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THE PI3K-AKT-mTOR PATHWAY IN INITIATION AND PROGRESSION OF THYROID TUMORS

Motoyasu Saji, M.D., Ph.D[1] and **Matthew D. Ringel, M.D**[1,2,3]

[1] Division of Endocrinology, Diabetes and Metabolism, The Ohio State University Medical Center, The Ohio State University and The Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University, Columbus, Ohio, USA

[2] Division of Oncology, The Ohio State University Medical Center, The Ohio State University and The Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University, Columbus, Ohio, USA

[3] Department of Internal Medicine & Molecular Virology, Immunology and Genetics, The Ohio State University Medical Center, The Ohio State University and The Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, The Ohio State University, Columbus, Ohio, USA

Abstract

The Phosphoinositide 3 (OH) kinase (PI3K) signaling cascade is involved in regulating glucose uptake and metabolism, growth, motility, and other essential functions for cell survival. Unregulated activation of this pathway commonly occurs in cancer through a variety of mechanisms, including genetic mutations of kinases and regulatory proteins, epigenetic alterations that alter gene expression and translation, and posttranslational modifications. In thyroid cancer, constitutive activation of PI3K signaling has been shown to play a role in the genetic predisposition for thyroid neoplasia in Cowden's syndrome, and is recognized to be frequently overactivated in sporadic forms of thyroid cancer including those with aggressive clinical behaviors. In this review, the key signaling molecules in the PI3K signaling cascade, the abnormalities known to occur in thyroid cancer, and the potential for therapeutic targeting of PI3K pathway members will be discussed.

INTRODUCTION

Over the past decade, evidence implicating an important role for phosphoinositide-3 (OH) kinase (PI3K) – Akt signaling in the formation and progression of a wide variety of tumors, including thyroid cancer, has accumulated [reviewed in (Testa et al., 2005; Shinohara et al., 2007; Paes et al., 2008; Chalhoub et al., 2009; Chin et al., 2009)]. The mechanisms by which aberrant activation of PI3K signaling occur in cancer are diverse, but all lead to similar downstream signaling events. Constitutive activation of PI3K-regulated intracellular signals is particularly relevant in thyroid cancer, as evidenced by; 1) the inclusion of thyroid neoplasia as a major criterion in the diagnosis of Cowden's syndrome, a disorder caused by inactivating mutations in the tumor suppressor *PTEN* (Liaw et al., 1997), 2) the high frequency of activating mutations and gene rearrangements in upstream signaling molecules such as RAS (Lemoine

Address for Correspondence: Matthew D. Ringel, M.D., 445D McCampbell Hall, 1581 Dodd Drive, Columbus, OH 43210, Tel: 614-292-3800, Fax: 614-292-1550, matthew.ringel@osumc.edu.

et al., 1988; Suarez et al., 1988) and RET/PTC (Grieco et al., 1990), and 3) the recent identification of mutations in *PIK3CA* (Garcia-Rostan et al., 2005; Hou et al., 2007) and *AKT1* (Ricarte-Filho et al., 2009). Because PI3K signaling is frequently overactivated in cancer, there have been intense efforts to develop compounds that inhibit proteins in this cascade, including some that are approved for use in the United States; some of which are being studied in preclinical thyroid cancer systems. In this review, recent findings highlighting PI3K-Akt-mTOR signaling in thyroid cancer oncogenesis and progression will be summarized.

PI3K-Akt-mTOR SIGNALING

Phosphoinositide-3 (OH)Kinase (PI3K)

PI3Ks represent a family of kinases that phosphorylate the 3 hydroxyl group in phosphatidylinositol inositides (PtdIns). Class I PI3Ks are comprised of two subunits, a regulatory subunit (p85α, p85β, and p55γ) and a p110 catalytic subunit (p110α, β, γ, and δ) [reviewed in (Stokoe, 2005; Vanhaesebroeck et al., 2005; Engelman et al., 2006)]. The most highly expressed regulatory subunit is p85α and amongst the three catalytic subunits, and β are ubiquitously expressed, and γ is expressed only in leukocytes. Class II and III PI3Ks are different from the Class I PI3Ks in structure and in functional substrate specificity. Class I PI3Ks catalyze the production of phosphatidylinositol 3-phosphate (PtdIns-3,4-P), phosphatidylinositol (3,4)-bisphosphate (PtdIns-3,4-P2), and phosphatidylinositol (3,4,5)-triphosphate (PtdIns-3,4,5-P3). Class II PI3Ks are involved in the production of PtdIns-3-P and PtdIns-3,4-P2, and class III PI3Ks catalyze the production of PtdIns-3,4-P2 (Stokoe, 2005; Vanhaesebroeck et al., 2005; Engelman et al., 2006).

PI3Ks bind to- and are activated by many tyrosine kinase receptors (RTK) either through direct interactions or indirectly through adaptor molecules, such as insulin receptor substrates (IRS) (Figure 1). PI3Ks can also be activated by G-protein couple receptors (Murga et al., 2000). For Class I PI3Ks, once upstream signals are stimulated, the regulatory subunit detaches from the catalytic subunit leading to activation of the catalytic subunit and consequent increases in PtdIns production. PtdIns-3,4-P2 and PtdIns-3,4,5-P3 that are produced by PI3K bind to the pleckstrin homology (PH) domains of a number PH domain-containing proteins, including 3-phosphoinositide-dependent protein kinase-1 (PDK-1) (Filippa et al., 2000;Storz et al., 2002) and Akt isoforms, leading to their recruitment to the cytosolic membrane (Andjelkovic et al., 1996;Kohn et al., 1996;Bellacosa et al., 1998). Co-recruitment of these molecules to the membrane results in their interaction and leads to a broad cascade of signaling involving many downstream targets including p21-activating kinase-1 (PAK1), p90 ribosomal S6 kinase (RSK), serum and glucocorticoid-inducible kinase (SGK), p70 S6 kinase (S6K1), and protein kinase C (PKC) that lead to cell proliferation, glucose uptake, migration, resistance to apoptosis, and other downstream events [reviewed in (Cully et al., 2006;Blanco-Aparicio et al., 2007)].

PI3K mutation and amplification in thyroid cancer

Constitutively activating mutations in the genes encoding the Class I PI3K catalytic subunit (*PIK3CA*) have been described in cancers (Samuels et al., 2004). Most of these mutations are found in p110α in hot spot regions (E542K, E545K, and H1047R) located in helical and kinase domains of the protein; less commonly, mutations in other regions have been reported (Vogt et al., 2007). These mutations result in the expression of p110α variants that are active independent of the regulatory subunit and capable of inducing cell proliferation, invasiveness, resistance to apoptosis, and malignant transformation *in vitro* (Vogt et al., 2007). *PIK3CA* mutation-induced transformation has been shown to be dependent on several PI3K-regulated signaling pathways *in vitro and in vivo* (Gustin et al., 2009). Indeed, Akt activation appears to be required for some, but not all of the effects initiated by expression of mutant *PI3K3CA*, as

emphasized in a recent report demonstrating that PI3K promotes transformation through both Akt-dependent and Akt-independent pathways (Vasudevan et al., 2009). To date, only mutations of the gene encoding the PI3K α subunit have been reported. However, recent studies suggest that mutations of the β, γ, δ and, also can result in constitutive PI3K signaling and malignant transformation in chicken embryo fibroblasts (Denley et al., 2008). Thus, it is possible that mutations of other PI3K subunits will be found in cancers in the future.

In thyroid cancers, *PIK3CA* mutations have been described by several groups (Hou et al., 2007; Wang et al., 2007; Santarpia et al., 2008; Ricarte-Filho et al., 2009). These occur more frequently in follicular and anaplastic thyroid cancers (FTC and ATC, respectively) in comparison to papillary thyroid cancer (PTC) and benign thyroid tumors. Moreover, one recent study demonstrated that *PIK3CA* mutations were identified in recurrent or metastatic lesions only from non-PTC forms of thyroid cancer (Ricarte-Filho et al., 2009). Consistent with the increased incidence of *PIK3CA* in poorly differentiated thyroid cancers, Santarpia, et. al. (Santarpia et al., 2008) demonstrated an increase incidence of *PIK3CA* mutations in the more poorly differentiated components of ATCs that had both well and poorly differentiated components identified in the same tumors. The association between *PIK3CA* mutations and more aggressive tumor behavior has also been reported in colon cancer (Ogino et al., 2009), suggesting this may be a more generalized relationship in solid tumors.

In addition to mutations, amplification of *PIK3CA* has been described to be a common event in thyroid tumors. When combining data reported in several recent studies, approximately 13% of follicular adenomas and 16% of PTCs showed gene amplification of *PIK3CA* while approximately 30% of FTC and 50% of ATC exhibited amplification, suggesting an increased frequency of *PIK3CA* amplification in more aggressive histological subtypes (Wu et al., 2005; Hou et al., 2007; Wang et al., 2007; Hou et al., 2008; Santarpia et al., 2008). Liu et. al. also reported that 25/55 (46%) FTCs and 16/42 (38%) ATCs have amplification of the gene encoding PI3K'p110 β (Liu et al., 2008). It is not yet known if *PIK3CA* mutations or amplification are sufficient to cause thyroid cancer *in vivo*. The capability is suggested in some *in vivo* model systems (Bader et al., 2006), but similar results have not been demonstrated for all tissues (Liang et al., 2009). Further studies will be required to fully characterize the role of this oncogene in thyroid cancer development and progression.

Mutations in the gene encoding the PI3K regulatory subunit, p85α that result in deletions proximal to the highly conserved serine 608 residue leading to constitutive PI3K signaling have been identified in human ovarian and colon cancer cells (Philp et al., 2001). The mechanism for this enhanced downstream signaling occurs presumably through reduced interactions between PI3K regulatory and catalytic subunits. While this mutation has not been described in thyroid cancer, one murine model of thyroid cancer in which thyroid tumors are induced by the homozygous genetic "knock-in" of the thyroid hormone β (TRβ) mutant PV may in part mimic this mechanism (Suzuki et al., 2002). The TRβPV mutant was identified in a patient with thyroid hormone resistance and has a unique insertion in the T3 receptor β gene. The altered T3 receptor is not only unable to bind T3, but also has dominant negative activity (Meier et al., 1993). While using this mutation to model thyroid hormone resistance, these mice were noted to develop thyroid cancer as they aged (Kaneshige et al., 2000; Suzuki et al., 2002). The tumors have phenotypic similarities to FTC, including a propensity for distant metastases, exhibit high degrees of Akt activation, and primary cultured cells from the tumors demonstrate Akt-dependent cell migration (Kim et al., 2005). Subsequent studies have shown that the TRβ can bind and sequester the p85 regulatory subunit of PI3K and that the TRβPV mutant has greater affinity than wild-type receptor for this effect (Furuya et al., 2006). Finally, inhibition of PI3K systemically using LY294002 inhibits both thyroid cancer development and metastasis and also reduces the degree of pituitary thyrotroph hyperplasia in these mice (Furuya et al., 2007). Recent data also demonstrate that crossing TRβPV mice with mice heterozygous

for *Pten* loss enhances tumor formation and increases the level of Akt activation in the tumors (Guigon et al., 2009). Thus, in this model it appears that the tumor induction and progression is likely dependent on PI3K signaling, although the high levels of TSH may provide additive or synergistic effects for either tumor formation or progression. Indeed, cooperative interactions between these pathways have been reported in several thyroid cell systems *in vitro* (Coulonval et al., 2000; Saito et al., 2001).

RET/PTC

RET/PTC oncogenes induce activation of a variety of signaling cascades, including MAPK-ERK (Santoro et al., 2006; Nikiforov, 2008) and PI3K-Akt (Miyagi et al., 2004). Primary cultured human thyroid epithelial cells infected with a retrovirus containing *RET/PTC1* develop an irregular nuclear contour and a euchromatic appearance that is reminiscent of human PTC (Fischer et al., 1998). The development of thyroid cancer in transgenic mice with thyroid-specific RET/PTC1 and RET/PTC3 overexpression confirm the thyroid oncogenic potential of RET/PTC *in vivo* (Jhiang et al., 1996; Santoro et al., 1996; Powell et al., 1998). Interestingly, conditional expression of RET/PTC1 and RET/PTC3 in rat PCCL3 cells leads to induction of DNA synthesis, and apoptosis, but not cell proliferation (Wang et al., 2003). The authors proposed that acute activation of RET/PTC may not be strongly oncogenic, but instead increases susceptibility to secondary genetic or epigenetic changes that may disable growth inhibitory or antiapoptotic signaling effectors. Studies performed to assess the association between *RET* oncogene expression and tumor aggressiveness suggest that while *RET/PTC* induces cancer, it does not appear to enhance tumor progression or be particularly associated with tumor recurrence, although each *RET/PTC* may not be identical in this regard (Basolo et al., 2001; Santoro et al., 2002; Nikiforov, 2008). Thus, the current model is that that *RET/PTC* rearrangements and consequent constitutive PI3K and MAPK signaling are early events in radiation-related thyroid tumorigenesis.

Phosphatase and Tensin Homolog Deleted on Chromosome Ten (PTEN)

PTEN is a dual function lipid and protein phosphatase that plays a central role as a negative regulator of PI3K-mediated signaling events (Li et al., 1997; Liaw et al., 1997; Steck et al., 1997). While PTEN has diverse functions in multiple subcellular locations (Gimm et al., 2000; Planchon et al., 2008), its best defined signaling activity is the phosphatase activity that leads to the removal of phosphates from the 3 position on phosphoinstides, thereby negatively regulating PI3K signaling. PTEN has been shown to function as a tumor suppressor through this activity (Di Cristofano et al., 2000). Clinically, the loss of *PTEN* expression in the germ line is the cause of the majority of cases of Cowden's syndrome, a multiple hamartomas syndrome that includes thyroid neoplasias (benign and malignant) as part of the phenotype (Liaw et al., 1997). In addition, the loss of *Pten* in mice has been shown to result in thyroid cancer and/or nodule development in both generalized and tissue-specific knock out models (Di Cristofano et al., 1998; Podsypanina et al., 1999; Yeager et al., 2007). Tumors from patients with *PTEN* loss have been shown to have enhanced Akt activation (Bruni et al., 2000) and the induction of tumors in mice heterozygous for *Pten* loss has been shown to be attenuated by loss of Akt 1 expression (Chen et al., 2006). Moreover, enhanced proliferation in the thyroid glands of *Pten* deficient mice is dependent on mTOR activity (Yeager et al., 2008). Thus, it appears that much of the tumor suppressor function of *PTEN* rests in its phosphatase activity which inhibits PI3K signaling. It is important to note that reduced levels of PTEN can occur through several mechanisms in human tumors, including gene mutations, promoter hypermethylation, and more recently reduced translation via micoRNA (miR21) overexpression (Meng et al., 2007).

Interest in *PTEN* as a tumor suppressor involved in thyroid neoplasia derived initially from the high frequency of follicular thyroid neoplasia in Cowden's syndrome (Liaw et al., 1997). In

more common sporadic thyroid cancers, *PTEN* mutations are relatively uncommon (Dahia et al., 1997; Halachmi et al., 1998), but *PTEN* gene methylation and reduced expression levels occur more commonly; particularly in follicular tumors (Bruni et al., 2000; Frisk et al., 2002; Alvarez-Nunez et al., 2006; Hou et al., 2008). Estrogen receptors may also be an important downstream modulator of *Pten* loss-related tumor formation, as suggested by studies that have identified a cooperative relationship between of estrogen receptor activation and *Pten* loss-related in endometrial and thyroid cancer models (Vilgelm et al., 2006; Yeager et al., 2007). These data may be important clinically considering the female preponderance of patients with thyroid cancer.

Although not yet studied in thyroid cancer, intriguing recent data have suggested a potential role for PTEN expression in the tumor-associated stroma in breast cancer behavior [(Trimboli et al., 2009) and reviewed in (Eng et al., 2009)]. While controversial, the clincal studies have indentified unique *PTEN* mutations in the microdissected stromal tissue that were not identified in the primary tumor tissue (Kurose et al., 2002). It is also possible that the cells in these clinical samples that harbor the *PTEN* mutations represent a subgroup of cancer cells that have undergone an epithelial-to-mesenchymal transition [EMT (Mani et al., 2008)], a feature identified frequently in locally invasive papillary thyroid cancers (Vasko et al., 2007). Recent functional data in which the selective loss of *Pten* in stromal fibroblasts of mice enhanced initiation, growth, and progression of breast cancers strongly supports a role for stromal cell PI3K activity in breast cancer progression (Trimboli et al., 2009). Whether the identified cells in human cancers are primary stromal cells or derived from cancer cells via EMT, these data demonstrate that signals from tumor-associated non-epithelial cells influence breast cancer progression and are regulated by PI3K/Akt signaling. In thyroid cancer, the derivation of cancer-associated stromal cells and the relationships between these cells and the epithelial thyroid cancer cells are incompletely defined.

PDK-1

PDK-1 is a PH domain-containing protein that is activated following PI3K activity that in turn phosphorylates Akt at threonine 308 along with a large variety of other AGC kinase substrates. Although this kinase has an important role in PI3K-Akt-mTOR signaling, activating mutations of the gene encoding PDK-1 have not been described. A recent study identified PDK-1 gene amplification in 14/58 (24%) FTC and 8/40 (20%) ATC, (Liu et al., 2008) suggesting that PDK-1 expression levels may regulate downstream signaling in thyroid cancer, but this has not been fully tested. Based on data demonstrating constitutive activation of PI3K signaling through mutations in *PIK3CA* or *PTEN*, it does not appear that PDK-1 is a site of major signaling down-regulation in thyroid cancers. PDK-1 activation is dependent on primarily on cytoplasmic membrane localization, and it is considered to be constitutively active. Thus, while it is unlikely that activating mutations in the kinase domain occur, it is possible that membrane-targeting PDK-1 mutations could result in pathway activation (Storz et al., 2002).

Akt

Akt, also known as protein kinase B (PKB), is a family of serine/threonine kinases comprised of three unique, but closely related isoforms, Akt 1, 2, and 3 (Staal, 1987; Jones et al., 1991; Masure et al., 1999; Nakatani et al., 1999). All Akt isoforms share structural homology, including PH domain, ATP binding site, and two phosphorylation sites. Similar to PDK-1, the PH domain of Akt binds with PtdIns-3,4-P2 and PtdIns-3,4,5-P3 leading to cytoplamic membrane recruitment (Kohn et al., 1996; Andjelkovic et al., 1997). Threonine 308 on Akt1 is phosphorylated by PDK-1 while the second critical phosphorylation site, Serine 473 on Akt1, can be phosphorylated by other kinases with so-called PDK-2 activity, such as Integrin-linked kinase, DNA-dependent kinase, protein kinase C, and the TORC2 complex (see below), and/or be autophosphorylated, resulting in maximum kinase activation (Alessi et al., 1996; Lynch

et al., 1999; Toker et al., 2000; Feng et al., 2004; Kawakami et al., 2004; Jacinto et al., 2006). Akt then phosphorylates a variety of downstream targets in the cytoplasm including glycogen systhesis kinase (GSK), Bad, forkhead family of transcription factor (FoxO), PAK1, cAMP response element-binding protein (CREB), p27, and Mdm2 (Brazil et al., 2004). Phosphorylation of downstream targets by Akt has been shown to increase cell proliferation, motility, protein synthesis, and gluconeogenesis as well as inhibit apoptosis. Akt has also been shown to target nuclear proteins directly, including transcription factors and nuclear receptors leading to a unique signaling network (Trotman et al., 2006). Akt signaling is extinguished by phosphatases, including PHLPP1 (Gao et al., 2005) and PP2A that may have isoform and localization specificity (Trotman et al., 2006). There are several possible determinants of Akt downstream effects, including the degree of activation, the subcellular localization of activated Akt, preferential activation of specific Akt isoforms, and levels of Akt expression. These will be discussed below.

In thyroid cancer, increased Akt activation has been reported, with greater degrees of activation in follicular and poorly differentiated thyroid cancers in comparison to papillary cancers; although activity has been reported in all subtypes (Bruni et al., 2000; Ringel et al., 2001; Miyakawa et al., 2003; Shin et al., 2004; Motti et al., 2005; Larson et al., 2007; Santarpia et al., 2008). It has also been demonstrated that higher levels of phosphorylated Akt (pAkt) occur in the invasive fronts of locally aggressive primary thyroid cancers (Vasko et al., 2004). Because of the tendency of immunoactive pAkt to be found in the nucleus in these invasive fronts, and those of other cancers, there has been an interest in identifying nuclear Akt downstream pathways (Trotman et al., 2006). Experimentally, it has been shown that nuclear-predominant Akt 1 activity is capable of regulating cell migration *in vitro* (Saji et al., 2005), suggesting a role for nuclear Akt 1 signaling in cancer invasion. Moreover, in T-cell lymphoma, the TCL-1 oncogene has been shown to enhance nuclear Akt1 important resulting in tumor formation (Pekarsky et al., 2000; Pekarsky et al., 2001). Despite these data, the precise mechanisms that regulate the nuclear transport of Akt is uncertain. Several Akt binding proteins involved in Akt nuclear import have been described, including heat shock protein 90 (HSP90) (Sato et al., 2000), T-cell lymphoma oncogene (Tcl)-1 (Pekarsky et al., 2000), adaptor protein containing PH domain, PTB domain and leucin-zipper motif (APPL) (Mitsuuchi et al., 1999), protein kinase C-related kinase (PRK) 2 (Koh et al., 2000), carboxyl-terminal modulator protein (CTMP) (Maira et al., 2001), mammalian homolog of Drosophila tribbles 3 (TRB3) (Du et al., 2003), and most recently p21 activated kinase 1 (PAK1) (Higuchi et al., 2008). Akt is exported from the nucleus by binding with chromosome region maintenance protein-1 (CRM-1), similar to many other proteins. Akt1 binds with CRM-1 at a nuclear export sequence (NES) located within its kinase domain (Saji et al., 2005). Pharmacological inhibition of CRM-1 binding and molecular disruption of binding between Akt1 and CRM-1 by mutation of the NES sequence lead to nuclear Akt 1 accumulation, supporting the functional role of this interaction in Akt subcellular shuttling. Finally, although the roles of Akt isoforms in cell growth and motility appear to be cell-type specific, in fibroblasts and mesenchymal cancer cells, overexpression of nuclear export deficient Akt1 results in increased cell motility in vitro (Saji et al., 2005).

In addition to nuclear Akt, activated Akt in the cytoplasmic membrane has also been shown to play an important role in enhancing cell motility (Higuchi et al., 2001; Kim et al., 2001). Transforming mutations of Akt1 in the pleckstrin homolog (PH) domain (Carpten et al., 2007) and replacement of the PH domain with a membrane-targeting myristoylated sequence both increase the association of Akt isoforms with the cytosolic membrane leading to constitutive activation and cellular proliferation, transformation, and motility, thereby confirming that important Akt cancer-promoting signaling events clearly occur in the cytosol (Mirza et al., 2000; Mende et al., 2001; Saji et al., 2005). Thus, Akt activity plays a role in cell migration through interactions with targets in both the nucleus and cytosol. These results

together demonstrate that subcellular localization of Akt activity may play an important role in defining its downstream biological consequences.

In addition to subcellular localization, there has been a strong interest in identifying Akt isoform-specific functions. This is particularly relevant in a translational manner due to efforts to develop Akt isoform-specific inhibitors for clinical trials. The lack of complete overlap in Akt isoforms function is highlighted by the selective knock out models of Akt 1 and 2. Akt 1 is expressed in almost all cells and generalized Akt 1 knock-out mice demonstrated a small size (Cho et al., 2001). Akt 2, while expressed in many cells, is preferentially expressed in insulin-responsive tissues and its knock-out mice develop type 2 diabetes without evidence of a rescue effect of compensatory Akt 1 overexpession (Cho et al., 2001; Garofalo et al., 2003). In the normal human thyroid, Akt 1 and 2 are the predominate isoforms expressed (Ringel et al., 2001), while Akt 3 expression is primarily detected in the brain, heart, and kidneys. Estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines also expressed Akt 3, suggesting that Akt 3 may have an important role in the malignant phenotype in these cancer lines (Nakatani et al., 1999). It is important to recognize that some Akt interacting proteins, such as PAK1 and TCl1, display isoform-specificity in Akt interactions, a feature that might be functionally important in defining isoform-specific roles for Akt in cancer (Pekarsky et al., 2000).

Interestingly, strikingly distinct cell context-specific differences in the roles of Akt isoforms in tumorigenesis, progression, and cell motility have recently been reported. For example, in certain breast cancer cells and in breast cancer *in vivo* models, Akt 1 appears to promote cell proliferation and tumor formation and inhibit cell motility and metastasis; while Akt 2 is primarily involved as a positive regulator of cell motility and metastases (Hutchinson et al., 2004; Irie et al., 2005; Yoeli-Lerner et al., 2005; Heron-Milhavet et al., 2006; Liu et al., 2006; Cheng et al., 2007; Dillon et al., 2009). By contrast, in poorly differentiated cancer cells and fibroblasts, Akt 1 functions as a positive regulator of motility, and its expression is both necessary and sufficient for cell motility (Saji et al., 2005; Zhou et al., 2006). These data may be of critical importance when considering Akt isoform inhibition as a modality for cancer therapy and suggests that isoform-specific inhibitors may need to be considered indepently. Finally, as noted above, Akt pathway activation in the tumor-related stroma may also modulate the aggressiveness of cancer behavior. Whether specific Akt isoforms are particularly relevant to these data is uncertain.

Akt activity levels in cancers may also be controlled by other factors. Gene amplification of Akt 1 and Akt 2 has recently been described to occur in a variety of tumors. In thyroid cancer, Liu et. al. recently reported that 5/61 (8%) FTC and 9/48 (19%) ATC had gene amplification of Akt 1, and that 13/58 (22%) FTC had gene amplification of Akt 2 (Liu et al., 2008). However, whether the amplification was associated with activated downstream signaling was not reported. Recently, gain-of function mutations of Akt isoforms have been reported in cancer. Carpten et. al. (Carpten et al., 2007) sequenced all Akt family genes in total over 150 of breast, colon, and ovarian cancers, and found a unique mutation at nucleotide 49 of Akt1 that results in a lysine substitution for glutamic acid at amino acid 17 (AKT1E17K) within PH domain in 5/61 (8%) breast, 3/51 (6%) colorectal and 1/50 (2%) ovarian cancers. The E17K substitution was shown to alter Akt1 PH domain conformation, allowing membrane recruitment of Akt1 independent of PtdIns binding, resulting in constitutive Akt kinase activity and signaling. AKT1E17K is a relatively uncommon gene mutation in many other cancers that have been studied, including lung, B-cell-derived lymphoid leukemias (0/87), and 1/26 urothelial carcinoma (Mahmoud et al., 2008; Malanga et al., 2008; Zilberman et al., 2009). In primary thyroid cancers, AKTE17K mutations appear to be uncommon as no Akt 1 or 2 PH-domain mutations were identified in 64 FTC, and 47 ATC samples (Liu et al., 2008). However, in a second study, Ricarte-Filho et. al. (Ricarte-Filho et al., 2009) reported recurrent or metastatic

lesions from 2/12 (17%) tall cell variant PTC, 1/3 (33%) Hürthle cell carcinoma, and 6/32 (19%) poorly differentiated PTC cases had AKT1K17E mutations, suggesting that Akt 1 mutations may have an important role as a "later" genetic alteration in tumor progression. In addition to Akt 1 mutations, similar mutations in the PH domain have recently been identified in the Akt 3 gene in malingnant melanoma (Davies et al., 2008). Akt 3 mutations have not been reported in thyroid cancer.

TSC1/2 (hamartin and tuberin)

TSC1 and 2 are important signaling molecules in PI3K-Akt-mTOR and MAP kinase signaling as described in detail above [Figure 2 and reviewed in (Guertin et al., 2007)]. Inactivating mutations in TSC1 and TSC2 result in constitutive mTOR activation and are the cause of types 1 and 2 tuberous sclerosis, a heritable disorder characterized by skin lesions, renal cysts, angiomyolipomas in multiple locations, astryctomas, and neuroendocrine tumors (Dworakowska et al., 2009). While thyroid cancer is not classically considered to be a component of tuberous sclerosis, inhibition of mTOR is one approach being evaluated with therapeutic intent (see below).

Mammalian target of rapamycin (mTOR)

mTOR, also known as FK506 binding protein 12-rapamycin associated protein (FRAP) 1, is a serine/threonine protein kinase in the PI3K cascade (Wullschleger et al., 2006; Abraham et al., 2007; Guertin et al., 2007). mTOR phosphorylates and activates S6K, and also inhibits eukaryotic translation initiation factor 4E binding protein (4E-BP), resulting in enhanced protein synthesis and cell proliferation. Although Akt activity enhances mTOR activity, the dynamic interplay between these two molecules is complex (Figure 2). Akt phosphorylates and inhibits tuberin sclerosis protein complexes (TSC1 and 2, also known as harmartin and tuberin, respectively) leading to an increase in Ras homolog enriched in brain (Rheb) activity and mTOR activation through interactions with the mTOR protein complexes (Astrinidis et al., 2005; Au et al., 2008), mTORC1 and mTORC2. mTORC1 is comprised of mTOR, Raptor, mLST8, and PRAS40, a mTOR inhibitor. Increased activity of Rheb and inhibition of PRAS40 by phosphorylation occurs once Akt inhbits TSC2, resulting in mTOR activation and subsequent downstream signaling. The activated mTORC1 complex has been shown to exert negative-feedback inhibition on PI3K signaling by downregulating more proximal signaling regulators such as IRS 1 (Takano et al., 2001). The mTORC2 protein complex contains mTOR, Rictor, mLST8, and mSIN1. mTORC2, when activated, has been convincingly shown to have PDK2 activity leading to serine 473 phosphorylation and activation of Akt (Sarbassov et al., 2005), thereby serving as a positive feedback loop. Other signaling pathways regulated by the mTORC2 complex are less well delineated.

Role of PI3K signaling in RAS Oncogene Activity

While Ras is known to signal through MAPK, this signaling molecule also has the ability to directly activated PI3K signaling (Cass et al., 2000). Gupta et. al. showed that expression of PI3K p110 mutants which are genetically modified to lose selectively binding with Ras resulted in reduced Ras-induced Akt activation and tumorigenesis (Gupta et al., 2007), suggesting that Ras-induced PI3K signaling is necessary for Ras-induced tumorigeneis. The relative contribution of these two pathways in RAS effects may be tissue or cell-type specific.

RAS mutations are most common in follicular thyroid tumors and in follicular variant of PTC (Nikiforov, 2008). In thyroid cells, *RAS* mutants have been shown to activate both the PI3K and MAPK signaling cascades. Recent in vivo data confirms that oncogenic transformation by mutant *KRAS* requires activation of both signaling cascades (Miller et al., 2009). Consistent with this observation is the relative resistance of thyroid cancer cells with RAS mutations to MEK inhibition in comparison to cell lines harboring BRAF V600E mutations (Ball et al.,

2007; Leboeuf et al., 2008). These data suggest that combined inhibition of MEK and PI3K signaling may be optimal for in vitro sensitivity of these cells.

Peroxisome Proliferators-activated receptor (PPAR) /Paired Box 8 (PAX8) Rearrangements

PPARγ/PAX8 rearrangements are reported to be found in a subset of benign and malignant follicular thyroid tumors (Kroll et al., 2000). The function of this rearranged protein is not entirely elucidated, but its protein functions appear to include a dominant-negative effect on PPAR activity (Reddi et al., 2007). Recent in vivo studies demonstrate that thyroid-specific expression of the protein results in thyroid hyperplasia (Diallo-Krou et al., 2009). Since PPAR has been shown to positively regulate *PTEN* gene expression, inhibition of PPAR would be predicted to suppress *PTEN* expression thereby increasing PI3k-Akt signaling (Farrow et al., 2003). Consistent with this mechanism, Vasko, *et. al.* identified a correlation between expression of *PPARγ/PAX8* and higher levels of immunoactive pAkt in human thyroid cancers (Vasko et al., 2004). Similarly, increased levels of immunoactive phosphorylated Akt were identified in the hyperplastic thyroid glands in the thyroid-specific *PPARγ/PAX8* mice and in *PPARγ/PAX8* -expressing human tumors were recently reported by Diallow-Krou, et al (Diallo-Krou et al., 2009). In addition, they also reported that the thyroid hyperplasia was more pronounced when the thyroid-specific *PPARγ/PAX8* mice were crossed with $Pten^{+/-}$ mice; although cancers were not identified.

Other Mechanisms for Enhanced PI3K Signaling in Thyroid Cancer

Other gain-of-function mutations and overexpression of tyrosine kinase receptors that potentially simulate PI3K-Akt-mTOR signaling can occur in thyroid cancer. Gene rearrangements involving the neurotrophic recoptor-tyrosine kinase (*NTRK1*) and overexpression of the hepatocyte growth factor receptor, c-MET, have been described in papillary thyroid cancer (Greco et al., 1992; Di Renzo et al., 1995). In addition, overexpression of EGF, VEGF, IGF-1, FGF receptors and others known to signal in part through PI3K are also common in thyroid cancer [reviewed in (Kondo et al., 2006)]. In addition to signaling in cancer cells, PI3K signaling may play a critical role in regulating the response of non-cancer cells in the associated stromal tissue to circulating factors, extracellular ligands, and tumor-derived factors. This cancer-related signaling may also play an important role in regulating cancer growth and progression.

PI3 KINASE AS A TARGET FOR TREATMENT OF PROGRESSIVE/POORLY DIFFERENTIATED THYROID CANCERS

Most patients with thyroid cancer are successfully treated with thyroidectomy, levothyroxine, and radioiodine in selected cases (Cooper et al., 2006). External beam radiation therapy may also be used to control local disease recurrence or to protect patients from local complications of metastatic lesions. Cytotoxic chemotherapy has been largely ineffective for patients with widespread progressive metastatic or poorly differentiated thyroid cancers. There has therefore been a major interest in developing and testing compounds for the treatment of patients with aggressive thyroid cancers. Due in part to the efficacy of targeted therapy with imatinib in BCR/Abl-positive chronic myelogeous leukemia and C-Kit positive gastrointestinal stromal tumor therapy, a plethora of compounds have been developed to target specific kinases or other signaling molecules known to be involved in cancer formation and progression. Because of the extent of knowledge of genetic and signaling abnormalities in thyroid cancer, it serves as an excellent model tumor for testing the efficacy of these “targeted” therapies.

To date, most of the compounds tested in treating thyroid cancer are competitive inhibitors of kinase activity that have some degree of specificity for tyrosine and serine/threonine kinases that share similar structures in their ATP binding pockets [reviewed in (Sherman, 2009)]. More

specific inhibitors that are being studied include allosteric inhibitors and specific agents designed to inhibit the extracellular activation of individual receptor kinases. In thyroid cancer, a group of compounds have been or are being studied that target, among other kinases, Ret, BRAF, VEGF receptors, cMet, and PDGF Receptors and are thus classified as multikinase inhibitors with varying degrees of activity and specificity for specific targets [reviewed in (Sherman, 2009)]. These include compounds, such as motesanib, sorafenib, and axitinib that have been shown to induce stable disease or partial remission in a high percentage of patients with aggressive thyroid cancers (Cohen et al., 2008; Gupta-Abramson et al., 2008; Sherman et al., 2008; Kloos et al., 2009). Others are currently being evaluated in Phase 2 and 3 studies. One common target of the active compounds proposed to be relevant for thyroid cancer treatment is the VEGF receptor.

Due to the high frequency of activation of PI3K and its downstream effectors in progressive, recurrent, and poorly differentiated thyroid cancers, this kinase represents particularly relevant and potentially important therapeutic target. One target in this pathway that has been exploited for treating other cancers is mTOR. Everolimus, a compound that targets mTOR, has recently been approved in the Unites States for clinical use in advanced treatment-resistant renal cell cancer. In addition rapamycin has been approved for use as an inhibitor of transplant rejection for a number of years. Rapamycin has been shown to inhibit *Pten* loss-related thyroid cell growth *in vivo* and (Yeager et al., 2008) and to inhibit thyroid cancer cell growth *in vitro* (Papewalis et al., 2009). In addition to inhibiting mTOR, PI3K itself also represents a therapeutic target for cancer. Indeed, in the laboratory, inhibition of PI3K is attained using several compounds including LY294002 and Wortmanin. Recently, oral PI3K inhibitors have been developed, including both generalized and isoform-specific compounds (Garcia-Echeverria et al., 2008; Jia et al., 2009). Similarly, Akt inhibitors are also being developed that inhibit all Akt isoforms. Several compounds inhibiting these targets are now in phase 1 clinical trials [reviewed in (Garcia-Echeverria et al., 2008)]. As noted above, care must be taken when considering isoform-specific Akt inhibitors due to the cell type-specific effects of Akt 1 as either an inhibitor or inducer of cell migration and metastases. Other non-specific approaches that inhibit PI3K signaling include using compounds that reduce protein stability either by disrupting protein-protein interactions with chaperones, such as HSP90 or by enhancing proteasomal degradation.

Because PI3K activation can occur in combination with constitutive activation of the MAPK pathway in progressive thyroid cancer and it has been described that mTOR inhibition may paradoxically increase Akt phosphorylation through feedback mechanisms (Sun et al., 2005), there has been an interest in combinatorial strategies to treat aggressive thyroid cancers, similar to lung cancer (Engelman et al., 2008). This approach may be particularly relevant for tumors with genetic alterations of both pathways, although recent data suggest that combined inhibition of mTOR and MEK may be effective in a broad array of thyroid cancer cell lines with different genetic abnormalities (Jin et al., 2009). Despite the potential for dose reductions of both agents through synergistic interactions, great care must be taken to carefully analyze for potential side effects of such combinatorial approaches.

PI3K signaling plays a critical role in a number of non-tumor tissues, and the benefits and concerns about globally inhibiting this pathway must be considered in clinical trials. For example, one can hypothesize that the effects of inhibiting PI3K on the tumor microenvironment and on recruiting cells to tumors, as recently highlighted in breast cancer models, may play an important role in the anti-tumor effects of these compounds (Trimboli et al., 2009). However, PI3K signaling is also crucial for essential cellular processes such as insulin signaling, neuron function, and endothelial function and inhibiting signaling in these cells may lead to toxicities. The relative specificity for tumor cells may be dose-related; however, careful scrutiny of safety parameters in clinical trials will be important, particularly

in combination studies. This may be particularly relevant for thyroid cancer patients, many of whom maintain an excellent quality of life even in the setting of metastatic disease.

CONCLUSIONS

Overactivation of PI3K signaling plays an important role in the development and progression of thyroid cancer. Clinical and functional data support a particularly important role for this cascade in follicular tumorigenesis. Clinical correlation data and *in vitro* studies support a role for PI3K signaling in tumor dedifferentiation and progression. Like all signaling cascades, the complexity of interactions between signaling molecules within this pathway and with other signaling cascades is now being elucidated. While the role of aberrant PI3K signaling as a mediator of thyroid cancer dedifferentiation and progression requires further experimental confirmation, this potential function suggests that critical signaling nodes in this pathway may be a particularly relevant therapeutic targets for patients with aggressive forms of the disease. Further studies to determine if anti-tumor activity can be predicted by the degree of pathway activation or by the presence of specific genetic abnormalities are needed to determine if this cascade represent an appropriate clinical therapeutic target.

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References

1. Abraham RT, Gibbons JJ. The mammalian target of rapamycin signaling pathway: Twists and turns in the road to cancer therapy. Clin Cancer Res 2007;13:3109–3114. [PubMed: 17545512]
2. Alessi DR, Andjelkovic M, Caudwell B, et al. Mechanism of activation of protein kinase b by insulin and igf-1. EMBO J 1996;15:6541–6551. [PubMed: 8978681]
3. Alvarez-Nunez F, Bussaglia E, Mauricio D, et al. Pten promoter methylation in sporadic thyroid carcinomas. Thyroid 2006;16:17–23. [PubMed: 16487009]
4. Andjelkovic M, Jakubowicz T, Cron P, et al. Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (rac-pk/pkb) promoted by serum and protein phosphatase inhibitors. Proc Natl Acad Sci U S A 1996;93:5699–5704. [PubMed: 8650155]
5. Andjelkovic M, Alessi DR, Meier R, et al. Role of translocation in the activation and function of protein kinase b. J Biol Chem 1997;272:31515–31524. [PubMed: 9395488]
6. Astrinidis A, Henske EP. Tuberous sclerosis complex: Linking growth and energy signaling pathways with human disease. Oncogene 2005;24:7475–7481. [PubMed: 16288294]
7. Au KS, Ward CH, Northrup H. Tuberous sclerosis complex: Disease modifiers and treatments. Curr Opin Pediatr 2008;20:628–633. [PubMed: 19005330]
8. Bader AG, Kang S, Vogt PK. Cancer-specific mutations in pik3ca are oncogenic in vivo. Proc Natl Acad Sci U S A 2006;103:1475–1479. [PubMed: 16432179]
9. Ball DW, Jin N, Rosen DM, et al. Selective growth inhibition in braf mutant thyroid cancer by the mitogen-activated protein kinase kinase 1/2 inhibitor azd6244. J Clin Endocrinol Metab 2007;92:4712–4718. [PubMed: 17878251]
10. Basolo F, Molinaro E, Agate L, et al. Ret protein expression has no prognostic impact on the long-term outcome of papillary thyroid carcinoma. Eur J Endocrinol 2001;145:599–604. [PubMed: 11720878]
11. Bellacosa A, Chan TO, Ahmed NN, et al. Akt activation by growth factors is a multiple-step process: The role of the ph domain. Oncogene 1998;17:313–325. [PubMed: 9690513]
12. Blanco-Aparicio C, Renner O, Leal JF, et al. Pten, more than the akt pathway. Carcinogenesis 2007;28:1379–1386. [PubMed: 17341655]
13. Brazil DP, Yang ZZ, Hemmings BA. Advances in protein kinase b signalling: Aktion on multiple fronts. Trends Biochem Sci 2004;29:233–242. [PubMed: 15130559]

14. Bruni P, Boccia A, Baldassarre G, et al. Pten expression is reduced in a subset of sporadic thyroid carcinomas: Evidence that pten-growth suppressing activity in thyroid cancer cells mediated by p27kip1. Oncogene 2000;19:3146–3155. [PubMed: 10918569]
15. Carpten JD, Faber AL, Horn C, et al. A transforming mutation in the pleckstrin homology domain of akt1 in cancer. Nature 2007;448:439–444. [PubMed: 17611497]
16. Cass LA, Meinkoth JL. Ras signaling through pi3k confers hormone-independent proliferation that is compatible with differentiation. Oncogene 2000;19:924–932. [PubMed: 10702801]
17. Chalhoub N, Baker SJ. Pten and the pi3-kinase pathway in cancer. Annu Rev Pathol 2009;4:127–50. [PubMed: 18767981]
18. Chen ML, Xu PZ, Peng XD, et al. The deficiency of akt1 is sufficient to suppress tumor development in pten+/− mice. Genes Dev 2006;20:1569–1574. [PubMed: 16778075]
19. Cheng GZ, Chan J, Wang Q, et al. Twist transcriptionally up-regulates akt2 in breast cancer cells leading to increased migration, invasion, and resistance to paclitaxel. Cancer Res 2007;67:1979–1987. [PubMed: 17332325]
20. Chin YR, Toker A. Function of akt/pkb signaling to cell motility, invasion and the tumor stroma in cancer. Cell Signal 2009;21:470–476. [PubMed: 19110052]
21. Cho H, Mu J, Kim JK, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase akt2 (pkb beta). Science 2001;292:1728–1731. [PubMed: 11387480]
22. Cho H, Thorvaldsen JL, Chu Q, et al. Akt1/pkbalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 2001;276:38349–38352. [PubMed: 11533044]
23. Cohen EE, Rosen LS, Vokes EE, et al. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: Results from a phase ii study. J Clin Oncol 2008;26:4708–4713. [PubMed: 18541897]
24. Cooper DS, Doherty GM, Haugen BR, et al. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2006;16:109–142. [PubMed: 16420177]
25. Coulonval K, Vandeput F, Stein RC, et al. Phosphatidylinositol 3-kinase, protein kinase b and ribosomal s6 kinases in the stimulation of thyroid epithelial cell proliferation by camp and growth factors in the presence of insulin. Biochem J 2000;348(Pt 2):351–358. [PubMed: 10816429]
26. Cully M, You H, Levine AJ, et al. Beyond pten mutations: The pi3k pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 2006;6:184–192. [PubMed: 16453012]
27. Dahia PLM, Marsh DJ, Zheng Z, et al. Somatic deletions and mutations in the cowden disease gene, *pten*, in sporadic thyroid tumors. Cancer Res 1997;57:4710–4713. [PubMed: 9354427]
28. Davies MA, Stemke-Hale K, Tellez C, et al. A novel akt3 mutation in melanoma tumours and cell lines. Br J Cancer 2008;99:1265–1268. [PubMed: 18813315]
29. Denley A, Kang S, Karst U, et al. Oncogenic signaling of class i pi3k isoforms. Oncogene 2008;27:2561–2574. [PubMed: 17998941]
30. Di Cristofano A, Pesce B, Cordon-Cardo C, et al. Pten is essential for embryonic development and tumour suppression. Nat Genet 1998;19:348–355. [PubMed: 9697695]
31. Di Cristofano A, Pandolfi PP. The multiple roles of pten in tumor suppression. Cell 2000;100:387–390. [PubMed: 10693755]
32. Di Renzo MF, Olivero M, Serini G, et al. Overexpression of the c-met/hgf receptor in human thyroid carcinomas derived from the follicular epithelium. J Endocrinol Invest 1995;18:134–139. [PubMed: 7629381]
33. Diallo-Krou E, Yu J, Colby LA, et al. Paired box gene 8-peroxisome proliferator-activated receptor-{gamma} fusion protein and loss of phosphatase and tensin homolog synergistically cause thyroid hyperplasia in transgenic mice. Endocrinology. 2009
34. Dillon RL, Marcotte R, Hennessy BT, et al. Akt1 and akt2 play distinct roles in the initiation and metastatic phases of mammary tumor progression. Cancer Res 2009;69:5057–5064. [PubMed: 19491266]
35. Du K, Herzig S, Kulkarni RN, et al. Trb3: A tribbles homolog that inhibits akt/pkb activation by insulin in liver. Science 2003;300:1574–1577. [PubMed: 12791994]

36. Dworakowska D, Grossman AB. Are neuroendocrine tumours a feature of tuberous sclerosis? A systematic review. Endocr Relat Cancer 2009;16:45–58. [PubMed: 18978035]
37. Eng C, Leone G, Orloff MS, et al. Genomic alterations in tumor stroma. Cancer Res 2009;69:6759–64. [PubMed: 19706759]
38. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006;7:606–619. [PubMed: 16847462]
39. Engelman JA, Chen L, Tan X, et al. Effective use of pi3k and mek inhibitors to treat mutant kras g12d and pik3ca h1047r murine lung cancers. Nat Med 2008;14:1351–1356. [PubMed: 19029981]
40. Farrow B, Evers BM. Activation of ppargamma increases pten expression in pancreatic cancer cells. Biochem Biophys Res Commun 2003;301:50–53. [PubMed: 12535639]
41. Feng J, Park J, Cron P, et al. Identification of a pkb/akt hydrophobic motif ser-473 kinase as DNA-dependent protein kinase. J Biol Chem 2004;279:41189–41196. [PubMed: 15262962]
42. Filippa N, Sable CL, Hemmings BA, et al. Effect of phosphoinositide-dependent kinase 1 on protein kinase b translocation and its subsequent activation. Mol Cell Biol 2000;20:5712–5721. [PubMed: 10891507]
43. Fischer AH, Bond JA, Taysavang P, et al. Papillary thyroid carcinoma oncogene (ret/ptc) alters the nuclear envelope and chromatin structure. Am J Pathol 1998;153:1443–1450. [PubMed: 9811335]
44. Frisk T, Foukakis T, Dwight T, et al. Silencing of the pten tumor-suppressor gene in anaplastic thyroid cancer. Genes Chromosomes Cancer 2002;35:74–80. [PubMed: 12203792]
45. Furuya F, Hanover JA, Cheng S-y. Activation of phosphatidylinositol 3-kinase signaling by a mutant thyroid hormone β receptor. Proc Natl Acad Sci USA 2006;103:1780–1785. [PubMed: 16446424]
46. Furuya F, Lu C, Willingham MC, et al. Inhibition of phosphatidylinositol 3′ kinase delays tumor progression and blocks metastatic spread in a mouse model of thyroid cancer. Carcinogenesis 2007;28:2451–2458. [PubMed: 17660507]
47. Gao T, Furnari F, Newton AC. Phlpp: A phosphatase that directly dephosphorylates akt, promotes apoptosis, and suppresses tumor growth. Mol Cell 2005;18:13–24. [PubMed: 15808505]
48. Garcia-Echeverria C, Sellers WR. Drug discovery approaches targeting the pi3k/akt pathway in cancer. Oncogene 2008;27:5511–5526. [PubMed: 18794885]
49. Garcia-Rostan G, Costa AM, Pereira-Castro I, et al. Mutation of the pik3ca gene in anaplastic thyroid cancer. Cancer Res 2005;65:10199–10207. [PubMed: 16288007]
50. Garofalo RS, Orena SJ, Rafidi K, et al. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking akt2/pkb beta. J Clin Invest 2003;112:197–208. [PubMed: 12843127]
51. Gimm O, Perren A, Weng LP, et al. Differential nuclear and cytoplasmic expression of pten in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. Am J Pathol 2000;156:1693–1700. [PubMed: 10793080]
52. Greco A, Pierotti MA, Bongarzone I, et al. Trk-t1 is a novel oncogene formed by the fusion of tpr and trk genes in human papillary thyroid carcinomas. Oncogene 1992;7:237–242. [PubMed: 1532241]
53. Grieco M, Santoro M, Berlingieri MT, et al. Ptc is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 1990;60:557–563. [PubMed: 2406025]
54. Guertin DA, Sabatini DM. Defining the role of mtor in cancer. Cancer Cell 2007;12:9–22. [PubMed: 17613433]
55. Guigon CJ, Zhao L, Willingham MC, et al. Pten deficiency accelerates tumour progression in a mouse model of thyroid cancer. Oncogene 2009;28:509–517. [PubMed: 18997818]
56. Gupta-Abramson V, Troxel AB, Nellore A, et al. Phase ii trial of sorafenib in advanced thyroid cancer. J Clin Oncol 2008;26:4714–4719. [PubMed: 18541894]
57. Gupta S, Ramjaun AR, Haiko P, et al. Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras- driven tumorigenesis in mice. Cell 2007;129:957–968. [PubMed: 17540175]
58. Gustin JP, Karakas B, Weiss MB, et al. Knockin of mutant pik3ca activates multiple oncogenic pathways. Proc Natl Acad Sci U S A 2009;106:2835–2840. [PubMed: 19196980]

59. Halachmi N, Halachmi S, Evron E, et al. Somatic mutatons of the pten/mmac1 tumor suppressor gene in sporadic follicular thyroid tumors. Genes Chromosomes Cancer 1998;23:239–243. [PubMed: 9790504]
60. Heron-Milhavet L, Franckhauser C, Rana V, et al. Only akt1 is required for proliferation, while akt2 promotes cell cycle exit through p21 binding. Mol Cell Biol 2006;26:8267–8280. [PubMed: 16982699]
61. Higuchi M, Masuyama N, Fukui Y, et al. Akt mediates rac/cdc42-regulated cell motility in growth factor-stimulated cells and in invasive pten knockout cells. Curr Biol 2001;11:1958–1962. [PubMed: 11747822]
62. Higuchi M, Onishi K, Kikuchi C, et al. Scaffolding function of pak in the pdk1-akt pathway. Nat Cell Biol 2008;10:1356–1364. [PubMed: 18931661]
63. Hou P, Liu D, Shan Y, et al. Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/akt pathway in thyroid cancer. Clin Cancer Res 2007;13:1161–1170. [PubMed: 17317825]
64. Hou P, Ji M, Xing M. Association of pten gene methylation with genetic alterations in the phosphatidylinositol 3-kinase/akt signaling pathway in thyroid tumors. Cancer 2008;113:2440–2447. [PubMed: 18831514]
65. Hutchinson JN, Jin J, Cardiff RD, et al. Activation of akt-1 (pkb-α) can accelerate erbb-2-mediated mammary tumorigenesis but suppresses tumor invasion. Cancer Res 2004;64:3171–3178. [PubMed: 15126356]
66. Irie HY, Pearline RV, Grueneberg D, et al. Distinct roles of akt1 and akt2 in regulating cell migration and epithelial-mesenchymal transition. J Cell Biol 2005;171:1023–1034. [PubMed: 16365168]
67. Jacinto E, Facchinetti V, Liu D, et al. Sin1/mip1 maintains rictor-mtor complex integrity and regulates akt phosphorylation and substrate specificity. Cell 2006;127:125–137. [PubMed: 16962653]
68. Jhiang SM, Sagartz JE, Tong Q, et al. Targeted expression of the ret/ptc1 oncogene induces papillary thyroid carcinomas. Endocrinology 1996;137:375–378. [PubMed: 8536638]
69. Jia S, Roberts TM, Zhao JJ. Should individual pi3 kinase isoforms be targeted in cancer? Curr Opin Cell Biol 2009;21:199–208. [PubMed: 19200708]
70. Jin N, Jiang T, Rosen DM, et al. Dual inhibition of mitogen-activated protein kinase kinase and mammalian target of rapamycin in differentiated and anaplastic thyroid cancer. J Clin Endocrinol Metab 2009;94:4107–4112. [PubMed: 19723757]
71. Jones PF, Jakubowicz T, Pitossi FJ, et al. Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. Proc Natl Acad Sci U S A 1991;88:4171–4175. [PubMed: 1851997]
72. Kaneshige M, Kaneshige K, Zhu X, et al. Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone. Proc Natl Acad Sci U S A 2000;97:13209–13214. [PubMed: 11069286]
73. Kawakami Y, Nishimoto H, Kitaura J, et al. Protein kinase c betaii regulates akt phosphorylation on ser-473 in a cell type- and stimulus-specific fashion. J Biol Chem 2004;279:47720–47725. [PubMed: 15364915]
74. Kim CF, Vasko VV, Kato Y, et al. Akt activation promotes metastasis in a mouse model of follicular thyroid carcinoma. Endocrinology 2005;146:4456–4463. [PubMed: 16002527]
75. Kim D, Kim S, Koh H, et al. Akt/pkb promotes cancer cell invasion via increased motility and metalloproteinase production. FASEB J 2001;15:1953–1962. [PubMed: 11532975]
76. Kloos RT, Ringel MD, Knopp MV, et al. Phase ii trial of sorafenib in metastatic thyroid cancer. J Clin Oncol 2009;27:1675–1684. [PubMed: 19255327]
77. Koh H, Lee KH, Kim D, et al. Inhibition of akt and its anti-apoptotic activities by tumor necrosis factor-induced protein kinase c-related kinase 2 (prk2) cleavage. J Biol Chem 2000;275:34451–34458. [PubMed: 10926925]
78. Kohn AD, Takeuchi F, Roth RA. Akt, a pleckstrin homology domain containing kinase, is activated primarily by phosphorylation. J Biol Chem 1996;271:21920–21926. [PubMed: 8702995]
79. Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. Nat Rev Cancer 2006;6:292–306. [PubMed: 16557281]
80. Kroll TG, Sarraf P, Pecciarini L, et al. Pax8-ppargamma1 fusion oncogene in human thyroid carcinoma. Science 2000;289:1357–1360. [PubMed: 10958784]

81. Kurose K, Gilley K, Matsumoto S, et al. Frequent somatic mutations in pten and tp53 are mutually exclusive in the stroma of breast carcinomas. Nat Genet 2002;32:355–357. [PubMed: 12379854]
82. Larson SD, Jackson LN, Riall TS, et al. Increased incidence of well-differentiated thyroid cancer associated with hashimoto thyroiditis and the role of the pi3k/akt pathway. J Am Coll Surg 2007;204:764–773. discussion 773–775. [PubMed: 17481480]
83. Leboeuf R, Baumgartner JE, Benezra M, et al. Brafv600e mutation is associated with preferential sensitivity to mitogen-activated protein kinase kinase inhibition in thyroid cancer cell lines. J Clin Endocrinol Metab 2008;93:2194–201. [PubMed: 18381570]
84. Lemoine NR, Mayall ES, Wyllie FS, et al. Activated ras oncogenes in human thyroid cancers. Cancer Res 1988;48:4459–4463. [PubMed: 3293774]
85. Li J, Yen C, Liaw D, et al. Pten, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943–1947. [PubMed: 9072974]
86. Liang S, Yang N, Pan Y, et al. Expression of activated pik3ca in ovarian surface epithelium results in hyperplasia but not tumor formation. PLoS One 2009;4:e4295.10.1371/journal.pone.0004295 [PubMed: 19172191]
87. Liaw D, Marsh DJ, Li J, et al. Germline mutations of the pten gene in cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997;16:64–67. [PubMed: 9140396]
88. Liu H, Radisky DC, Nelson CM, et al. Mechanism of akt1 inhibition of breast cancer cell invasion reveals a protumorigenic role for tsc2. Proc Natl Acad Sci U S A 2006;103:4134–4139. [PubMed: 16537497]
89. Liu Z, Hou P, Ji M, et al. Highly prevalent genetic alterations in receptor tyrosine kinases and phosphatidylinositol 3-kinase/akt and mitogen-activated protein kinase pathways in anaplastic and follicular thyroid cancers. J Clin Endocrinol Metab 2008;93:3106–3116. [PubMed: 18492751]
90. Lynch DK, Ellis CA, Edwards PA, et al. Integrin-linked kinase regulates phosphorylation of serine 473 of protein kinase b by an indirect mechanism. Oncogene 1999;18:8024–8032. [PubMed: 10637513]
91. Mahmoud IS, Sughayer MA, Mohammad HA, et al. The transforming mutation e17k/akt1 is not a major event in b-cell-derived lymphoid leukaemias. Br J Cancer 2008;99:488–490. [PubMed: 18665177]
92. Maira SM, Galetic I, Brazil DP, et al. Carboxyl-terminal modulator protein (ctmp), a negative regulator of pkb/akt and v-akt at the plasma membrane. Science 2001;294:374–380. [PubMed: 11598301]
93. Malanga D, Scrima M, De Marco C, et al. Activating e17k mutation in the gene encoding the protein kinase akt1 in a subset of squamous cell carcinoma of the lung. Cell Cycle 2008;7:665–669. [PubMed: 18256540]
94. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704–715. [PubMed: 18485877]
95. Masure S, Haefner B, Wesselink JJ, et al. Molecular cloning, expression and characterization of the human serine/threonine kinase akt-3. Eur J Biochem 1999;265:353–360. [PubMed: 10491192]
96. Meier CA, Parkison C, Chen A, et al. Interaction of human β 1 thyroid hormone receptor and its mutants with DNA and retinoid x receptor β. T3 response element-dependent dominant negative potency. J Clin Invest 1993;92:1986–1993. [PubMed: 8408652]
97. Mende I, Malstrom S, Tsichlis PN, et al. Oncogenic transformation induced by membrane-targeted akt2 and akt3. Oncogene 2001;20:4419–4423. [PubMed: 11466625]
98. Meng F, Henson R, Wehbe-Janek H, et al. Microrna-21 regulates expression of the pten tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133:647–658. [PubMed: 17681183]
99. Miller KA, Yeager N, Baker K, et al. Oncogenic kras requires simultaneous pi3k signaling to induce erk activation and transform thyroid epithelial cells in vivo. Cancer Res 2009;69:3689–3694. [PubMed: 19351816]
100. Mirza AM, Kohn AD, Roth RA, et al. Oncogenic transformation of cells by a conditionally active form of the protein kinase akt/pkb. Cell Growth Differ 2000;11:279–292. [PubMed: 10910095]

101. Mitsuuchi Y, Johnson SW, Sonoda G, et al. Identification of a chromosome 3p14.3-21.1 gene, appl, encoding an adaptor molecule that interacts with the oncoprotein-serine/threonine kinase akt2. Oncogene 1999;18:4891–4898. [PubMed: 10490823]
102. Miyagi E, Braga-Basaria M, Hardy E, et al. Chronic expression of ret/ptc 3 enhances basal and insulin-stimulated pi3 kinase/akt signaling and increases irs-2 expression in frtl-5 thyroid cells. Mol Carcinog 2004;41:98–107. [PubMed: 15378648]
103. Miyakawa M, Tsushima T, Murakami H, et al. Increased expression of phosphorylated p70s6 kinase and akt in papillary thyroid cancer tissues. Endocr J 2003;50:77–83. [PubMed: 12733712]
104. Motti ML, Califano D, Troncone G, et al. Complex regulation of the cyclin-dependent kinase inhibitor p27kip1 in thyroid cancer cells by the pi3k/akt pathway: Regulation of p27kip1 expression and localization. Am J Pathol 2005;166:737–749. [PubMed: 15743786]
105. Murga C, Fukuhara S, Gutkind JS. A novel role for phosphatidylinositol 3-kinase beta in signaling from g protein-coupled receptors to akt. J Biol Chem 2000;275:12069–12073. [PubMed: 10766839]
106. Nakatani K, Sakaue H, Thompson DA, et al. Identification of a human akt3 (protein kinase bg) which contains the regulatory serine phosphorylation site. Biochem Biophys Res Commun 1999;257:906–910. [PubMed: 10208883]
107. Nakatani K, Thompson DA, Barthel A, et al. Up-regulation of akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines. J Biol Chem 1999;274:21528–21532. [PubMed: 10419456]
108. Nikiforov YE. Thyroid carcinoma: Molecular pathways and therapeutic targets. Mod Pathol 2008;21 (Suppl 2):S37–43. [PubMed: 18437172]
109. Ogino S, Nosho K, Kirkner GJ, et al. Pik3ca mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol 2009;27:1477–1484. [PubMed: 19237633]
110. Paes JE, Ringel MD. Dysregulation of the phosphatidylinositol 3-kinase pathway in thyroid neoplasia. Endocrinol Metab Clin North Am 2008;37:375–387. [PubMed: 18502332]
111. Papewalis C, Wuttke M, Schinner S, et al. Role of the novel mtor inhibitor rad001 (everolimus) in anaplastic thyroid cancer. Horm Metab Res 2009;41:752–756. [PubMed: 19513966]
112. Pekarsky Y, Koval A, Hallas C, et al. Tcl1 enhances akt kinase activity and mediates its nuclear translocation. Proc Natl Acad Sci U S A 2000;97:3028–3033. [PubMed: 10716693]
113. Pekarsky Y, Hallas C, Croce CM. The role of tcl1 in human t-cell leukemia. Oncogene 2001;20:5638–5643. [PubMed: 11607815]
114. Philp AJ, Campbell IG, Leet C, et al. The phosphatidylinositol 3′-kinase p85alpha gene is an oncogene in human ovarian and colon tumors. Cancer Res 2001;61:7426–7429. [PubMed: 11606375]
115. Planchon SM, Waite KA, Eng C. The nuclear affairs of pten. J Cell Sci 2008;121:249–253. [PubMed: 18216329]
116. Podsypanina K, Ellenson LH, Nemes A, et al. Mutation of pten/mmac1 in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci U S A 1999;96:1563–1568. [PubMed: 9990064]
117. Powell DJ, Russell J, Nibu K, et al. The ret/ptc3 oncogene: Metastatic solid-type papillary carcinomas in murine thyroids. Cancer Res 1998;58:5523–5528. [PubMed: 9850089]
118. Reddi HV, McIver B, Grebe SKG, et al. The paired box-8/peroxisome proliferator-activated receptor-g oncogene in thyroid tumorigenesis. Endocrinology 2007;148:932–935. [PubMed: 16946003]
119. Ricarte-Filho JC, Ryder M, Chitale DA, et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for braf, pik3ca, and akt1. Cancer Res 2009;69:4885–4893. [PubMed: 19487299]
120. Ringel MD, Hayre N, Saito J, et al. Overexpression and overactivation of akt in thyroid carcinoma. Cancer Res 2001;61:6105–6111. [PubMed: 11507060]
121. Saito J, Kohn AD, Roth RA, et al. Regulation of frtl-5 thyroid cell growth by phosphatidylinositol (oh) 3 kinase-dependent akt-mediated signaling. Thyroid 2001;11:339–351. [PubMed: 11349832]
122. Saji M, Vasko V, Kada F, et al. Akt1 contains a functional leucine-rich nuclear export sequence. Biochem Biophys Res Commun 2005;332:167–173. [PubMed: 15896313]

123. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the pik3ca gene in human cancers. Science 2004;304:554. [PubMed: 15016963]
124. Santarpia L, El-Naggar AK, Cote GJ, et al. Phosphatidylinositol 3-kinase/akt and ras/raf-mitogen-activated protein kinase pathway mutations in anaplastic thyroid cancer. J Clin Endocrinol Metab 2008;93:278–284. [PubMed: 17989125]
125. Santoro M, Chiappetta G, Cerrato A, et al. Development of thyroid papillary carcinomas secondary to tissue-specific expression of the ret/ptc1 oncogene in transgenic mice. Oncogene 1996;12:1821–1826. [PubMed: 8622903]
126. Santoro M, Papotti M, Chiappetta G, et al. Ret activation and clinicopathologic features in poorly differentiated thyroid tumors. J Clin Endocrinol Metab 2002;87:370–363. [PubMed: 11788678]
127. Santoro M, Melillo RM, Fusco A. Ret/ptc activation in papillary thyroid carcinoma: European journal of endocrinology prize lecture. Eur J Endocrinol 2006;155:645–653. [PubMed: 17062879]
128. Sarbassov DD, Guertin DA, Ali SM, et al. Phosphorylation and regulation of akt/pkb by the rictor-mtor complex. Science 2005;307:1098–1101. [PubMed: 15718470]
129. Sato S, Fujita N, Tsuruo T. Modulation of akt kinase activity by binding to hsp90. Proc Natl Acad Sci U S A 2000;97:10832–10837. [PubMed: 10995457]
130. Sherman SI, Wirth LJ, Droz JP, et al. Motesanib diphosphate in progressive differentiated thyroid cancer. N Engl J Med 2008;359:31–42. [PubMed: 18596272]
131. Sherman SI. Advances in chemotherapy of differentiated epithelial and medullary thyroid cancers. J Clin Endocrinol Metab 2009;94:1493–1499. [PubMed: 19258410]
132. Shin E, Hong SW, Kim SH, et al. Expression of down stream molecules of ret (p-erk, p-p38 mapk, p-jnk and p-akt) in papillary thyroid carcinomas. Yonsei Med J 2004;45:306–313. [PubMed: 15119004]
133. Shinohara M, Chung YJ, Saji M, et al. Akt in thyroid tumorigenesis and progression. Endocrinology 2007;148:942–947. [PubMed: 16946008]
134. Staal SP. Molecular cloning of the akt oncogene and its human homologues akt1 and akt2: Amplification of akt1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci U S A 1987;84:5034–5037. [PubMed: 3037531]
135. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, mmac1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15:356–362. [PubMed: 9090379]
136. Stokoe D. The phosphoinositide 3-kinase pathway and cancer. Expert Rev Mol Med 2005;7:1–22. [PubMed: 15938760]
137. Storz P, Toker A. 3′-phosphoinositide-dependent kinase-1 (pdk-1) in pi 3-kinase signaling. Front Biosci 2002;7:d886–902. [PubMed: 11897568]
138. Suarez HG, Du Villard JA, Caillou B, et al. Detection of activated ras oncogenes in human thyroid carcinomas. Oncogene 1988;2:403–406. [PubMed: 3283656]
139. Sun SY, Rosenberg LM, Wang X, et al. Activation of akt and eif4e survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. Cancer Res 2005;65:7052–7058. [PubMed: 16103051]
140. Suzuki H, Willingham MC, Cheng SY. Mice with a mutation in the thyroid hormone receptor beta gene spontaneously develop thyroid carcinoma: A mouse model of thyroid carcinogenesis. Thyroid 2002;12:963–969. [PubMed: 12490073]
141. Takano A, Usui I, Haruta T, et al. Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. Mol Cell Biol 2001;21:5050–5062. [PubMed: 11438661]
142. Testa JR, Tsichlis PN. Akt signaling in normal and malignant cells. Oncogene 2005;24:7391–7393. [PubMed: 16288285]
143. Toker A, Newton AC. Akt/protein kinase b is regulated by autophosphorylation at the hypothetical pdk-2 site. J Biol Chem 2000;275:8271–8274. [PubMed: 10722653]
144. Trimboli AJ, Cantemir-Stone CZ, Li F, et al. Pten in stromal fibroblasts suppresses mammary epithelial tumours. Nature 2009;461:1084–1091. [PubMed: 19847259]

145. Trotman LC, Alimonti A, Scaglioni PP, et al. Identification of a tumour suppressor network opposing nuclear akt function. Nature 2006;441:523–527. [PubMed: 16680151]

146. Vanhaesebroeck B, Ali K, Bilancio A, et al. Signalling by pi3k isoforms: Insights from gene-targeted mice. Trends in Biochemical Sciences 2005;30:194–204. [PubMed: 15817396]

147. Vasko V, Saji M, Hardy E, et al. Akt activation and localization correlate with tumor invasion and oncogene expression in thyroid cancer. J Mol Genet 2004;41:161–170.

148. Vasko V, Espinosa AV, Scouten W, et al. Gene expression and functional evidence of epithelial-to-mesenchymal transition in papillary thyroid carcinoma invasion. Proc Natl Acad Sci U S A 2007;104:2803–2808. [PubMed: 17296934]

149. Vasudevan KM, Barbie DA, Davies MA, et al. Akt–independent signaling downstream of oncogenic pik3ca mutations in human cancer. Cancer Cell 2009;16:21–32. [PubMed: 19573809]

150. Vilgelm A, Lian Z, Wang H, et al. Akt-mediated phosphorylation and activation of estrogen receptor alpha is required for endometrial neoplastic transformation in pten+/− mice. Cancer Res 2006;66:3375–3380. [PubMed: 16585156]

151. Vogt PK, Kang S, Elsliger MA, et al. Cancer-specific mutations in phosphatidylinositol 3-kinase. Trends Biochem Sci 2007;32:342–349. [PubMed: 17561399]

152. Wang J, Knauf JA, Basu S, et al. Conditional expression of ret/ptc induces a weak oncogenic drive in thyroid pccl3 cells and inhibits thyrotropin action at multiple levels. Mol Endocrinol 2003;17:1425–1436. [PubMed: 12690093]

153. Wang Y, Hou P, Yu H, et al. High prevalence and mutual exclusivity of genetic alterations in the pi3k/akt pathway in thyroid tumors. J Clin Endocrinol Metab 2007;92:2387–2390. [PubMed: 17426084]

154. Wu G, Mambo E, Guo Z, et al. Uncommon mutation but common amplifications of the pik3ca gene in thyroid tumors. J Clin Endocrinol Metab 2005:4688–4693. [PubMed: 15928251]

155. Wullschleger S, Loewith R, Hall MN. Tor signaling in growth and metabolism. Cell 2006;124:471–484. [PubMed: 16469695]

156. Yeager N, Klein-Szanto A, Kimura S, et al. Pten loss in the mouse thyroid causes goiter and follicular adenomas: Insights into thyroid function and cowden disease pathogenesis. Cancer Res 2007;67:959–966. [PubMed: 17283127]

157. Yeager N, Brewer C, Cai KQ, et al. Mammalian target of rapamycin is the key effector of phosphatidylinositol-3-oh initiated proliferative signals in the thyroid follicular epithelium. Cancer Res 2008;68:444–449. [PubMed: 18199538]

158. Yoeli-Lerner M, Yiu GK, Rabinovitz I, et al. Akt blocks breast cancer cell motility and invasion through the transcription factor nfat. Mol Cell 2005;20:539–550. [PubMed: 16307918]

159. Zhou GL, Tucker DF, Bae SS, et al. Opposing roles for akt1 and akt2 in rac/pak signaling and cell migration. J Biol Chem 2006;281:36443–36453. [PubMed: 17012749]

160. Zilberman DE, Cohen Y, Amariglio N, et al. Akt1 e17 k pleckstrin homology domain mutation in urothelial carcinoma. Cancer Genet Cytogenet 2009;191:24–27.

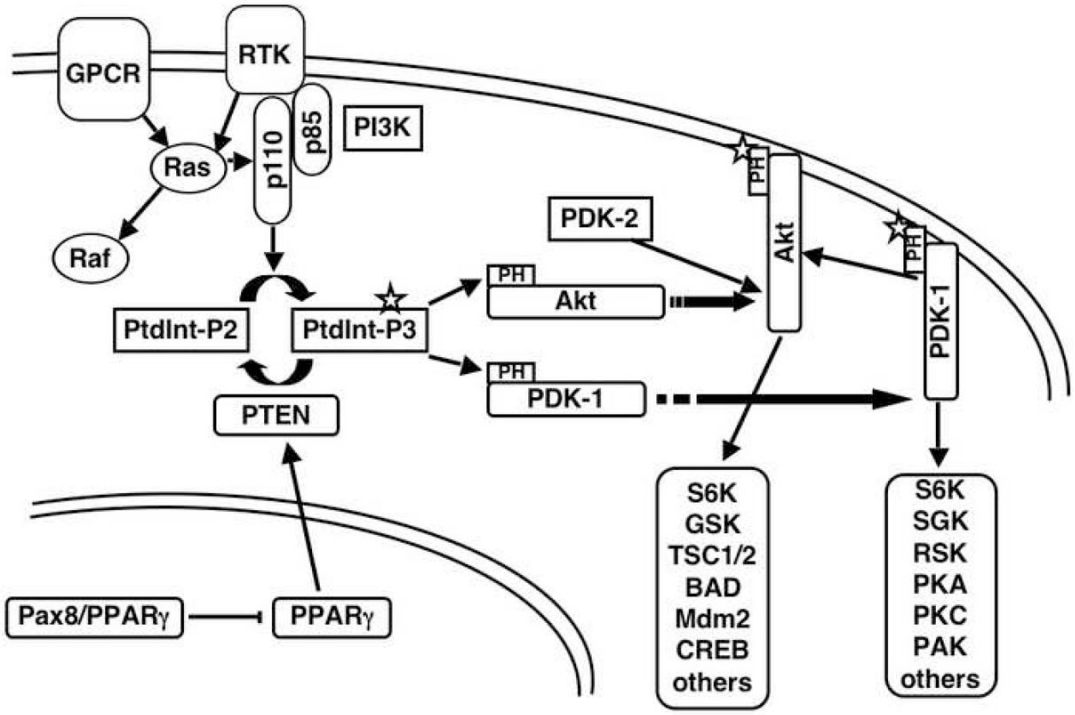

Figure 1.
Schematic presentation of PI3K-Akt signaling. PI3K is activated by RTK and GPCR stimulation, and subsequently phosphorylates and activates PDK-1 and Akt. [Adapted with permission from (Paes et al., 2008)].

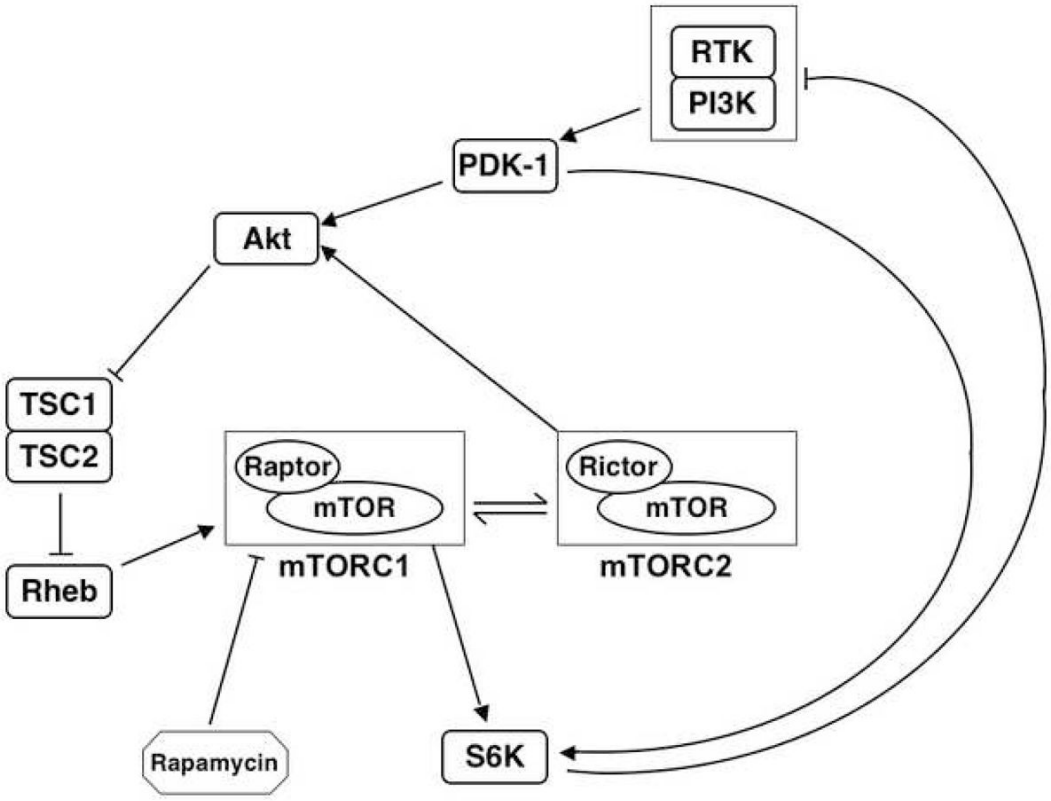

Figure 2.
Schematic presentation of Akt-mTOR signaling. Akt stimulates mTORC1 and 2 complex activities which leads to downstream signaling as well as both negative (mTORC1) and positive (mTORC2) feedback loops.