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PERSPECTIVE

From man to mouse and back again: advances in defining tumor AKTivities in vivo

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AKT hyperactivation is a common event in human cancers, and inhibition of oncogenic AKT activation is a major goal of drug discovery programs. Mouse tumor models that replicate AKT activation typical of human cancers provide a powerful means by which to investigate mechanisms of oncogenic signaling, identify potential therapeutic targets and determine treatment regimes with maximal therapeutic efficacy. This Perspective highlights recent advances using in vivo studies that reveal how AKT signaling supports tumor formation, cooperates with other mutations to promote tumor progression and facilitates tumor-cell dissemination, focusing on well-characterized prostate carcinoma mouse models that are highly sensitive to AKT activation. The implications of these findings on the therapeutic targeting of AKT and potential new drug targets are also explored.

Introduction

The AKT [also known as protein kinase B (PKB)] signaling pathway is dysregulated in diverse disease processes, ranging from neurodegenerative disorders to diabetes and cancer. AKT is a protein kinase with three isoforms [AKT1, AKT2 and AKT3 (also known as PKBα, PKBβ and PKBγ, respectively)], which influence cell survival, growth, proliferation and insulin signaling. Hyperactive AKT signaling, in many cases via alterations in phosphoinositol-3 kinase (PI3K) and phosphatase and tensin homolog (PTEN), is common in many pathologies, particularly cancer.

Inhibiting hyperactivated AKT might help to treat cancer, in which the PI3K-PTEN-AKT pathway is one of the most commonly mutated signaling pathways. Therefore, upstream regulators or downstream effectors of AKT are desirable therapeutic targets. For example, humanized monoclonal antibodies specific for the upstream epidermal growth factor receptor family, or inhibitors of the downstream mammalian target of rapamycin complex 1 (mTORC1), are FDA approved, including for the treatment of some cancer types. This suggests the potential for further manipulation of AKT signaling for anti-oncogenic treatments and has promoted extensive research into AKT activation and signaling, as is evident from the growing number of related clinical trials (LoPiccolo et al., 2008; Klein and Levitzki, 2009).

There are several mouse models of cancer that provide a malleable in vivo environment in which to study the role of the AKT pathway in tumorigenesis, and to predict the efficacy, selectivity and side effects that novel therapies will have in patients. In this Perspective article, we review new developments in this field that have enabled important insights into the role of AKT in cancer and, by focusing on AKT mouse models of prostate carcinoma (CaP), explore how these advances should facilitate more effective, tailored cancer treatments for patients in the future.

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PI3K-PTEN-AKT pathway signaling and its activation in human tumors

The PI3K-PTEN-AKT signaling pathway transduces signals from membrane receptors to its major effector molecule, AKT (Fig. 1). This pathway is conserved in lower organisms and is ubiquitous in mammalian cells, in which it promotes cell growth, proliferation and survival, as well as mediates hormone metabolism, immune responses and angiogenesis (for a review, see Alessi, 2001; Brazil and Hemmings, 2001; Altomare and Testa, 2005; Manning and Cantley, 2007; Bozulic and Hemmings, 2009). Receptor tyrosine kinase stimulation activates AKT via a tightly controlled multi-step process (Fig. 1). Activated receptors stimulate class 1A PI3K directly or via adapter molecules such as the insulin receptor substrate (IRS) proteins. Class 1A PI3Ks bind via one of their five regulatory subunits (p85α, p85β, p55α, p55γ or p50α), which in turn binds to one of three catalytic subunits [p110α, p110β or p110δ (in leukocytes)], allowing conversion of phosphatidylinositol (3,4)-bisphosphate [PtdIns(3,4)P_2] lipids to phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] at the plasma membrane. AKT binds to PtdIns(3,4,5)P_3 at the plasma membrane, where 3-phosphoinositide-dependent protein kinase 1 (PDK1) can then access the 'activation loop' of AKT to phosphorylate threonine 308 (Thr308), leading to partial AKT activation (Alessi et al., 1997). This AKT modification is sufficient to activate mTORC1 by directly phosphorylating and inactivating proline-rich AKT substrate of 40 kDa (PRAS40) and tuberous sclerosis protein 2 (TSC2). These phosphorylation events release the kinase mammalian target of rapamycin (mTOR) that is bound to PRAS40, prevent TSC2 GTPase activity and allow active, GTP-bound Rheb to activate mTORC1. mTORC1 substrates include the eukaryotic translation initiation factor, 4E, binding protein 1 (4EBP1) and ribosomal protein S6 kinase, 70 kDa, polypeptide 1 (S6K1), which in turn phosphorylates the ribosomal protein S6 (S6; also known as RPS6), promoting protein synthesis and cellular proliferation.

Phosphorylation of AKT at Ser473 in the C-terminal hydrophobic motif, either by mTOR associated with mTOR complex 2 (mTORC2) (Sarbassov et al., 2005) or by DNA-dependent protein kinase (DNA-

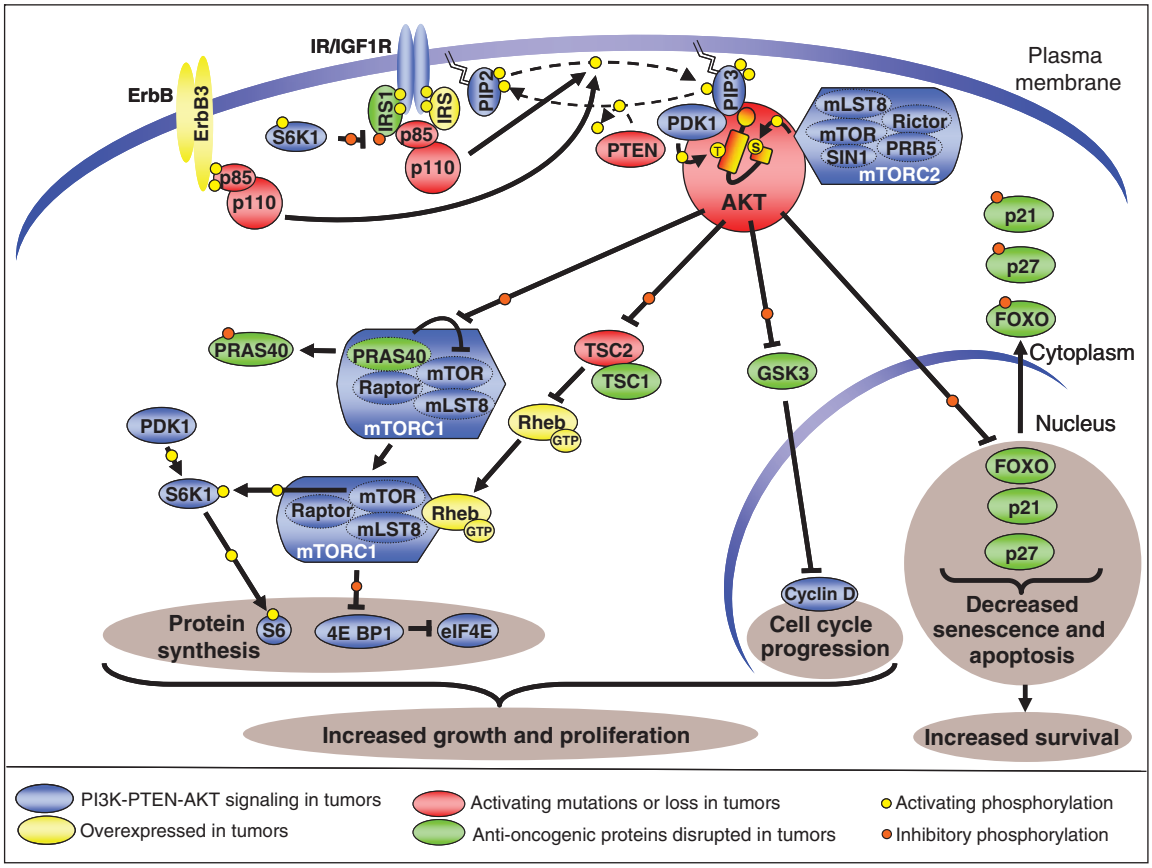

Fig. 1. The PI3K-PTEN-AKT signaling pathway and the causes of its hyperactivation in tumorigenesis. Increased AKT activation can occur through overexpression of pathway components (yellow) or inhibitory mutation or complete loss of components (red). These events can lead to decreased activation of anti-oncogenic proteins (green) and increased growth, proliferation and survival signals to promote tumorigenesis. eIF4E, eukaryotic translation initiation factor 4E; LST8, target of rapamycin complex subunit LST8; PRR5, proline-rich protein 5; SIN1, SAPK-interacting protein 1; PIP2, PtdIns(3,4)P_2; PIP3, PtdIns(3,4,5)P_3.

PK) (Feng et al., 2004) stimulates full AKT activity. Full activation of AKT leads to additional substrate-specific phosphorylation events, including inhibitory phosphorylation of the proapoptotic FOXO proteins. Dephosphorylation of Ser473 by the PH-domain leucine-rich repeat-containing protein phosphatases PHLPP1 and PHLPP2, and the conversion of PtdIns(3,4,5)P_3 to PtdIns(3,4)P_2 by PTEN, inhibits AKT signaling.

Human tumors commonly display amplification or overexpression of cell-surface receptors or signaling molecules that activate the PI3K-PTEN-AKT pathway, activating mutations of PI3K, loss of expression of the negative regulator PTEN and/or mutation of AKT (Fig. 1). These mutations account for findings that the AKT pathway is activated in a high proportion of tumors, in a wide variety of tissues; a selection of these findings are summarized in Table 1.

From man to mouse: elucidating oncogenic AKT signaling in mice

Mouse models are invaluable tools for understanding how mutations in PI3K-PTEN-AKT signaling contribute to tumorigenesis in human cancer. In humans, mild mutations in PTEN, TSC1 or TSC2 result in familial tumor-susceptibility syndromes, and a similar neoplasia is seen when the mild mutations are modeled in mice. By contrast, human biopsies of spontaneous tumors that display PTEN, TSC1 or TSC2 loss have increased AKT signaling compared with biopsies of tumors from patients with familial syndromes. This increased AKT signaling and the corresponding more severe tumor development are reflected in mouse models that have heterozygous and homozygous loss of PTEN, TSC1 or TSC2. These studies highlight the contribution that mouse models of AKT activation can make in elucidating oncogenic AKT signaling in familial and spontaneous neoplasia.

Human tumor-susceptibility syndromes and neoplasia phenotypes in mice

In humans, mutations in PTEN (which is upstream of AKT), or in TSC1 or TSC2 (which are downstream of AKT), result in complex disease syndromes such as Cowden disease or tuberous sclerosis (Table 2). These diseases display a variety of symptoms (for reviews, see Eng, 2003; Zhou et al., 2003; Crino et al., 2006), because various point mutations or partial deletions in these genes cause diverse effects on the levels of functional protein, thereby affecting AKT-related signaling (Zhou et al., 2003; Trotman et al., 2007). Interestingly, *PTEN*$^{+/-}$, *TSC1*$^{+/-}$ or *TSC2*$^{+/-}$ mice do not show the same spectrum of symptoms as patients with these syndromes, which might reflect the fact that mutated forms of these proteins

Table 1. Common upstream AKT-activating mutations and somatic AKT mutations found in tumors

Gene	Mutation	Affected tissue	Incidence (%) (samples)	References
ErbB2	Point insertions	Breast	4 (4/94)	Stephens et al., 2004; Lee et al., 2006; Forbes et al., 2010
		Lung	4 (5/120)	
		Stomach	5 (9/180)	
		Colorectal	3 (3/104)	
ErbB2	Amplification	Breast	18-40 (19/103, 110/245, 34/86)	Slamon et al., 1987; Slamon et al., 1989; Reichelt et al., 2007; Marx et al., 2009
		Ovary	26 (31/120)	
		Stomach	16 (27/166)	
		Oesophogeal	5-15 (7/145, 16/110)	
IRS2	Amplification	Colon	2 (3/146)	Knobbe and Reifenberger, 2003; Parsons et al., 2005
		Brain	2 (2/103)	
p85 (PI3K)	Deletions	Ovary	4 (3/80)	Philp et al., 2001; Mizoguchi et al., 2004; Parsons et al., 2008
		Colon	2 (1/60)	
		Brain	3-10 (1/30, 9/91)	
p110α (PI3K)	Various (especially point mutants E542K, E545K and H1047R)	Colon	19-32 (6/32, 74/199)	Bachman et al., 2004; Campbell et al., 2004; Samuels et al., 2004; Hartmann et al., 2005; Lee et al., 2005; Levine et al., 2005; Oda et al., 2005; Buttitta et al., 2006; Gallia et al., 2006; Velasco et al., 2006; Parsons et al., 2008
		Brain	7-27 (5/70, 10/105, 11/73, 4/15)	
		Stomach	4-25 (4/94, 12/185, 3/12)	
		Breast	18-40 (13/53, 13/72, 19/92, 25/93, 28/70)	
		Liver	36 (26/73)	
		Lung	4 (1/24)	
		Ovary	6-12 (11/167, 24/198)	
		Uterus	36 (24/66)	
p110α (PI3K)	Amplification	Lung	33 (46/139)	Shayesteh et al., 1999; Campbell et al., 2004; Wu et al., 2005; Yamamoto et al., 2008
		Ovary	25-58 (83/341, 7/12)	
		Breast	9 (9/92)	
K-Ras	Point mutant (especially G12D)	Pancreas	75-95 (5/6, 12/16, 28/30, 21/22)	Almoguera et al., 1988; Smit et al., 1988; Suzuki et al., 1990; Burmer et al., 1991; Boughdady et al., 1992; Lemoine et al., 1992; Rodenhuis and Slebos, 1992
		Colon	30-60 (10/29, 14/40, 37/61)	
		Lung	15-25 (22/129, 43/181)	
PTEN	Promoter methylation	Brain	35-37 (22/60, 27/77)	Salvesen et al., 2001; Baeza et al., 2003; Garcia et al., 2004; Khan et al., 2004; Wiencke et al., 2007
		Breast	34-48 (15/44, 43/90)	
		Uterus	19 (26/138)	
PTEN	Deletions, point mutants, LOH	Most tissues:		Rasheed et al., 1997; Tashiro et al., 1997; Feilotter et al., 1998; Zhou et al., 1999; Kondo et al., 2001; Forbes et al., 2010
		Brain	16-31 (14/91, 13/42)	
		Prostate	49 (25/51)	
		Uterus	50 (16/32)	
		Colon	25 (14/57)	
PDK1	D527E	Colon	<1 (1/204)	Parsons et al., 2005
	T354M	Colon	1 (2/204)	
AKT1	E17K	Breast	4-8 (4/93, 5/61)	Carpten et al., 2007; Davies et al., 2008; Kim et al., 2008; Malanga et al., 2008; Shoji et al., 2009
		Colorectal	6 (3/51)	
		Ovary	2 (1/50)	
		Endometrium	2 (2/89)	
		Skin	<1 (1/137)	
		Lung	6 (2/36)	
AKT1	Amplification	Stomach	20 (1/5)	Staal, 1987; Knobbe and Reifenberger, 2003
		Brain	1 (1/103)	
AKT2	S302G	Colon	<1 (1/204)	Parsons et al., 2005; Soung et al., 2006
	R371H	Colon	<1 (1/204)	
	A377V	Lung	1 (1/79)	
AKT2	Amplification	Colon	1 (2/146)	Cheng et al., 1992; Bellacosa et al., 1995; Ruggeri et al., 1998; Snijders et al., 2003; Parsons et al., 2005; Pedrero et al., 2005; Nakayama et al., 2006; Nakayama et al., 2007; Yu et al., 2009
		Breast	3 (3/106)	
		Ovary	12-18 (16/132, 12/66)	
		Head and neck	30 (12/40)	
		Pancreas	20 (7/35)	
AKT3	E17K	Skin	2 (2/137)	Davies et al., 2008
AKT3	G171R	Brain	11 (1/9)	Hunter et al., 2006
AKT3	Amplification	Brain	4-14 (4/230, 29/206)	Hashimoto et al., 2004; CGARN, 2008; Ichimura et al., 2008
		Liver	30 (6/19)	

PDK1, AKT2 and the AKT3 G171R somatic point mutants were detected in tumor samples and are hypothesized to promote activation due to the mutations occurring in kinase domains; however, their activating potential has yet to be characterized. Genes are listed in the order that their encoded proteins act in the PI3K-PTEN-AKT signaling pathway (from receptor activation to AKT activity). Studies first reporting the indicated mutations, and those with large datasets, are referenced. LOH, loss of heterozygosity.

in the human syndrome can affect regulation of the AKT pathway even without the large decreases in protein levels that are present in the heterozygous mouse models. However, increased neoplasia formation in multiple organs is a feature common to both the human syndromes and mice with the corresponding gene disruptions (Table 2). This suggests that a conserved mechanism underlying the neoplasia phenotype is increased AKT signaling.

Increases in AKT signaling correlate with both the severity of neoplasia and PTEN, TSC1 or TSC2 dysregulation both in neoplasms derived from the human familial syndromes and in the corresponding mouse models, as well as in spontaneous tumor formation. Cowden disease patients with mutations that decrease PTEN levels have a corresponding increase in AKT activity and exhibit increased formation of gastrointestinal polyps (Trotman et

Table 2. Phenotypes of mouse models representing common human familial tumor syndromes

Mutated protein	Human syndrome	Human presentation of syndrome	Mouse phenotype upon deletion of associated gene
PTEN	Cowden disease, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, Proteus-like syndrome	Breast, thyroid and uterine neoplasia, lipomas, macrocephaly, hamartomatous polyps of the gastrointestinal tract, mucocutaneous lesions	Homozygous lethal; conditional deletion in tissues generally results in tumors; heterozygotes develop a range of neoplasms (adrenal, thyroid, uterine, breast, prostate, gastrointestinal tract)
TSC1 or TSC2	Tuberous sclerosis	Hamartomata and cysts in multiple organ systems, polycystic renal disease, renal carcinoma	Homozygous lethal; heterozygotes develop renal cystadenomas, liver hemangiomas, lung adenomas

All data taken from Online Mendelian Inheritance in Man (OMIM), McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2009 (http://www.ncbi.nlm.nih.gov/omim/ and http://www.informatics.jax.org/).

al., 2007). In mice, mutations that affect the regulation of PTEN or TSC2 display abnormal activation of AKT signaling and develop a neoplasia phenotype that is reminiscent of the human syndromes (Pollizzi et al., 2009; Alimonti et al., 2010b; Wang et al., 2010), which is milder than that observed in *PTEN*$^{+/-}$ or *TSC1/2*$^{+/-}$ mice. In the case of spontaneous tumor formation, it is homozygous loss of PTEN or the TSC proteins that is seen in tumor progression. These observations suggest that mouse models in which AKT signaling is activated are relevant to both familial and spontaneous neoplasia formation in humans.

Modeling human tumors with activating PI3K-PTEN-AKT pathway mutations

Mutations in components of the PI3K-PTEN-AKT pathway in human tumors (Table 1) lead to the development of tumors that have activated AKT and increased downstream oncogenic signaling. Accordingly, several mouse models have shown that AKT activation is crucial for tumorigenesis. These models demonstrate that, in various tissues, tumor phenotypes are induced by AKT activation and can be reversed by preventing AKT activation through its simultaneous deletion (Table 3).

These models have assisted in elucidating the contribution of AKT signaling in specific tumor tissue settings, as was the case in mammary-specific *ErbB2*-overexpressing mouse models that represent the common *ErbB2* amplification found in human breast tumors. However, owing to the selective activation of AKT, three mouse models of AKT activation – conditional *PTEN*-null, *PTEN*$^{+/-}$ and transgenic mice conditionally expressing a myristoylated form of AKT (myr-AKT) – are the models of choice for studying the contribution of specific AKT signaling to tumorigenesis. myr-AKT expression results in the translocation of constitutively active AKT to the plasma membrane, inducing neoplasia (Staal, 1987). Importantly, although myr-AKT models drive neoplasia development via a non-physiological, modified form of AKT, neoplasia development in these mice mimics the phenotype and AKT activation pattern seen in mice with heterozygous *PTEN* loss, an event that is common in many human tumors (Majumder et al., 2008; Gray et al., 1995). Conditional ablation of *PTEN* results in a more aggressive phenotype, consistent with the observation that homozygous *PTEN* loss is a late event in many human cancers and therefore making it an attractive model for testing therapies for the most refractory tumors (Trotman et al., 2003; Wang et al., 2003; Komiya et al., 1996). *PTEN* loss promotes activation of PDK1, thereby potentially activating multiple signaling pathways via the phosphorylation of over 20 protein kinases, including AKT (Mora et al., 2004). However, *AKT1* ablation on a *PTEN*$^{+/-}$ background inhibits neoplasia formation, indicating that AKT1 and not alternative PDK1 signaling is responsible for neoplasia (Chen et al., 2006). Furthermore, *PTEN*$^{+/-}$ mice with hypomorphic *PDK1* alleles that cause 80-90% reduction in PDK1 expression show reduced tumor formation that is proportional to the loss of PDK1-mediated phosphorylation, which is required for AKT activation (Bayascas et al., 2005). Therefore, neoplasia development correlates with the upregulation of AKT activity in *PTEN* and myr-AKT models, making them particularly useful for determining how alterations in AKT signaling can affect neoplasia development.

Prostate cancer: an example of the involvement of AKT in tumorigenesis

In humans, premalignant proliferation of the epithelium in the prostate gland is commonly referred to as prostatic intraepithelial neoplasia (PIN) and is considered a precursor lesion to CaP. PIN displays decreasing PTEN expression with progression to CaP, and PTEN expression is completely lost in late-stage advanced CaP (McMenamin et al., 1999; Schmitz et al., 2007). PTEN loss correlates with AKT activation and tumor grade, indicating that PTEN contributes to prostate tumorigenesis via loss of its function as a negative regulator of AKT activation (Malik et al., 2002). In mouse models, PTEN loss or AKT activation in the prostate induces PIN and progression to CaP, and increases in the level of phosphorylation of AKT Ser473 parallel the reduction in PTEN levels and correspond with increased incidence, onset and progression to CaP (Di Cristofano et al., 2001; Kwabi-Addo et al., 2001; Trotman et al., 2003; Wang et al., 2003). Mice lacking PTEN expression in the prostate display features that resemble advanced CaP in humans, including local invasion, metastasis and androgen independence. Therefore, in humans and mice, similar molecular pathology seems to underpin CaP development.

The similar pathological features of CaP development in mice and humans, and the importance of AKT in the process, make this an excellent setting in which to dissect how AKT signaling supports tumorigenesis and to determine how it could be therapeutically inhibited to treat cancer. Accordingly, the following sections focus on recent advances in mouse models of CaP that have defined fundamental concepts on how AKT signaling contributes to neoplasia, progression and acquisition of malignancy in CaP (summarized in Fig. 2).

Neoplasia is initiated by AKT signaling to mTORC1

One of the earliest events in human CaP is loss of expression of NK3 transcription factor related, locus 1 (Nkx3.1), which leads to aberrant gene expression (Bethel et al., 2006). This is seen from early PIN, when increased cellular proliferation and moderate activation of AKT is observed (Renner et al., 2007). A connection between

Table 3. Defining mouse models of AKT activation and signaling in tumorigenesis

Gene					
Primary mutation	Secondary mutation	Type of mutation	Effect	Phenotype	References
ErbB2	–	Tg-MG	O/E	Mammary tumors	Muller et al., 1988
	PTEN	cKO-MG	Loss	Acceleration of tumors	Dourdin et al., 2008
	Myr-AKT1	Tg-MG	O/E	Acceleration of tumors	Young et al., 2008
	AKT1	KO	Loss	Inhibition of tumors and of metastasis	Ju et al., 2007; Maroulakou et al., 2007
	AKT2	KO	Loss	Acceleration of tumors	Maroulakou et al., 2007
	AKT3	KO	Loss	No observable effect on tumorigenesis	Maroulakou et al., 2007
PolyMidT	–	Tg-MG	O/E	Mammary tumors	Guy et al., 1992
	IRS1	KO	Loss	Mammary tumors and metastasis	Ma et al., 2006
	IRS2	KO	Loss	Decreased number of mammary tumors	Nagle et al., 2004
	AKT1	KO	Loss	Inhibition of tumors	Maroulakou et al., 2007
	AKT2	KO	Loss	Acceleration of tumors	Maroulakou et al., 2007
	AKT3	KO	Loss	No observable effect on tumorigenesis	Maroulakou et al., 2007
IRS1	–	KO	Loss	Insulin resistance, reduced growth	Araki et al., 1994; Tamemoto et al., 1994
	–	Tg-MG	O/E	Mammary tumors and metastasis	Dearth et al., 2006
IRS2	–	KO	Loss	Diabetes	Withers et al., 1998
	–	Tg-MG	O/E	Mammary tumors and metastasis	Dearth et al., 2006
K-ras^{G12D}	–	KI-PtMt	G12D	Lung tumors	Johnson et al., 2001
	p85α$^{T208D/K227A}$	KI-PtMt	T208D and K227A	Resistant to Ras binding and Ras-induced lung tumorigenesis	Gupta et al., 2007
Myr-p110α	–	Tg-Pr	O/E	Hyperplasia	Renner et al., 2007
p85α	–	cKO-Pr	Loss	No observable tumor phenotype	Jia et al., 2008
p85β	–	cKO-Pr	Loss	No observable tumor phenotype	Jia et al., 2008
PTEN	–	Tg-Hy	Hy/+	Neoplasia after long latency	Alimonti et al., 2010b
	–	Tg-Hy	Hy/–	Increased neoplasia, decreased latency	Trotman et al., 2003
	–	KO	Ht	MG, adrenal, thyroid, colon, B-cell, uterine, prostate neoplasia	Di Cristofano et al., 1998; Suzuki et al., 1998; Podsypanina et al., 1999
	IRS2	KO	Loss	Decreased number of tumors in multiple tissues	Szabolcs et al., 2009
	p85α$^{+/-}$	KO	Ht	Increased number of GI polyps, PIN unaffected	Luo et al., 2005
	p85β$^{-/-}$	KO	Loss	Decreased PIN	Luo et al., 2005
	p85α$^{+/-}$β$^{-/-}$	KO	Ht/Loss	Increased number of GI polyps, PIN unaffected	Luo et al., 2005
	PDK1	KO	Hy/–	Inhibition of PTEN-driven tumors	Bayascas et al., 2005
	AKT1	KO	Loss	Inhibition of PTEN-driven tumors	Chen et al., 2006
	–	cKO-Sk	Loss	Susceptibility to carcinogens	Inoue-Narita et al., 2008
	B-Raf^{V600E}	Tg-Sk	O/E	Metastatic melanoma	Dankort et al., 2009
	–	cKO-Pr	Loss	Metastatic prostate tumors	Trotman et al., 2003; Wang et al., 2003
	P110α$^{-/-}$	cKO-Pr	Loss	No effect on PTEN tumorigenesis	Jia et al., 2008
	P110β$^{-/-}$	cKO-Pr	Loss	Loss of PTEN tumorigenesis	Jia et al., 2008
	mTOR	cKO-Pr	Loss	Inhibition of tumors	Nardella et al., 2009
	rictor	cKO-Pr	Loss	Inhibition of tumors	Guertin et al., 2009
AKT1	–	KO	Loss	Small, partial lethality	Chen et al., 2001; Cho et al., 2001a
Myr-AKT1	–	Tg-Pr	O/E	High-grade PIN; 100% penetrance	Majumder et al., 2003
	p27	cKO	Loss	Progression to cancer	Majumder et al., 2008
	–	Tg-Lv	O/E	Insulinomas	Alliouachene et al., 2008
	S6K1	KO	Loss	Inhibition of insulinomas	Segrelles et al., 2007
	–	Tg-Sk	O/E	Skin carcinomas, DMBA sensitive	Malstrom et al., 2001
	–	Tg-Tc	O/E	Thymic lymphoma with short latency	Rathmell et al., 2003
	–	Tg-MG	O/E	With DMBA: ER+ mammary tumors	Blanco-Aparicio et al., 2007
Myr-AKT$^{\Delta 11\text{-}60}$	–	Tg-Br	O/E	No tumor phenotype	Holland et al., 2000
	K-Ras^{G12D}	PtMt-Br	O/E	Glioblastoma	Holland et al., 2000
	B-Raf^{V600E}	PtMt-Br	O/E	Gliomas	Robinson et al., 2010
AKT1^{E40K}	–	Tg-Tc	E40K	Peripheral lymphoma with long latency	Malstrom et al., 2001
AKT2	–	KO	Loss	Diabetes	Cho et al., 2001b
Myr-AKT2	–	Tg-Tc	O/E	Thymic lymphoma after long latency	Mende et al., 2001
AKT3	–	KO	Loss	Small brain	Easton et al., 2005; Tschopp et al., 2005
DNAPK$_{cs}$	–	KO	Loss	Thymic lymphomas	Jhappan et al., 1997
	AKT1	KO	Loss	Inhibition of DNAPK$_{cs}$-driven thymic lymphomas	Surucu et al., 2008

Br, brain; cKO, conditional tissue deletion; DMBA, 7,12-dimethylbenz[a]anthracene; DNAPK$_{cs}$, DNA-dependent protein kinase catalytic subunit; ER+, estrogen receptor positive; GI, gastrointestinal; Ht, heterozygous loss of protein; Hy, hypomorphic gene modification; Hy/–, hypomorphic and deleted allele; Hy/+, hypomorphic and wild-type allele; KI, knock-in gene mutation; KO, whole body knockout; Loss, complete protein loss; Lv, liver; MG, mammary gland; O/E, protein overexpression; PolyMidT, polyoma middle T oncoprotein; Pr, prostate; PtMt, genetic point mutant; Sk, skin; Tc, T-cell; Tg, transgenic. Proteins are listed in the order that they act in the PI3K-PTEN-AKT signaling pathway (from receptor activation to AKT activity).

Nkx3.1 and AKT is illustrated by the fact that mice lacking Nkx3.1 expression display cellular proliferation and low-grade PIN, together with increased PI3K signaling to AKT (Abdulkadir et al., 2002; Gary et al., 2004; Song et al., 2009). The onset of PIN also correlates with phosphorylation of the mTORC1 target 4EBP1 (Kremer et al., 2006), indicating that AKT-mediated activation of mTORC1 is involved in this process. In addition, increased AKT activation in myr-AKT or *PTEN*$^{+/-}$ mice leads to the development of high-grade PIN (Di Cristofano et al., 1998; Majumder et al., 2003; Wang et al., 2003; Ratnacaram et al., 2008). Knocking out *AKT1* in *PTEN*$^{+/-}$ mice prevents PIN development, illustrating that this process depends on AKT1 signaling (Chen et al., 2006). Furthermore, AKT signaling to mTORC1 is crucial for PIN development, because inhibition of mTORC1 signaling with a specific inhibitor, RAD001, in myr-AKT1 mice abolished mTOR signaling and cellular proliferation, and restored normal prostatic gland architecture (Majumder et al., 2004).

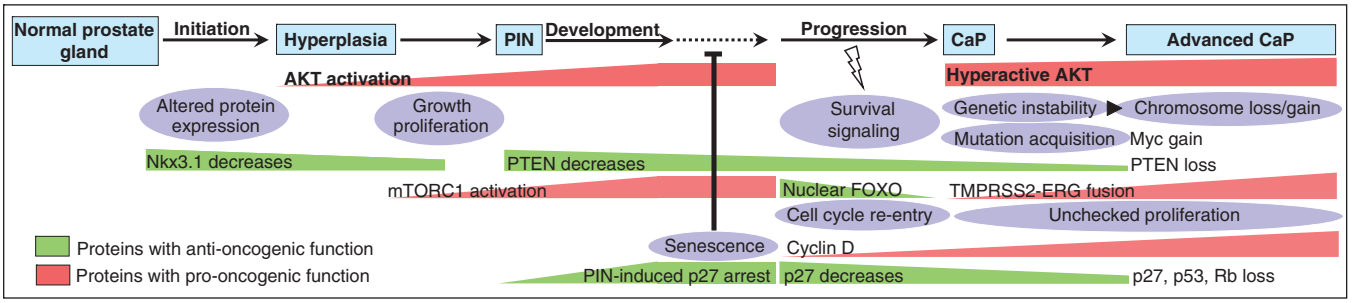

Fig. 2. AKT activation and associated events during tumor development in the prostate. Initiation of tumorigenesis and hyperplasia occur through altered protein expression, which promotes AKT activation, mTORC1 activation and PIN development. p27-induced senescence prevents progression to CaP, which is overcome by AKT signaling combined with changes in the expression and/or activity of other proteins and genes. CaP displays high AKT activation, supporting proliferation, survival and acquisition of mutations with increasing genetic instability, leading to the gross chromosomal losses and gains that are characteristic of advanced malignant CaP.

The role of mTORC1 in proliferation and PIN development is further highlighted by mouse models in which TSC2 (Ma et al., 2005) and Rheb (Nardella et al., 2008) expression is manipulated. Mouse prostates overexpressing Rheb promote activation of mTORC1 and S6K1, and the consequent phosphorylation of their respective targets, 4EBP1 and S6. Prostates in these mice display mild increases in proliferation and low-grade PIN, albeit with long latency (~10 months) and low penetrance (20-30%). Conversely, in *TSC2*$^{+/-}$ mouse prostates, mTOR phosphorylation is insufficient to trigger downstream signaling and phosphorylation of S6. In this case, neither increased proliferation nor PIN development is observed. These studies complement the RAD001 findings, indicating that the activation of mTORC1 and downstream signaling is necessary and sufficient to induce cellular proliferation and initiate PIN. Importantly, PIN develops in the Rheb-overexpressing prostates in the presence of low AKT activation and signaling, owing to a negative feedback loop inhibiting PI3K via S6K1 and IRS1 (Nardella et al., 2008). Thus, independent of other AKT-mediated signaling, activation of mTORC1 signaling seems to be the essential component of PIN development in prostates exhibiting activated AKT.

Senescence responses prevent progression from PIN to CaP

Prostates expressing myr-AKT1 or Rheb express the senescence markers senescence-associated β-galactosidase (SA-βgal) (Majumder et al., 2008; Nardella et al., 2008) and heterochromatin protein 1 (HP1) (Majumder et al., 2008), and exhibit increasing nuclear localization of the cell-cycle inhibitor p27, during PIN development. Cellular growth arrest and reduced incorporation of BrdU (a reagent used to track proliferating cells), indicate a functional and effective senescence checkpoint in affected PIN epithelium (Majumder et al., 2008). Importantly, SA-βgal (Chen et al., 2005; Majumder et al., 2008), HP1 (Majumder et al., 2008) and p27 nuclear accumulation (Di Cristofano et al., 2001; Majumder et al., 2008) are also found in human PIN samples. p27 accumulation is also observed during PIN in *PTEN*$^{+/-}$ mice and in an unrelated mouse model of CaP in which the *Myc* oncogene is expressed in the prostate, suggesting that senescence is a specific response to PIN induction and not to AKT activation or signaling (Majumder et al., 2008).

The relationships between senescence induction, PIN development and mTORC1 activation are illustrated by inhibition of mTORC1 with RAD001 in myr-AKT1 mice. RAD001 does not affect the levels of AKT Ser473 phosphorylation, but does decrease the phosphorylation of the downstream mTORC1 target S6 within 2 days of treatment. However, reduction of p27 nuclear accumulation and expression of HP1 was not observed until after 14 days of treatment, when normal prostatic gland architecture was restored (Majumder et al., 2004). Therefore, senescence is a response to loss of normal prostatic gland architecture rather than to increased mTORC1 signaling, which favors proliferation.

Prostatic glands displaying PIN and senescence have disrupted basement membrane (BM) attachments. E-cadherin mediates crucial attachment to the BM and is reduced in human CaP (Umbas et al., 1992). Knockdown of E-cadherin expression, or culturing isolated myr-AKT mouse prostate epithelial cells or myr-AKT-transfected human prostate epithelial cells in low adherence conditions, compromises BM contacts and induces p27 nuclear accumulation (Majumder et al., 2008). Thus, the loss of BM attachment observed in PIN morphology induces p27-mediated senescence that prevents progression from PIN to CaP in *PTEN*$^{+/-}$ and myr-AKT models.

Overcoming p27-mediated cell-cycle arrest

Loss of p27 expression and cell-cycle dysregulation might be mechanisms by which activated AKT signaling overcomes p27-mediated senescence in the prostate and induce CaP. In human CaP, increasing loss of p27 (Cordon-Cardo et al., 1998; Fernandez et al., 1999; Di Cristofano et al., 2001; Majumder et al., 2008) or activation of the protein that degrades p27, Skp2, is often observed (Yang et al., 2002). In myr-AKT1 or *PTEN*$^{+/-}$ mouse prostates, a gene-dose effect on development of CaP is seen with *p27* loss, with CaP cells exhibiting decreased senescence markers and reactivation of cell cycling (Di Cristofano et al., 2001; Majumder et al., 2008). Reduction of p27 levels is seen when Skp2 is overexpressed in mouse prostate, with low-grade PIN to low-grade CaP lesions developing relative to the levels of Skp2 expressed (Shim et al., 2003). Conversely, loss of Skp2 on a *PTEN*-null background triggers senescence with increased expression of p27 and the other cell-cycle inhibitors p21 and p19^{Arf} (Lin et al., 2010). p27 can inhibit cell cycling by binding to cyclin D, a function also executed by the

cell-cycle inhibitor p18. Similarly to p27, decreased p18 in conjunction with PTEN heterozygosity accelerates the progression to high-grade PIN, whereas complete loss of p18 expression leads to invasive carcinoma that exhibits increased AKT phosphorylation (Bai et al., 2006). Thus, activated AKT can overcome p27-mediated senescence when combined with cellular changes that affect either p27 expression levels or cell-cycle activation.

Alternative signaling inputs can overcome p27-mediated senescence by affecting the interplay between AKT activation, p27 levels, glandular architecture and cell-cycle control. TSC2 inhibits mTORC1 and Wnt signaling via Rheb and β-catenin, respectively. Promoting mTORC1 signaling alone by crossing *PTEN*$^{+/-}$ with Rheb-overexpressing mice results in high-grade PIN with 100% penetrance (Nardella et al., 2008). However, if *PTEN*$^{+/-}$ mice lose a single allele of *TSC2*, PIN develops, similar to when Rheb is overexpressed in *PTEN*$^{+/-}$ mice, but in 75% of mice it progresses to CaP (Ma et al., 2005). This indicates that CaP development can occur via dysregulation of TSC2-mediated control of Wnt signaling. In mice and humans, *TSC2* loss stabilizes β-catenin and increases transcription of the cyclin D gene to promote cell-cycle progression (Mak et al., 2005). However, *TSC2* loss can also affect β-catenin–E-cadherin complexes to impair BM–E-cadherin signaling; this signaling is crucial for prostatic p27-mediated senescence. Indeed, nuclear β-catenin accumulation and decreased E-cadherin is observed in human CaP (Jaggi et al., 2005), and expression of dominant-stabilized nuclear β-catenin in the prostate results in CaP via increased Wnt signaling and disruption of cell contacts (Pearson et al., 2009). Therefore, signaling pathways such as Wnt might promote cell cycling or disrupt senescence signaling to p27 to enable neoplastic cells with activated AKT to progress to human CaP.

AKT antiapoptotic and survival signaling in the progression to CaP

Full activation of AKT occurs via mTORC2-mediated phosphorylation of Ser473, which promotes cell survival by inhibiting the activity of proapoptotic proteins such as the FOXO proteins. Mouse embryonic fibroblasts lacking components of mTORC2 lack AKT Ser473 phosphorylation but exhibit phosphorylation of Thr308 (Guertin et al., 2006; Jacinto et al., 2006; Shiota et al., 2006). mTORC1 activity is unaffected by mTORC2 disruption, but phosphorylation of FOXO1 and FOXO3a are reduced, increasing apoptosis in conditions of stress. Apoptosis is reversed by reconstitution of mTORC2 (Shiota et al., 2006). The nuclear proapoptotic activity of FOXO proteins is inhibited by AKT-mediated phosphorylation, which sequesters them in the cytoplasm. In mice displaying ~60% loss of PTEN expression, cytoplasmic localization of FOXO proteins is observed. Approximately 20% of these animals form CaP lesions with increased Ser473 signaling and decreased p27, but surprisingly with no significant increase in mTORC1 activation compared with *PTEN*$^{+/-}$ PIN lesions (Trotman et al., 2003). Further increases in Ser473 AKT phosphorylation resulting from a complete loss of PTEN expression correlate with additional decreases in p27 expression and nuclear FOXO1 levels without affecting the activation of mTORC1, indicating that these actions on p27 and FOXO1 occur independently of mTORC1 activation (Trotman et al., 2003). Consistent with this, knockout of the mTORC2 component rictor does not affect mTORC1 signaling but does abolish Ser473 phosphorylation, maintaining a strong nuclear accumulation of FOXO1 and preventing the progression to CaP, even in *PTEN*-null prostates (Guertin et al., 2009). This indicates that increased signaling to AKT substrates downstream of Ser473 phosphorylation promotes antiapoptosis and survival to overcome senescence and facilitate the progression of tumors.

Unchecked cell cycling and genetic instability promotes CaP malignancy

Unchecked cell cycling and increased survival signaling in tumor cells promotes the acquisition of mutations that cause genetic instability (GI) and gross genetic aberrations, such as rearrangements and chromosomal loss or gain. GI is detected in ~60% of prostate biopsies from patients with CaP (Thuret et al., 2005), whereas fusions of genes encoding transmembrane protease, serine 2 (TMPRSS2) and ETS-related gene (ERG) transcription factor (*TMPRSS2-ERG*) are seen in up to 65% of cases of human prostate neoplasia (Perner et al., 2006; King et al., 2009). In vitro and in vivo evidence supports the idea that *TMPRSS2-ERG* fusion is an event that occurs in early CaP that promotes malignancy by contributing to migration, invasion and metastasis (Tomlins et al., 2008; Carver et al., 2009; Yu et al., 2010). In mice, TMPRSS2-ERG or ERG overexpression promotes PIN, and the progression from PIN to CaP in both of these cases specifically involves AKT (Furusato et al., 2008; Klezovitch et al., 2008; Carver et al., 2009; King et al., 2009; Zong et al., 2009). In human samples, TMPRSS2-ERG is found in regions of copy-number loss (including of PTEN) (Taylor et al., 2010). Recent data from studies in mice suggest that progression to CaP when ERG is overexpressed and AKT is hyperactivated is supported by increased androgen receptor (AR) signaling (Goldstein et al., 2010). Interestingly, another study, which involved genomic profiling of 218 human prostate tumors, illustrated that, although AR abnormalities were exclusive to metastatic samples, increased signaling via the AR pathway was found in 56% of non-metastatic samples (Taylor et al., 2010).

PTEN heterozygosity is common in human CaP, with complete loss (via deletion of a region of chromosome 10q) occurring only in 30-60% of advanced CaP cases (Gray et al., 1995; Komiya et al., 1996). This suggests that progression to CaP via alternative mechanisms that cooperate with PTEN heterozygosity might be selected for by neoplastic cells. Analysis of *PTEN*-null prostates demonstrated that they exhibit a strong cellular senescence response mediated by p53, with an increase in the expression of cell-cycle inhibitors p19 and p21 (Chen et al., 2005). Maintaining p53 levels in *PTEN*-null prostates by administration of Nutlin-3, the small-molecule inhibitor of p53 degradation by the E3 ubiquitin-protein ligase Mdm2 (Mdm2), results in >50% reduction in tumor volume, with glands showing significantly increased senescence (Alimonti et al., 2010a). Hence, in human tumors in which the loss of a *PTEN* allele occurs in two distinct steps, overcoming a p27- and then p53-mediated senescence might impair tumor survival, suggesting that PTEN loss is observed only in advanced CaP, when additional mutations prevent an effective p53-mediated senescence response.

In advanced CaP, chromosomal regions are frequently lost [such as Ch17q (which encodes p53) and Ch13q (which encodes the tumor suppressor protein Rb)] (Carter et al., 1990; von Knobloch et al., 2004) or amplified [such as Ch8q (which encodes Myc)] (Jenkins et al., 1997; Qian et al., 2002). Similarly to PTEN loss, p53

deletion occurs late in human CaP, promoting malignancy (Qian et al., 2002). In mice, loss of p53 has little effect on the prostate, although *PTEN*-null prostates that mimic the loss of PTEN in late human CaP display invasion and metastasis, which are features of advanced CaP. However, when *p53* is knocked out in *PTEN*$^{-/-}$ prostates, aggressive and lethal CaP develops (Chen et al., 2005). Thus, complete loss of PTEN and p53 is consistent with the concept that, at late stages of CaP, malignancy is promoted by gross chromosomal abnormalities that arise from genetic instability.

From mice to man: targeting AKT in anticancer therapies

The mouse models discussed in the earlier sections illustrate that the alterations in PI3K-PTEN-AKT signaling that are associated with CaP progression in mice are similar to those seen in human biopsies. These studies highlight which members of the pathway might be valid therapeutic targets, and at which stage of the disease current or developing therapies would be most effective (see Table 4). In addition, the studies demonstrate that a crucial aspect of AKT-mediated tumor progression in CaP is the involvement of cooperating mutations (see Table 5), which should direct the development of new combinational therapeutic regimes. Importantly, AKT activation in the prostate affects conserved pro- and anti-oncogenic signaling, which is often disrupted in tumors of other tissues, suggesting that the findings in the prostate are applicable to tissues outside the prostate. The following section explores the potential of current and future strategies by which to control AKT signaling in tumors, including monotherapies and combination therapies.

Inhibiting AKT activation and signaling

PI3K inhibitors

The potential benefits of PI3K inhibition in treating cancer are supported by the finding that PI3K-activating mutations in p110α are common in human tumors, and that the inhibitors LY294002 and wortmannin, which primarily target PI3K, potently inhibit AKT activation in cancer cell lines. Toxicity of these early PI3K inhibitors prompted the development of new, more specific PI3K inhibitors (for reviews, see Brachmann et al., 2009; Maira et al., 2009), including isoform-specific inhibitors that were developed to prevent induction of insulin resistance while retaining anti-tumor efficacy. These might be particularly effective in tumors in non-insulin-sensitive tissues, because deletion of certain isoforms of the p85 or p110 subunits of PI3K has shown that these subunits operate in tumors in a tissue-specific manner (Luo et al., 2005; Jia et al., 2008). Indeed, in the prostate, p110β selectively mediates tumorigenic signaling (Jia et al., 2008). However, pan-PI3K inhibitors and dual PI3K and mTOR inhibitors block tumor growth in mouse models without overt effects on glucose levels (Folkes et al., 2008; Maira et al., 2008; Serra et al., 2008; Liu, T. J. et al., 2009). Interestingly, helical-domain mutations of p110α require Ras binding for AKT activation (Zhao and Vogt, 2010), and Ras binding to p110α is also required for Ras-mediated tumorigenesis (Gupta et al., 2007). Although Ras binding and signaling is unaffected by current ATP-competitive PI3K inhibitors, combination therapy with MEK inhibitors in mice shows strong synergy in inhibiting tumors (Engelman et al., 2008). Thus, PI3K inhibitors might yet prove effective in either single or combinational therapeutic regimes.

AKT inhibitors

Pan and isoform-specific inhibition of AKT isoforms are potential anti-tumor therapies, particularly in tumors that have lost PTEN expression. AKT1 is necessary for tumor progression in *PTEN*$^{+/-}$ mice in multiple organs, including the prostate (Chen et al., 2006), and the loss of AKT1 was found to reduce neoplasia without compensatory AKT2 or AKT3 upregulation. AKT1 is also a promising target because *PTEN*$^{+/-}$ neoplasia development in mice is significantly reduced when AKT1 levels are decreased by 50% (e.g. in heterozygous AKT1 deletions), a decrease in activity that is therapeutically more achievable than complete inhibition. Specific inhibitors of AKT2 or AKT3 could also be effective in the treatment of tumors such as melanomas (AKT3) (Stahl et al., 2004) or ovarian carcinomas (AKT2) (Cheng et al., 1992), in which these

Table 4. Patterns of PI3K-PTEN-AKT signaling in human and mouse CaP, and current therapies

Protein modification	Species	Stage of prostate neoplasia progression				Drug target	References
		BH	PIN	CaP	Metastasis		
PI3K activation	M	√	X	X	X	CT	Renner et al., 2007; Zhu et al., 2008; Brachmann et al., 2009; Maira et al., 2009
	H	X	X	√	√		
PTEN loss	M (+/–)	√	√	X	X	*	Di Cristofano et al., 2001; Kwabi-Addo et al., 2001; Trotman et al., 2003; Wang et al., 2003; Kremer et al., 2006; Ratnacaram et al., 2008
	H (+/–)	X	X/√	√	X/√		
	M (–/–)	√	√	√	√		
	H (–/–)	X	X	√	√		
AKT activation	M	√	√	X	X	CT	Malik et al., 2002; Majumder et al., 2003; Li et al., 2007; Renner et al., 2007
	H	√/X	√	√	√	In dev	
TSC2 loss	M	√	√	X	X	*	Ma et al., 2005
	H	nd	nd	nd	nd		
Rheb activation	M	√	√	X	X	*	Nardella et al., 2008
	H	nd	nd	nd	nd		
mTOR loss	M	X	X	X	X	Avail[a]	Kremer et al., 2006; Apsel et al., 2008; Maira et al., 2008; Guertin et al., 2009; Nardella et al., 2009; Thoreen et al., 2009
	H	X	X	X	X	CT	
mTOR activation	M	nd	nd	nd	nd	In dev	
	H	√/X	√	√	√		
4EBP1 activation	M	nd	nd	nd	nd	*	Kremer et al., 2006; Hsieh et al., 2010
	H	X	√	√	√		

[a]mTORC1 inhibitors (rapalogs). √, observed; X, not observed; *, not currently in development; Avail, approved for use; BH, benign hyperplasia; CT, in clinical trials; H, human samples; In dev, currently under development; M, mouse model; ND, not determined. Proteins are listed in the order that they act in the PI3K-PTEN-AKT signaling pathway (from receptor activation to AKT activity).

Table 5. Oncogenic events in human CaP that have been shown to cooperate with AKT activation in mice and are potential drug targets

Gene			Stage of prostate neoplasia progression					
Primary mutation	Secondary mutation	Species	BH	PIN	CaP	Metastasis	Drug target	References
Nkx3.1 loss	–	M	√	√	X	X	–	Abdulkadir et al., 2002; Kim et al., 2002; Gary et al., 2004; Bethel et al., 2006; Zong et al., 2009
	PTEN$^{+/-}$	M	√	√	√	X		
	–	H	√	√	√	√		
ERG gain	–	M	√	√	X	X	–	Petrovics et al., 2005; Rostad et al., 2007; Klezovitch et al., 2008; Carver et al., 2009
	PTEN$^{+/-}$	M	√	√	√	X		
	–	H	√	√	√	√		
PAR4 loss	–	M	√	X	X	X	–	Fernandez-Marcos et al., 2009
	PTEN$^{+/-}$	M	√	√	√	X		
	–	H	X	X	√	(X)		
FGF8b gain	–	M	√	√	X	X	–	Gnanapragasam et al., 2003; Zhong et al., 2006
	PTEN$^{+/-}$	M	√	√	√	√		
	–	H	X	X	√	√		
TRMSS2-ERG fusion	–	M	√	X	X	X	–	Tomlins et al., 2008; Carver et al., 2009; King et al., 2009
	PTEN$^{+/-}$	M	√	√	√	X		
	–	H	X	√/X	√	√		
p27 loss	–	M	√	X	X	X	CT	Cordon-Cardo et al., 1998; Di Cristofano et al., 2001; Majumder et al., 2008; Dickson and Schwartz, 2009
	PTEN$^{+/-}$	M	√	√	√	X	In dev*	
	–	H	X	X	√	√		
p18 loss	–	M	√	X	X	X	CT	Bai et al., 2006; Dickson and Schwartz, 2009
	PTEN$^{+/-}$	M	√	√	√	X	In dev*	
	–	H	X	X	√	√		
ErbB2 gain	–	M	√	√	X	X	Avail	Kuhn et al., 1993; Morote et al., 1999; Casimiro et al., 2007; Rodriguez et al., 2009
	PTEN$^{+/-}$	M	√	√	√	X		
	–	H	X	X	√	√		

*Cyclin dependant kinase inhibitors; CT, in clinical trials; H, human; M, mouse; (X), Not examined. See Table 4 footnote for abbreviations. Protein modifications are listed in order of reported occurrence in patient biopsies during the development from benign hyperplasia to advanced CaP.

isoforms are specifically increased. However, as with PI3K inhibitors, inhibition of AKT2 activity could promote insulin resistance. In such a case, pan-AKT inhibitors could circumvent off-target effects of AKT2 inhibition. In tumors with supraphysiological levels of AKT activation and a dependency on AKT for tumorigenesis, pan-AKT inhibitors could significantly reduce the levels of activated AKT within the tumor while minimizing adverse drug reactions, such as insulin resistance, in response to complete inhibition of a single AKT isoform in normal tissues. The pan-AKT inhibitor GSK690693 was shown to act in this manner in a mouse xenograph model and is now in clinical trials (Rhodes et al., 2008). These inhibitors could also enable broad-spectrum inhibition and allow targeting of tumors irrespective of the predominant AKT isoform involved.

mTOR inhibitors

The mTORC1 complex was first successfully inhibited by rapamycin, and related 'rapalogs' such as RAD001 that have more favorable pharmacokinetics and tolerance are used in various clinical settings. The ability of RAD001 to reverse PIN in the mouse prostate indicates that rapalogs can effectively target this process, although detection of such early abnormalities in patients is difficult. However, it is worth noting that cellular proliferation mediated by mTORC1 contributes to the development of pre-neoplastic lesions in over 60% of endometrial hyperplasia cases (Milam et al., 2008) and precancerous intestinal polyps from Peutz-Jeghers Syndrome patients (Shackelford et al., 2009). In mouse models of these pathologies, progression can be inhibited with mTORC1 inhibitors (Milam et al., 2007; Shackelford et al., 2009).

In advanced cancers, the rapalog RAD001 is approved as a single agent for renal cell carcinomas that depend on mTORC1-mediated translation of hypoxia-inducible factor 1 (HIF1). Tumor progression after resection has also been shown to be reduced in some gliomas treated with rapamycin in a phase 1 trial (Cloughesy et al., 2008). However, clinical studies suggest that the effectiveness of mTORC1 inhibition is exceptional: in most tumor settings, the anti-tumorigenic effects of mTORC1 inhibition are outweighed by increased AKT-Ser473-mediated pro-survival and antiapoptotic signaling that occurs because of loss of the negative feedback regulation of the PI3K pathway by S6K1 (Shi et al., 2005; O'Reilly et al., 2006; Cloughesy et al., 2008). Indeed, in Rheb-overexpressing mouse prostates, treatment with RAD001 showed loss of phosphorylated S6 (downstream of mTORC1) but increased AKT Ser473 phosphorylation. This suggests that therapeutic efficacy requires rapalogs used in combination with therapies that disrupt the feedback regulation of AKT Ser473 phosphorylation, such as PI3K or mTORC2 inhibitors.

mTORC2 inhibitors should prevent pro-survival and antiapoptotic functions. Indeed, loss of the mTORC2 component rictor prevents CaP in PTEN-deficient mouse prostates (Guertin et al., 2009). Inhibiting mTORC2 activity would be an effective way to target the wide variety of tumors that have high phosphorylation of Ser473, via PI3K activation or reduced activity of PHLPP1 or PHLPP2 (PHLPP1/2; the phosphatases responsible for dephosphorylation of AKT Ser473). In human colon cancer, expression of PHLPP1/2 is lost or reduced in ~75% of tumor samples (Liu, J. et al., 2009). In addition, a significant decrease in the levels of mRNA encoding FKBP51 (the protein that enables docking of PHLPP1/2 to AKT) was reported in pancreatic tumor tissue, and a decrease or loss of FKBP51 protein expression was found in pancreatic and breast cancer cell lines (Pei et al., 2009).

Dual mTORC1 and mTORC2 inhibitors that target the mTOR kinase (Apsel et al., 2008; Maira et al., 2008; Thoreen et al., 2009) are currently in clinical trials for their potential to inhibit tumor

proliferation and survival signals (www.clinicaltrial.gov/). In support of these agents as effective therapies, conditional ablation of *mTOR* in the *PTEN*-null mouse prostate blocks cellular proliferation and the development of PIN and CaP (Nardella et al., 2009). Use of mTOR inhibitors or specific mTORC2 inhibitors could prove particularly useful in settings of advanced cancer, such as CaP, in which PTEN expression has been lost, making AKT refractory to treatment by upstream receptor and PI3K inhibitors.

Combining inhibition of AKT signaling with additional therapeutics

ErbB2 inhibition

Effective inhibition of AKT signaling by ErbB2-specific monoclonal antibodies is the primary strategy in treating ErbB2-expressing breast tumors. Although such treatment can lead to tumor remission (Vogel et al., 2002), resistance occurs in ~50% of patients as a result of downstream mutations in genes encoding oncogenic proteins such as Ras and Src, or via loss of PTEN, leading to AKT activation (Berns et al., 2007). Loss of PTEN or an increase in activated AKT in ErbB2-overexpressing mouse mammary glands accelerates tumor formation in mice, whereas ablation of AKT1 or rapamycin-mediated inhibition of mTORC1 inhibits tumor progression (Mosley et al., 2007). In the prostate, ErbB2 can also cooperate with AKT activation in promoting CaP development (Rodriguez et al., 2009). This supports the use of ErbB2-specific antibodies together with rapalogs or AKT inhibitors to treat ErbB2-expressing tumors, including advanced CaP, in which ErbB2 is associated with androgen independence (Signoretti et al., 2000), increased tumor grade (Ross et al., 1993), anueploidy (Ross et al., 1993) and metastasis (Morote et al., 1999).

Raf, MEK and ERK inhibitors

Activation of the Ras-Raf-MEK-ERK signaling cascade by mutation or overexpression of extracellular receptors such as ErbB2 is common in many cancers. In advanced human CaP, mutations in Ras (7%) or Raf (10%) have been reported (Cho et al., 2006). In mouse models, ERK activation is associated with androgen independence, and simultaneous inhibition of ERK and AKT signaling has shown enhanced tumor inhibition (Gao et al., 2006; Kinkade et al., 2008). A relationship between mTORC1 inhibition and ERK activation has also been observed in the clinic when mTORC1 activity is inhibited with RAD001. In these cases, ERK activation occurs when mTORC1 is blocked (Carracedo et al., 2008), suggesting that blocking AKT signaling might result in compensatory rewiring of proliferation and survival signals through ERK. Positive outcomes after simultaneous ERK and PI3K signaling inhibition were observed in studies of mouse models of hepatocellular carcinoma (HCC) and ErbB2-overexpressing breast tumors; the use of MEK and mTORC1 inhibitors in HCC (Huynh, 2010), or a Raf inhibitor and blocking of PI3K signaling through neutralizing antibodies to ErbB2 in ErbB2-overexpressing breast tumors (Hausherr et al., 2006), improved the extent of tumor inhibition in both cases.

MEK-ERK activation also occurs as a result of the V600E B-Raf mutation in over 60% of pre-malignant melanocytic nevi, leading to increased ERK phosphorylation. Downstream signaling cascades inhibit TSC2 and increase cyclin D levels (Zheng et al., 2009), although melanoma progression is prevented by activation of an oncogene-induced senescence program. This senescence is relieved by increased AKT3 levels and signaling that cooperates with ERK to increase proliferative and survival signaling (Stahl et al., 2004; Cheung et al., 2008). Promotion to malignant melanoma occurs in up to 60% of cases, owing to loss of a portion of chromosome 10 that contains *PTEN* (Herbst et al., 1994; Robertson et al., 1998; Stahl et al., 2003). Highlighting the importance of AKT activation in melanoma, V600E B-Raf expression in *PTEN*-null mice leads to malignant melanoma formation (Dankort et al., 2009). Together, these findings suggest that simultaneous targeting of the ERK and AKT signaling pathways could be an effective way to treat tumors that commonly have Ras and Raf mutations.

Biguanides

Biguanides (AMPK activators) inhibit the activity of mitochondrial respiratory chain complex I, thereby reducing ATP levels and activating AMPK signaling. AMPK negatively regulates the cell cycle and prevents pro-oncogenic signaling by both Wnt and mTORC1 by activating TSC2 (Inoki et al., 2003) and inhibiting the Rag family of GTPases that are required for mTORC1 activation (Kalender et al., 2010). Decreased AMPK activity is observed in human breast cancer samples (Hadad et al., 2009). The AMPK activator metformin is well tolerated as an insulin-sensitizing agent and has also been shown to increase latency and reduce tumors in a mouse ErbB2 mammary model (Anisimov et al., 2005), possibly by inhibiting S6K1 activity and decreasing ErbB2 expression (Vazquez-Martin et al., 2009). Metformin also impairs the ability of p53-negative tumor cells to form tumors in mice (Buzzai et al., 2007) and, combined with doxorubicin, selectively kills cancer stem cells (Hirsch et al., 2009). However, in a mouse estrogen-receptor-negative mammary model of cancer, metformin promoted angiogenesis and supported tumors (Phoenix et al., 2009), suggesting that the drug might be effective only in conjunction with other chemotherapeutic agents. Studies of CaP support this: metformin significantly inhibits tumor growth, but does not induce apoptosis of prostate cancer cells when injected into mice, despite the fact that tumors from treated mice showed a cell cycle block with decreased cyclin D, activation of Rb and increased p27 levels (Ben Sahra et al., 2008). These features of metformin treatment could prove beneficial in conjunction with AKT inhibitors, because increased cyclin D levels (Rodriguez et al., 2009), inactivated Rb or decreasing p27 all cooperate with AKT activation in the mouse prostate to allow PIN progression to CaP. Thus, if metformin can hinder these cooperating alterations, it might prove effective in inhibiting tumor progression and induce apoptosis when used in conjunction with agents that inhibit AKT survival signaling.

Conclusions and future directions

The broad incidence of activating AKT mutations in tumors from diverse tissues indicates a crucial role for AKT signaling in tumor development and progression. In this Perspective, we have discussed recent work on mouse models that has helped to define how AKT signaling contributes to tumorigenesis at different stages, and through different downstream signaling pathways, to facilitate the proliferation, survival and progression of tumors. The findings from mouse models are consistent with analyses of patient tumors, providing validation that mouse experiments are relevant to the

human disease. Although we focused here on CaP, it is clear that the disruption of the PI3K-PTEN-AKT pathway, or of the mTOR complexes, is also associated with tumors in other tissues (Table 3). This suggests that at least the core members of this pathway contribute to oncogenic signaling – and particularly to proliferation – in the prostate, as well as in tumors of other tissues. This is promising from a therapeutic perspective because several of these pathway components are targets of anti-oncogenic therapies that have already been approved for the clinic. Therefore, these therapies might by broadly applicable for treating several different types of cancer.

An interesting finding in studies of CaP is that tumor progression generally requires cooperation of secondary mutations with activated AKT (Table 5). Many mutations that cooperate with AKT hyperactivation in the prostate – such as increased ErbB2 (and ERK activation), loss of p27 or loss of p18 – promote cell cycle progression and are also associated with tumors of a wide variety of tissues. This has important implications for treating tumors of tissues in which mutations in ErbB2 or cell cycle proteins are common because it suggests that the presence of even moderate AKT activation could have significant effects on progression. Inhibition of AKT-mediated survival and antiapoptotic signaling both alone and in conjunction with cell cycle inhibition might be a powerful regime for treating CaP and other tumors with cooperating mutations. Notably, some of these secondary mutations are the target of existing therapies. For example, tumor cells treated with the common anti-diabetic treatment metformin show inhibited cyclin D levels. The cell cycle is also controlled with cyclin-dependent kinase inhibitors that are in clinical trials, raising the possibility that their use together with inhibitors of AKT signaling could provide a well-tolerated therapeutic regime effective for treating a broad spectrum of tumor types. Alternatively, supporting the induction of senescence responses could also prove effective. Proof of this concept was recently shown by the finding that Skp2 inhibition promoted p27-, p21- and $p19^{Arf}$-mediated senescence in a preclinical study (Lin et al., 2010).

A number of areas still remain to be defined with respect to their contribution to AKT signaling in tumors. These include identifying mechanisms of PTEN regulation (Poliseno et al., 2010) and activities independent of its effects on AKT1 (Mounir et al., 2009) that could be compromised during tumorigenesis and therefore be valid therapeutic targets. Similarly, an understanding of how Ser473-specific PHLPP phosphatases are regulated is still to be fully explored. Investigation into both of these areas has the potential to identify novel targets involved in tumor progression.

Finally, with the ongoing progress on strategies to therapeutically inhibit AKT, the compensatory rewiring by tumors will become increasingly relevant in terms of resistance to future AKT signaling inhibitors. As discussed earlier, rewiring to activate ERK signaling is observed upon inhibition of mTORC1 (Carracedo et al., 2008). Similarly, 'PI3K-addicted' cells have been shown to be able to survive AKT inhibition by signaling through PDK1 (Vasudevan et al., 2009). Interestingly, CaP in *ErbB2;PTEN*$^{+/-}$ mice, and PIN in *LKB1*$^{+/-}$ mice, showed signaling downstream of mTORC1 without mTORC1 activation, suggesting alternative activation pathways (Pearson et al., 2008; Rodriguez et al., 2009). Interestingly, in both cases, activated PDK1 was observed and proposed to be mediating this signaling. Understanding whether PDK1 or other proteins can sustain tumors that have inhibited AKT activity, and via which downstream substrates and signaling pathways this can occur, are among the many issues that will be addressed in the next generation of PI3K-PTEN-AKT mouse tumor models.

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COMPETING INTERESTS

The authors declare no competing interests.

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