is accelerated by Tsc2 heterozygous loss (**a**) as well as Rheb transgenic expression (**b**), whereas it is opposed by loss of *mTOR* (**c**)

on mTORC1 (reviewed in Guertin and Sabatini 2007). When active, mTORC1 promotes cell growth through phosphorylation of various regulators of translation including the well-characterized ribosomal S6K1 which activates the S6 ribosomal protein (S6), and the eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) which leads to its uncoupling from the elongation initiation factor 4E (eIF4E; Wullschleger et al. 2006).

Several mouse models have validated the importance of the mTOR pathway in further promoting tumorigenesis driven by *Pten* loss. For instance, oncogenic events proximal to mTOR activation such as *Tsc2* heterozygosity as well as *Rheb* overexpression cooperate with *Pten* haploinsufficiency to accelerate tumorigenesis (Ma et al. 2005b; Manning et al. 2005; Nardella et al. 2008) (Fig. 1a, b). A definitive proof of principle for a major role of mTOR in prostate tumorigenesis driven by *Pten*-loss was uncovered by the conditional inactivation of *mTor* in *Pten* null prostates. In these prostates, *mTor* deletion markedly suppressed the tumor initiation and progression observed in *Pten*-null mice (Nardella et al. 2009) (Fig. 1c). These findings are corroborated by the work of Guertin et al, which showed that Rictor, one of the components of the mTORC2 multiprotein complex, is also required for *Pten*-loss induced tumorigenesis in the mice (Guertin et al. 2009).

Collectively, these data have important therapeutic application in the treatment of cancer triggered by loss of PTEN, since mTOR is a kinase with activity that is amenable to pharmacological inhibition. Indeed, efforts have been placed to develop and improve upon drugs that inhibit mTOR activity. The first generation

of mTOR inhibitors directed solely against mTORC1, such as Rapamycin have had limited success in the clinic. We now await the development and testing of drugs targeting both mTORC1 and mTORC2 for the treatment of tumors triggered by PTEN deficiency and aberrant PI3K-AKT-mTOR signaling.

5 PTEN Network: Linking the PI3K Signaling Cascade to Other Oncogenic Pathways Through In Vivo Genetic Analysis

It is now well established that the PI3K pathway is intimately linked to several other oncogenic events including activated MAPK signaling, ETS-related gene (ERG) overexpression, and loss of the tumor suppressor gene p53. Below, we will describe the efforts to generate faithful mouse models of human cancers which recapitulate critical cooperative events identified in human cancer.

5.1 PTEN-MAPK Pathway

The RAS oncogene and the PTEN tumor suppressor are upstream of two of the most predominant oncogenic signaling pathways, MAPK and PI3K, respectively (Dhillon et al. 2007; Engelman et al. 2006). The signaling emanating from these two pathways is however complicated by a remarkable number of interconnections (Carracedo et al. 2008).

Ras is the upstream regulator of the MAPK pathway and frequently activated in cancer through mutations (G12D, G12V) which are sufficient to initiate cancer in the mouse (Fisher et al. 2001; Johnson et al. 2001). Ras mutations lead to hyperactivation of the MAPK pathway, which cross-talks with the PI3K cascade through the regulation of common targets such as BAD and TSC2 (Datta et al. 1996; Fang et al. 1999; Gupta et al. 2007; Ma et al. 2005a). In addition, activated Ras leads to loss of PTEN expression through c-Jun-mediated transcriptional events (Vasudevan et al. 2007). Consistent with this notion, mutations in RAS and PTEN in cancer tend to be mutually exclusive RAS mutations are prevalent in pancreatic, lung, and colon cancers, but not in glioblastomas whereas the opposite is true for PTEN mutation (Liu et al. 1997; Simpson and Parsons 2001).

Downstream of RAS, RAF (BRAF, CRAF, and ARAF) serine/threonine protein kinases regulate MAPK signaling (Balmanno and Cook 2009; Moodie et al. 1993). BRAF is also frequently found mutated in cancer (principally V600E mutation), most frequently in melanoma where BRAF mutation is observed in 50–70% of cell lines and tumors, and does not overlap with RAS mutations (Halilovic and Solit 2008). Unlike RAS, concomitant genetic alterations in PTEN and BRAF have been found in melanoma and shown to be cooperative events in the mouse (see below), therefore reinforcing the complexity of the interaction between components within these two pathways (PTEN-PI3K and MAPK) in cancer.

Combining PTEN and BRAF mutations to model human metastatic melanoma in mouse. Mutant activated BRAF ($BRAF^{V600E}$) can induce senescence in cultured melanocytes providing an explanation for the high frequency of BRAF mutations in benign nevi (Denoyelle et al. 2006; Dhomen et al. 2009; Michaloglou et al. 2005; Taube et al. 2009). Hence, overcoming oncogene-induced senescence may be critical for melanomagenesis. Progression to malignant melanoma is invariably accompanied by silencing of one or more tumor suppressor genes, most commonly PTEN or CDKN2A (Chin et al. 2006; Garraway et al. 2005). Additionally, the combination of mutated BRAF and silencing of PTEN expression is observed in 20% of human melanomas (Backman et al. 2004). While *Pten*-loss or $BRAF^{V600E}$ activation alone did not have a dramatic consequence for melanoma onset and progression in mice, compound $BRaf^{V600E}$-*Pten*-Null mice succumbed to an aggressive form of metastatic melanoma (Dankort et al. 2009) (Fig. 2a). Of note, combinatorial inhibition of MAPK and mTORC1 led to the reduction of melanoma

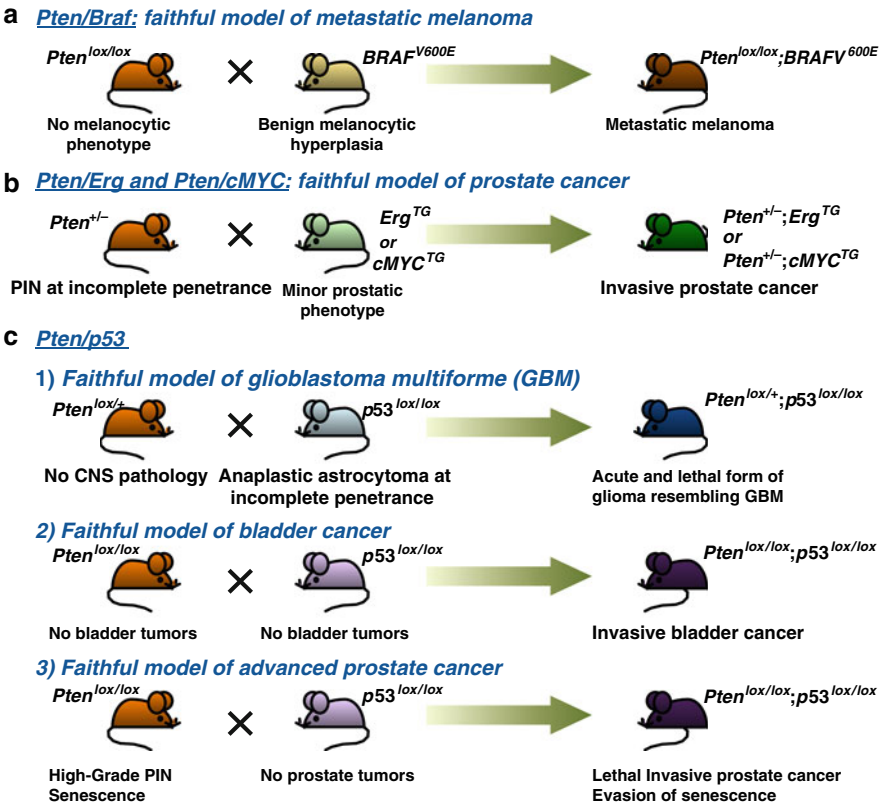

Fig. 2 Genetic interventions in the mouse that have allowed the generation of faithful mouse models resembling human disease. Faithful mouse models of human melanoma (**a**), prostate cancer onset and progression (**b**), glioblastoma multiforme, bladder cancer, and advanced prostate cancer (**c**)

formation, implying that this pharmacological approach may be an effective therapeutic avenue in the treatment of this type of cancer. Therefore, the *Pten*$^{-/-}$; BRaf^{V600E} melanoma model, together with other recently developed genetically modified melanoma mouse models (Goel et al. 2009), represent an invaluable tool for modeling of melanoma in the mouse and the evaluation of therapeutic approaches in the treatment of this deadly disease. Importantly, whether PTEN is required for the bypass and senescence induced by BRAF mutation in melanoma remains to be determined, and might add complexity to the already diverse tissue-specific outcomes of *Pten*-loss in vivo (see below).

5.2 *Pten and Transcriptional Regulators: Erg and Myc*

As mentioned above, PTEN is frequently lost or downregulated in prostate cancer (Salmena et al. 2008). Recently, the translocation of an ETS transcription factor gene (ERG or ETV1) to the TMPRSS2 gene promoter region, which contains androgen responsive elements, has been identified in prostate tumors (Tomlins et al. 2005). TMPRSS2-ERG is the first recurrent translocation event to be described in human tumors. It occurs in approximately 40% of prostate tumors and results in an aberrant androgen-regulated expression of ERG (Perner et al. 2007). In mice, transgenic ERG expression in the prostate leads to an unremarkable phenotype (Carver et al. 2009a), suggesting that ERG overexpression is not an initiating event in prostate cancer, in line with the notion that TMPRSS22-ERG translocation is rarely found in early lesions (Balmanno and Cook 2009; Carver et al. 2009b).

On the other hand, genetic lesions such as amplification and polymorhisms at 8q24, where c-MYC is located, are robustly associated with prostate cancer risk (Amundadottir et al. 2006; Bubendorf et al. 1999; El Gedaily et al. 2001; Gudmundsson et al. 2007; Haiman et al. 2007; Qian et al. 1997; Tsuchiya et al. 2002; Witte 2007; Yeager et al. 2007). Furthermore, recent studies have shown that over-expression of c-MYC is not restricted to advanced/late prostate cancer lesions, but occurs also in early lesions (Gurel et al. 2008). Additional studies are needed in order to determine the mechanisms underlying the frequent over-expression of c-MYC in prostate cancer, and to assess whether this is also a consequence of 8q24-polymorphism-relates transcriptional events or may in fact be the result of post-transcriptional mechanisms (Pomerantz et al. 2009).

Seeking a faithful model of prostate cancer in the mouse: Combinatorial mutation of PTEN with ERG and MYC. Loss of PTEN is frequently accompanied by the translocation of TMPRSS22-ERG in prostate cancer (Balmanno and Cook 2009; Carver et al. 2009a). Modeling the compound loss of *Pten* and *Erg* overexpression (Probasin-transgenic Erg, Erg^{TG}) in the mouse prostate has uncovered a strong cooperativity between these two genetic events (Balmanno and Cook 2009; Carver et al. 2009a) (Fig. 2b). Whereas *Pten* heterozygosity leads to high-grade prostate intraepithelial neoplasia (HGPIN) lesions starting at the age of 9 months, compound

Pten$^{+/-}$-Erg^{TG} mutants develop HGPIN by 2 months of age, which progresses to invasive cancer by the age of 6 months.

c-MYC transgenic expression in the prostate leads to hyperproliferation and PIN (Ellwood-Yen et al. 2003; Kim et al. 2009; Zhang et al. 2000). Moreover, Pten-heterozygous loss cooperated with c-MYC to induce high-grade prostatic intraepithelial neoplasia (HGPIN)/cancer lesions, which harbor loss of the wild type *Pten* allele (Kim et al. 2009) (Fig. 2b).

Overall, *Pten*$^{+/-}$-Erg^{TG} and *Pten*$^{+/-}$; cMYC^{TG} prostate cancer models likely represents the most faithful models of prostate cancer initiation and progression to date, since they recapitulate the precise sequence of mutagenic events occurring in a large fraction of human prostate cancers. However, unlike human prostate cancer, which exhibits a highly metastatic tropism to the bone in later stages, mouse models have thus far failed to recapitulate these late events faithfully. Hence, additional genetic studies and novel combinatorial efforts in the mouse are required to generate better models of human prostate cancer progression to metastasis.

5.3 Pten/p53

In terms of overall frequency, p53 is undoubtedly the most frequently mutated tumor suppressor gene in human cancers (Levine et al. 2004; Vogelstein et al. 2000) with PTEN following in second (Cantley and Neel 1999; Simpson and Parsons 2001). The spectrum of human cancers associated with p53 and PTEN mutation are very different. (Fujisawa et al. 2000; Kato et al. 2000; Koul et al. 2002; Kurose et al. 2002). Mutations of p53 occur at high frequencies in lung, colon and breast cancers, whereas PTEN mutations are mostly found in glioblastoma, endometrial cancer, malignant melanoma, and prostate cancer. However, compound loss of *PTEN* and *p53* has been reported in glioblastoma (Han et al. 2008; Salmena et al. 2008), bladder cancer (Puzio-Kuter et al. 2009) and advanced/metastatic prostate cancer (Chen et al. 2009).

Mouse models of combined loss of Pten and p53 in glioblastoma, bladder and prostate cancer. In glioblastoma, the impact of combined *Pten* and *Trp53* loss has been recently reported in a model of concomitant deletion of *Pten* (in heterozygosity) and *Trp53* in the GFAP+ cell lineage. These mice develop an acute and lethal form of glioblastoma multiforme (Fig. 2c). Importantly, this mouse model displays features reminiscent of the pathological lesions observed in human glioblastoma multiforme (Zheng et al. 2008). Mechanistically, compound loss of *Pten* and *Tp53* in neural stem cells leads to increased cell renewal and decreased differentiation in a MYC-dependent fashion (Zheng et al. 2008).

Bladder cancer is a major cause of cancer morbidity and mortality (Jemal et al. 2005). Combined *p53* and *PTEN* losses have been identified in invasive bladder cancer and are reportedly causal factors that predict poor outcome (Puzio-Kuter et al. 2009). Indeed, combined loss of *Trp53* and *Pten* in mice results in lesions with

characteristics of human carcinoma in situ with complete penetrance at 6 months of age(Puzio-Kuter et al. 2009) (Fig. 2c).

In prostate cancer, partial loss of the *PTEN* tumor suppressor gene is a prevalent event (see above). However, complete loss of *PTEN* is infrequent in early lesions and is restricted to advanced cancers. Through the analysis of acute complete conditional loss of *Pten* in the prostatic epithelium, we found that one plausible explanation for this phenomenon is the fact that complete acute loss of *Pten* elicits a p53-dependent failsafe senescence response which opposes tumor progression, (Chen et al. 2005). In agreement with this notion and the fact that in human prostate cancer *p53* loss is a late event observed prevalently in advanced lesions, compound loss of *Pten* and *Trp53* in the mouse prostate leads to a lethal form of advanced prostate cancer where the senescence response has been evaded (Chen et al. 2005). In spite of the local aggressiveness of these tumors, *Pten/Trp53* compound mutants, surprisingly, do not develop metastasic prostate cancer (Chen et al. 2005).

Although *p53* and *PTEN* represent the most frequently lost of all tumor suppressors, further studies are required to precisely determine the frequency and the timing of their loss, and the specific tissues where it occurs. However, modeling these mutations in the mouse has already allowed the generation of faithful models of advanced prostate, bladder cancers, and glioblastoma that will prove extremely valuable to study the biology of these cancers and to test novel therapeutic modalities in preclinical studies.

6 Context-Dependent Differential Outcomes Triggered by Loss of *PTEN*

The large number of studies reporting phenotypes of *Pten* conditional knockout mice has highlighted the function of *Pten* in different cell and tissue types.

Although the PI3K pathway is ubiquitous, PTEN-mediated regulation of the PI3K/AKT pathway results in cell context-dependent outcomes such as cell size, proliferation, survival and senescence. Furthermore, there is a growing body of evidence suggesting that differential outcomes can be due to differential timing of *Pten* loss in specific stages of the development within the same tissue.

Cell size. Conditional knock-out mice show that loss of *Pten* may influence cell size or cell number depending on the specific context. The brain is an example of where selective deletion of *Pten* in specific cell types such as granule neurons of the cerebellum and dentate gyrus, cerebellar precursor cells and Purkinje neurons results in a cell-autonomous size increase in Pten-deficient cells (Backman et al. 2001; Kwon et al. 2001; Marino et al. 2002).

Cell number. In other settings, the consequences of *PTEN* loss determines changes in cell number, due to the combined effects of proliferation and cell survival, rather than aberrant cell size. Conditional deletion of *Pten* caused increased proliferation, decreased apoptosis and tumorigenesis, as exemplified in keratinocytes (Backman et al. 2004; Suzuki et al. 2004), prostatic epithelium

(Backman et al. 2004; Wang et al. 2003), mammary epithelium (Li et al. 2002), germ cells (Kimura et al. 2003) and hepatocytes (Horie et al. 2004). Overall, these examples suggest that the cellular context strongly influences the specific outcome of *PTEN* deficiency.

Cellular senescence. PTEN does not exert its tumor suppressive function in isolation, but cross-talks extensively with other tumor suppressors, including p53. Therefore, the status of the p19ARF/p53 network in the different tissues can also affect the differential context-dependent outcomes dictated by the loss of *Pten*.

This is nicely exemplified by the response of the prostatic epithelium upon complete inactivation of *Pten*. Surprisingly, Chen et al. showed that complete acute loss of *PTen* in the prostate did not provide a proliferative advantage as would be expected, but instead promoted a strong p53-dependent senescence response that opposed tumor progression (Chen et al. 2005). As predicted from these findings, combined inactivation of *Pten* and *Trp53* leads to unconstrained tumor growth as demonstrated by the generation of massive invasive prostate tumors. This implies that complete ablation of *PTEN* can be detrimental to tumor growth in the absence of p53 mutations and highlights the importance of haploinsufficiency or partial *PTEN* impairment in tumor progression. Clinically, these findings provide an explanation as to why complete *PTEN* loss is not frequently observed at cancer presentation.

Yilmaz et al. (2006) suggested that the hematopoietic stem cell (HSC) compartment may also be a tissue where complete loss of *Pten* triggers a senescence response (Yilmaz et al. 2006). Deletion of *Pten* in the adult HSCs results in a different outcome in normal hematopoietic stem cells versus leukemia-initiating cells (Yilmaz et al. 2006). Specifically, the authors show that deletion of *Pten* results in the generation of leukemic stem cells and concomitant depletion of normal HSCs. The mechanism responsible for the depletion of Pten-deficient HSCs remains to be elucidated but it has been speculated that *Pten* deficiency induces a senescence response in HSCs whereas the leukemia-initiating cells might acquire secondary mutations that inactivate the senescence response (Yilmaz et al. 2006).

Differential outcomes. In certain tissues, p53 mutations are not required for tumors to progress upon *Pten* loss because p53 is repressed through different mechanisms, and consequently cellular senescence is not observed. This is well exemplified by the deletion of *Pten* in smooth muscle, which results in the development of leiomyosarcomas with very high penetrance (80%) (Hernando et al. 2007). In response to loss of *Pten* the authors observed a substantial upregulation of p19Arf in the sarcoma cells, without concomitant induction of p53. In addition, they observed no evidence of cellular senescence either in the hyperplastic tissue or in the sarcomas. However, marked Mdm2 levels in leiomyosarcoma cells compared to normal smooth muscle of *Pten*-null mice, which kept p53 functionally repressed, thus reducing the need for p53 mutations usually required for tumor progression. In sum, Mdm2 stabilization promoted by Akt phosphorylation seems to prevail over Mdm2 inhibition by p19Arf in *Pten*-null smooth muscle cells, resulting in p53 functional inactivation and thereby tumor development (Hernando et al. 2007).

7 Conclusion

Tremendous technology advances have allowed us to gain powerful insight into the molecular and genetic determinants that drive cancer. Mouse models have been at the forefront of this revolution of information that has allowed us to faithfully recapitulate the features of tumor initiation and progression observed in human cancer. Mouse models of *Pten* loss have shed light on the critical roles of Pten in tumor suppression, specifically as a regulator of cell size, proliferation rate, and failsafe responses, such as senescence, in specific tissues. As one of the "most modeled" of all human cancer genes, Pten mouse models are exemplary of the power of genetic modeling and the success that can be achieved through such studies.

Further insight into the function of PTEN genetic mutations will rely upon the generation of specific point mutations knock-in mice models, which can inform us not only about canonical PTEN function, but the ever-increasing role of PI3K and AKT independent functions of PTEN. These models will provide further understanding of the regulatory mechanisms that affect the role of this protein in normal development and tumorigenesis.

Translation of the information acquired in mice has been and will be extremely useful for the preclinical evaluation of targeted therapeutic anti-cancer agents thereby dramatically improving our ability to cure this and other diseases.

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