



Published in final edited form as:

Adv Cancer Res. 2009 ; 102: 19–65. doi:10.1016/S0065-230X(09)02002-8.

PI3K/PTEN Signaling in Angiogenesis and Tumorigenesis

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Abstract

Phosphatidylinositol 3-kinase (PI3K) and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) signaling pathway play an important role in multiple cellular functions such as cell metabolism, proliferation, cell-cycle progression, and survival. PI3K is activated by growth factors and angiogenesis inducers such as vascular endothelial growth factor (VEGF) and angiopoietins. The amplification and mutations of PI3K and the loss of the tumor suppressor PTEN are common in various kinds of human solid tumors. The genetic alterations of upstream and downstream of PI3K signaling molecules such as receptor tyrosine kinases and AKT, respectively, are also frequently altered in human cancer. PI3K signaling regulates tumor growth and angiogenesis by activating AKT and other targets, and by inducing HIF-1 and VEGF expression. Angiogenesis is required for tumor growth and metastasis. In this review, we highlight the recent studies on the roles and mechanisms of PI3K and PTEN in regulating tumorigenesis and angiogenesis, and the roles of the downstream targets of PI3K for transmitting the signals. We also discuss the crosstalk of these signaling molecules and cellular events during tumor growth, metastasis, and tumor angiogenesis. Finally, we summarize the potential applications of PI3K, AKT, and mTOR inhibitors and their outcome in clinical trials for cancer treatment.

I. INTRODUCTION OF PI3K/PTEN SIGNALING PATHWAY

The phosphatidylinositol 3-kinases (PI3Ks) in mammalian cells form a family that can be divided into three classes, class I, II, and III, based on their structure, substrate, distribution, mechanism of activation, and functions (Domin and Waterfield, 1997; Walker *et al.*, 1999). Among these classes, class I PI3Ks are the best understood to play vital roles in regulating cell proliferation, growth, and survival initiated by many growth and survival factors (Cantley, 2002; Fruman *et al.*, 1999; Morita *et al.*, 1999). Based on different associated adaptors, class I PI3Ks are divided into class IA and IB PI3Ks. Class IA PI3Ks are activated by receptor tyrosine kinases (RTKs), while class IB PI3Ks are activated by G-protein-coupled receptors (GPCRs) (Engelman *et al.*, 2006; Vanhaesebroeck *et al.*, 1997). Class IA PI3Ks consist of the heterodimers of a p110 catalytic subunit and a p85 regulatory subunit, and use phosphatidylinositol, phosphatidylinositol-4-phosphate (PIP), and phosphatidylinositol-4,5-bisphosphate (PIP₂) as substrates. Three isoforms of p110, p110 α , p110 β , and p110 δ are encoded by *PIK3CA*, *PIK3CB*, and *PIK3CD*, respectively. There are also three isoforms of p85 subunit: p85 α , p85 β , and p85 γ that are encoded by *PIK3R1*, *PIK3R2*, and *PIK3R3*, respectively. Class IB PI3Ks are composed of the heterodimers of a p110 γ catalytic subunit and a p101 regulatory subunit or its homologues p84 or p87PIKAP (PI3K γ adaptor protein of 87 kDa). Class II PI3Ks include PIK3C2 α , PIK3C2 β , and PIK3C2 γ , all of them are characterized by containing a common C2 domain at the C-terminus. Class II PI3Ks can also be activated by RTKs, cytokine receptors and integrins, and use phosphatidylinositol and PIP as substrates (Arcaro *et al.*, 2000; Falasca and Maffucci, 2007; MacDougall *et al.*, 2004;

Wheeler and Domin, 2001). But the specific functions of class II PI3Ks in response to these activators are poorly understood. Class III PI3Ks are composed of the heterodimers of catalytic and adaptor subunits. This class of PI3Ks only uses phosphatidylinositol as a substrate (e.g., mammalian PI3K and yeast Vps34p). The structure of PI3K family is shown in Box 1. It has been indicated that class III PI3Ks are involved in the regulation of mammalian target of rapamycin (mTOR) activity in response to amino acid levels, and the regulation of autophagy in response to cellular stress (Gulati *et al.*, 2008; Tassa *et al.*, 2003). The class III PI3K Vps34 is present in all eukaryotic organisms, while both class I and II PI3Ks only exist in multicellular organisms.

The two subfamilies of class IA and IB PI3Ks have evolved in mammals. Class I, especially class IA PI3Ks, are the most extensively investigated in regulating cellular functions such as cell proliferation, growth, and survival. Class I PI3Ks catalyze the conversion of PIP₂ at the D-3 position to phosphatidylinositol-3,4,5-trisphosphate (PIP₃) via its regulatory subunit p85 linking to upstream receptors that are activated by growth factors or hormones (Cantley, 2002; Luo *et al.*, 2006; Zhao *et al.*, 2006). RTKs, such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and insulin-like growth factor 1 receptor (IGF-1R), can interact with the p85 regulatory subunit to activate PI3K (Hu *et al.*, 1992; McGlade *et al.*, 1992; Zhu *et al.*, 1992), while Ras protein directly interacts with the p110 catalytic subunit of PI3K in a GTP-dependent manner (Peyssonnaud *et al.*, 2000; Rodriguez-Viciana *et al.*, 1996). In addition, p85 subunit also binds to the intracellular proteins such as protein kinase C, SHP1, Rac, Rho, hormonal receptors, mutated Ras and Src, providing an integration point for p110 activation (Hennessy *et al.*, 2005). It has been demonstrated that PI3K can be regulated by the molecular switch, which is formed by a GTPase-responsive domain and an inhibitory domain on p85 regulatory subunit of PI3K. H-Ras and Rac1 activate PI3K by targeting the GTPase-responsive domain and the stimulatory effects can be blocked by the inhibitory domain, which functions by binding to tyrosine-phosphorylated molecules (Chan *et al.*, 2002).

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which is also known as MMAC1 or TEP1, was named due to its sequence homology with phosphatases and the cytoskeletal protein tensin (Dahia *et al.*, 1997; Li *et al.*, 1997b; Maehama and Dixon, 1998). PTEN is a tumor suppressor commonly mutated in many human cancers (Salmena *et al.*, 2008). PTEN locates on 10q23.3, which encodes a 403-residue dual-specificity phosphatase that has protein phosphatase activity, and lipid phosphatase activity that antagonizes PI3K activity (Maehama and Dixon, 1998). Since the product of p110 α , PIP₃, is a second messenger for promoting cell proliferation, growth, metabolism, and survival, PTEN hydrolyzes the 3-phosphate on PIP₃ to generate PIP₂, and negatively regulates PIP₃-mediated signaling pathways. Thus, PTEN plays an important role in phosphatidylinositol homeostasis (Maehama and Dixon, 1998). It has been demonstrated that PTEN can be upregulated by early growth regulated transcription factor 1 (EGR1) through direct binding to the PTEN promoter. In addition, peroxisome proliferator activated receptor γ (PPAR γ), p53, and activating transcription factor 2 (ATF2) can also transcriptionally upregulate PTEN by binding to its promoter (Patel *et al.*, 2001; Shen *et al.*, 2006; Stambolic *et al.*, 2001), while transforming growth factor (TGF)- β , nuclear factor kappaB (NF- κ B), and Jun negatively regulate PTEN mRNA expression (Hettinger *et al.*, 2007; Mahimainathan *et al.*, 2006; Xia *et al.*, 2007). Recently, it has been found that some microRNAs such as miR-21, miR-19a, and miR-214 inhibit PTEN through targeting the 3'-untranslated region (UTR) of PTEN, leading to inhibition of PTEN translation (Meng *et al.*, 2007; Pezzolesi *et al.*, 2008; Yang *et al.*, 2008). PTEN activity can also be regulated by the posttranslational regulation including phosphorylation, acetylation, oxidation, and control of its localization (Gericke *et al.*, 2006; Ikenoue *et al.*, 2008; Leslie, 2006; Planchon *et al.*, 2008; Tamguney and Stokoe, 2007).

Serine–threonine protein kinase AKT (also known as protein kinase B) is initially found to be the cellular homolog of AKT8 retroviral oncogene (Bellacosa *et al.*, 1991). AKT is one of the most important downstream targets of PI3K. Human AKT has three isoforms: AKT1, AKT2, and AKT3 (also known as PKB α , PKB β , and PKB γ , respectively). The product of PI3K, PIP3, binds to AKT and leads to the membrane recruitment of AKT, and also binds to phosphoinositide-dependent kinase 1 (PDK1) via their pleckstrin homology (PH) domains (Downward, 1998; Engelman *et al.*, 2006), then PDK1 phosphorylates AKT in the kinase domain (Thr 308 in AKT1). For the full activation of AKT, the phosphorylation within the carboxyl-terminal hydrophobic motif (Ser 473 in AKT1) of AKT by PDK2 is required (Hresko *et al.*, 2003; Sarbassov *et al.*, 2005; Stokoe *et al.*, 1997). Once activated, AKT moves to the cytoplasm and nucleus, where it phosphorylates, activates, or inhibits many downstream targets to regulate various cellular functions including cell metabolism, protein synthesis, cell survival/inhibition of apoptosis, and cell-cycle progression (Box 1). In this review, we will focus on the roles of class IA PI3Ks, PTEN, and AKT in tumor growth and angiogenesis.

II. ANGIOGENESIS REGULATED BY VEGF, ANGIOPOIETINS, AND PI3K ACTIVATION

Angiogenesis is the process by which new blood capillaries are generated from the preexisting vasculature. It is essential for the embryo development, female reproduction, tissue repair, inflammatory diseases, tumor growth, and metastasis. Tumor angiogenesis occurs by sprouting the new vessels from preexisting blood vessels or by inserting interstitial tissue columns into the lumen of preexisting vessels (Carmeliet and Jain, 2000). This process can be triggered by extracellular signals such as growth factors, by genetic alterations such as activation of oncogenes including PI3K, and by mutations of tumor suppressor genes such as PTEN and p53 (Carmeliet and Jain, 2000; Folkman, 1995). Among all the proangiogenic factors, vascular endothelial growth factor (VEGF) and angiopoietins (Ang) and their receptors—VEGF and Tie [tyrosine kinase with immunoglobulin (Ig) and EGF homology domains] receptors play important roles during tumor growth and angiogenesis.

VEGFR family and the Tie receptor family are expressed specifically in endothelium. The VEGF family members are secreted, dimeric glycoproteins. In mammals, VEGF family members consist of VEGF-A, -B, -C, -D, and placenta growth factor (PLGF) (Olsson *et al.*, 2006). VEGF-A plays a key role in vasculogenesis and angiogenesis. Genetic studies have demonstrated that *VEGF-A* gene knockout mice either homozygotes or heterozygotes die in the embryonic stage due to the defects in vasculature (Carmeliet *et al.*, 1996; Ferrara *et al.*, 1996). There are five human isoforms of VEGF-A: VEGF121, VEGF145, VEGF165, VEGF189, and VEGF206. Among them, VEGF121, VEGF165, and VEGF189 are the dominant subtypes based on the amount and biological activity (Olsson *et al.*, 2006; Shibuya, 2008). VEGF receptors have three family members: VEGFR1 (fms-like tyrosine kinase, Flt-1), VEGFR2 (Flk-1/KDR), and VEGFR3 (Flt-4). All three VEGF receptors contain tyrosine phosphorylation sites with regulatory and signaling functions. These receptors play critical role in promoting vasculogenesis during normal embryogenesis and pathologic angiogenesis. VEGF-A binds to both VEGFR1 and VEGFR2 to regulate tumorigenesis and angiogenesis, while VEGF-B and PLGF bind to VEGFR1. Under pathological conditions, the increased PLGF and VEGF-A can recruit monocytes/macrophages via VEGFR1 to cancer tissues or inflammatory lesions, and significantly induce angiogenesis (Brown *et al.*, 2001; Murakami *et al.*, 2008). VEGF-C and -D mainly bind to VEGFR3, and stimulate lymphangiogenesis.

VEGFR1 binds to the p85 regulatory subunit of PI3K on Tyr1213 and 1333 and has crosstalk with VEGFR2 in controlling cell migration, differentiation, and angiogenesis (Autiero *et al.*, 2003; Cunningham *et al.*, 1995). VEGFR2 is the predominant receptor in angiogenic signaling since it regulates endothelial cell migration, proliferation, differentiation and survival, as well

as vessel permeability and dilation (Cebe-Suarez *et al.*, 2006). It has been demonstrated that tyrosines 799 and 1173 of VEGFR2 are binding sites for the p85 subunit, and that activation of PI3K is responsible for endothelial cell proliferation (Dayanir *et al.*, 2001). Previous study showed that VEGFR2 was associated with p85 regulatory subunit of PI3K to phosphorylate p85 subunit, resulting in increased PI3K and AKT activities *in vitro* (Gerber *et al.*, 1998). Grb2-adaptor binder 1 (Gab1) PH domain serves as a primary actor in coupling VEGFR2 to PI3K through an amplification loop involving PIP3 and its PH domain (Dance *et al.*, 2006; Laramée *et al.*, 2007). VEGF-induced endothelial cell survival was blocked by PI3K inhibitors, wortmannin and LY294002, and by overexpression of a dominant-negative form of AKT (AKT-DN) (Gerber *et al.*, 1998). VEGFR3 is expressed in developing veins and lymphatics, in blood vessels in the vicinity of tumors, and in several benign and malignant tumor cells (Cebe-Suarez *et al.*, 2006). VEGFR3 can promote cell migration and survival in lymphatic endothelial cells by activating PI3K and mitogen-activated protein kinase (MAPK) pathways (Lin *et al.*, 2005; Makinen *et al.*, 2001).

The angiopoietins are a family of secreted proteins including three human angiopoietins (Ang-1, Ang-2, and Ang-4), and one mouse angiopoietin, Ang-3. Ang-1 is an angiogenic growth factor with a central role in promotion of structural integrity in the vasculature. Both Ang-1 and Ang-2 can bind to Tie2 receptor. Ang-1 is a Tie2 agonist, while Ang-2 could act as either a context-dependent competitive antagonist or an agonist depending on cell type and microenvironmental conditions (Davis *et al.*, 1996; Maisonpierre *et al.*, 1997). Transgenic overexpression of both Ang-1 and Ang-2 led to vascular defects (Sato *et al.*, 1995). Ang-3 is moderately expressed in multiple mouse tissues, and functions as a Tie2 activator or as a Tie2 antagonist. Ang-4 mRNA is abundantly expressed in human lungs, and functions as a Tie2 agonist (Jones *et al.*, 2001; Makinde and Agrawal, 2008). The Tie receptor family is comprised of Tie1 and Tie2/Tek. Ang-1, 2, 3, and 4 are specific ligands for Tie2. The specific ligand for Tie1 is unknown. The phosphorylation of Tie1 is dependent on Tie2 activation, suggesting that Tie2 tyrosine kinase domain may be responsible for phosphorylating Tie1 as a result of heterodimerization (Yuan *et al.*, 2007). Tie2 is expressed not only in vascular cells, but also in cancer cells. Several tumor cells express high levels of Ang-1, indicating an autocrine/paracrine loop of Ang-1-Tie2 signaling in the tumor. Genetic studies have showed that deletion of Ang-1 or Tie2 genes led to severe defects in the vasculature and subsequent lethality, suggesting that Ang-1/Tie2 signaling pathway is required in microvascular development (Makinde and Agrawal, 2008). There are several lines of evidence suggesting that PI3K/AKT signaling plays a major role in Ang-1-mediated cell migration, survival, and angiogenesis: (1) Ang-1 was shown to induce phosphorylation of Tie2, then recruited and interacted with p85 subunit of PI3K in a phosphotyrosine-dependent manner through their Src homology 2 (SH2) domains, resulting in the induction of PI3K activities and activation of AKT (Jones *et al.*, 1999); (2) Ang-1 induced survival, migration, and sprouting of endothelial cells through PI3K and AKT activation (Jones *et al.*, 1999; Kanda *et al.*, 2005; Kim *et al.*, 2000); (3) *In vivo* studies also showed that Ang-1 induced angiogenesis through increasing AKT phosphorylation and PI3K-mediated endothelial nitric oxide synthase (eNOS) activation (Babaei *et al.*, 2003; Cho *et al.*, 2004).

III. GENETIC ABERRATIONS OF PI3K, PTEN, AND AKT IN CANCER

PI3K activation is implicated to be involved in oncogenesis by the observation that PI3K is associated with the Src and the middle T oncoproteins (Sugimoto *et al.*, 1984; Whitman *et al.*, 1985). The activation of PI3K is through the interaction with p85 regulatory subunit of PI3K, which contains SH2 domains that bind to phosphotyrosines, and localize PI3K to the plasma membrane (Otsu *et al.*, 1991). The p110 α catalytic subunit of PI3K was initially identified as an oncogene from the spontaneous chicken tumor (Chang *et al.*, 1997). The expression of active PI3K by avian retrovirus induced the transformation of chick embryo

fibroblasts *in vitro*, and induced tumor in chicken (Chang *et al.*, 1997). Abnormalities of PI3K upstream molecules are common in cancer and this cascade has a role in tumorigenesis and neoplastic transformation. PI3K is also frequently mutated in various kinds of human cancers such as ovarian, breast, gastric, bowel, brain, colon, and hepatocellular carcinomas (Engelman *et al.*, 2006; Hennessy *et al.*, 2005; Jiang and Liu, 2008). The amplification of *PIK3CA*, the gene encoding p110 α catalytic subunit of PI3K, was observed in ovarian, cervical, gastric, and breast cancers (Engelman *et al.*, 2006; Hennessy *et al.*, 2005; Jiang and Liu, 2008). In addition, the somatic missense mutations of *PIK3CA* are the most frequently genetic aberrations in breast cancer, especially in HER2-amplified and hormone-receptor-positive breast cancers (Paradiso *et al.*, 2007). The mutations of *PIK3CA* were also found in colorectal, gastric, lung, ovarian, hepatocellular, thyroid, endometrial cancers, glioblastomas, acute leukemia, as well as in malignancies of the central nervous system (Campbell *et al.*, 2004; Jiang and Liu, 2008; Samuels *et al.*, 2004). The p85 regulatory subunit dimerizes with p110 catalytic subunit, and inhibits PI3K activity in normal cells. The deletion of p85 protein that lacks the inhibitory domain, and loss of the autophosphorylation site at the p85 inhibitory domain, commonly increases PI3K activity. The deletion and somatic mutations of p85 α regulatory subunit (*PIK3R1*) were rare, and occurred in primary human glioblastoma, colon, ovarian cancers, and lymphoma (Jucker *et al.*, 2002; Philp *et al.*, 2001). Recent study has demonstrated that *PIK3CA*-knockout mouse embryonic fibroblasts are deficient in cellular signaling in response to various growth factors, unable to differentiate into adipocytes, and are resistant to oncogenic transformation induced by RTKs (Zhao *et al.*, 2006). Another genetic study indicated that the kinase activity of p110 β was required for GPCR signaling triggered by lysophosphatidic acid and had a function in oncogenic transformation.

PTEN was first discovered as the tumor suppressor on human chromosome 10q23 in 1997 (Li *et al.*, 1997a; Steck *et al.*, 1997). *PTEN* is highly susceptible to deletion or mutation in many human malignancies including brain, breast, kidney, and prostate cancers (Li *et al.*, 1997a; Steck *et al.*, 1997). A series of studies have shown that the tumor suppressor *PTEN* is frequently mutated or lost in many kinds of human primary cancers including glioblastomas, kidney and uterine endometrioid carcinomas, breast cancer, lung cancer, colon cancer, and melanoma (Jiang and Liu, 2008; Salmena *et al.*, 2008; Steck *et al.*, 1997). In addition, the decreasing levels of *PTEN* expression are correlated with the progressive outcome of solid cancers, including ovarian, prostate, and cervical cancers (Harima *et al.*, 2001; Yoshimoto *et al.*, 2007). *PTEN* germline mutations lead to a group of autosomal dominant syndromes including Cowden syndrome, Lhermitte–Duclos disease, Bannayan–Riley–Ruvalcaba syndrome, and Proteus and Proteus-like syndromes characterized by developmental disorders, neurological deficits, multiple hamartomas, and an increased risk of breast, thyroid, and endometrial cancers (Liaw *et al.*, 1997; Marsh *et al.*, 1997; Tsou *et al.*, 1997; Tsuchiya *et al.*, 1998). Mice with *PTEN* deletion and mutation are highly susceptible to tumor induction and conditional knockout of *PTEN* leads to neoplasia in multiple organs such as the mammary gland, skin, and prostate (Backman *et al.*, 2004; Li *et al.*, 2002; Suzuki *et al.*, 1998). In an animal model of prostate tumor induced by *PTEN* loss, ablation of p110 β impeded tumorigenesis with a concomitant diminution of AKT phosphorylation (Jia *et al.*, 2008), indicating the important role of p110 β in cell transformation and tumorigenesis. These studies demonstrate the key roles of PI3K and *PTEN* in cancer development. The transgenic ablation models of PI3K and *PTEN* in tumorigenesis are summarized in Table I.

IV. ROLES OF PI3K AND AKT IN REGULATING ANGIOGENESIS

PI3K/AKT signaling pathway also plays an important role in regulating the vasculature and angiogenesis. In zebrafish, K-ras/PI3K/AKT signaling is essential for hematopoiesis and angiogenesis (Liu *et al.*, 2008a). The direct evidence of PI3K and AKT involvement in regulating angiogenesis *in vivo* was initially observed by the forced expression of PI3K and

AKT using RCAS retroviral vector system (Jiang *et al.*, 2000). Overexpression of PI3K or AKT induced angiogenesis, while overexpression of PTEN or of dominant-negative constructs of PI3K inhibited angiogenesis in chicken embryos, suggesting that PI3K signaling is required for normal embryonic angiogenesis (Jiang *et al.*, 2000). Mice deficient in the p110 α catalytic subunit of PI3K displayed multiple vascular defects, including dilated vessels in the head, reduced branching morphogenesis in the endocardium, lack of hierarchical order of large and small branches in the yolk sac, impaired development of anterior cardinal veins, and significant decrease of Tie2 protein level (Lelievre *et al.*, 2005). In mice deficient in p110 γ , the vascular permeability response to both Ras and VEGF was significantly diminished, suggesting that PI3K γ is necessary and sufficient for vascular permeability (Serban *et al.*, 2008). Endothelial cell-specific-p110 α ^{-/-} led to embryonic lethality at mid-gestation due to severe defects in angiogenic sprouting and vascular remodeling (Graupera *et al.*, 2008). Knockout of p85 α /p55 α /p50 α caused perinatal lethality with bleeding into the blebs during the turning process (Brachmann *et al.*, 2005). Muscle-specific pan-p85 α ^{-/-} p85 β ^{-/-} mice exhibited reduced heart size and altered cardiac gene expression (Luo *et al.*, 2005). Mutated p110 α proteins show a gain of enzymatic function *in vitro*. Recent studies show that three prevalent mutants of p110 α , E542K, E545K, and H1047R, are oncogenic *in vivo* (Bader *et al.*, 2006). These tumors are marked by increased angiogenesis and the activation of AKT pathway (Bader *et al.*, 2006).

AKT was initially found to be the homolog of a viral oncogene (Bellacosa *et al.*, 1991). In various kinds of tumors, AKT is also overexpressed or amplified, with elevated level of AKT phosphorylation (Hennessy *et al.*, 2005; Jiang and Liu, 2008). There are several reports showing the genetic amplification of AKT isoforms. *AKT1* amplification has been observed in gastric adenocarcinoma, glioblastoma, gliosarcoma, and high-grade gliomas (Jiang and Liu, 2008; Liaw *et al.*, 1997; Sasaki *et al.*, 2003; Staal, 1987). *AKT2* amplification or mutations are found in head and neck squamous cell carcinoma, pancreatic, ovarian, breast, and colorectal cancers (Hennessy *et al.*, 2005; Jiang and Liu, 2008). Increased *AKT3* mRNA level is correlated to breast and prostate cancers (Nakatani *et al.*, 1999). Recent studies have shown that *AKT1*^{-/-} mice are resistant to ErbB2-or MMTV-v-H-Ras-induced carcinogenesis, indicating the key role of AKT1 in oncogenesis (Ju *et al.*, 2007; Skeen *et al.*, 2006). Among three isoforms of AKT, AKT1 shows closely related with vasculature during animal development and pathological angiogenesis. *AKT1*^{-/-} mice have defects in both fetal and postnatal growth into adulthood with smaller litter sizes and reduced fetal weight (Chen *et al.*, 2001; Cho *et al.*, 2001b). Since AKT1 is widely expressed in placenta including all types of trophoblast and vascular endothelial cells, *AKT1*^{-/-} mice exhibited a higher fetal mortality due to the impaired extraembryonic vascularization and placental hypotrophy, indicating the significant role of AKT1 in fetal development and vascularization (Yang *et al.*, 2003). AKT1 is the predominant isoform in vascular cells. *AKT1*^{-/-} mice showed impaired vascular maturation due to reduced activation of eNOS and the major phenotypic changes in vascular permeability and angiogenesis with decreased expression of thrombospondins 1 and 2 (TSP-1 and TSP-2) (Chen *et al.*, 2005). AKT1 is critical for ischemic-and VEGF-induced angiogenesis. *AKT1*^{-/-} mice exhibited defective ischemia-and VEGF-induced angiogenesis and showed severe peripheral vascular disease. In response to ischemia, *AKT1*^{-/-} mice had much less endothelial progenitor cell (EPC) mobilization. Intravenous administration of EPCs from wild-type AKT1 mice, but not EPCs isolated from *AKT1*^{-/-} mice, into mice improved limb blood flow, increased the migration of fibroblasts and endothelial cells after femoral ligation. These results indicate that AKT1 is sufficient and essential for regulating ischemia-induced angiogenesis (Ackah *et al.*, 2005). *AKT2*^{-/-} mice displayed normal cardiac growth in response to provocative stimulation, and were sensitized to cardiomyocyte apoptosis in response to ischemic injury (DeBosch *et al.*, 2006). The studies on transgenic models related to vasculature and angiogenesis are summarized in Table I.

V. PI3K/PTEN CONTROLS ANGIOGENESIS THROUGH INCREASING HIF-1 AND VEGF EXPRESSION

Hypoxia is an integral characteristic of the tumor microenvironment, associated with accelerated neoplastic growth. Hypoxia-inducible factor 1 (HIF-1) is a heterodimer consisting of HIF-1 α and HIF-1 β [also known as the aryl hydrocarbon nuclear translocator (ARNT)] subunits, and acts as a mediator of transcriptional activation in responses to hypoxia (Wang *et al.*, 1995). HIF-1 α is rapidly degraded under normoxic conditions by hydroxylation at several proline residues, and acetylation at lysine 5328 (Jeong *et al.*, 2002; Semenza, 2000). The von Hippel-Lindau tumor suppressor gene product, pVHL, functions as the substrate recognition component of an E3-ubiquitin ligase, which targets the oxygen-sensitive HIF alpha-subunit for rapid proteasomal degradation under normoxic conditions and as such plays a central role in oxygen sensing (Maxwell *et al.*, 1999). Hypoxia or loss of pVHL inhibits prolyl-hydroxylation, leading to accumulation of HIF-1 α protein in the cytoplasm (Kapitsinou and Haase, 2008). Growth factors, cytokines, and other signaling molecules stimulate HIF-1 α synthesis via activation of PI3K or MAPK pathways (Mazure *et al.*, 1997; Zhong *et al.*, 2000). HIF-1 regulates VEGF expression by binding to the hypoxia responsive element (HRE) of VEGF promoter (Levy *et al.*, 1995; Wang *et al.*, 1995). HIF-1 can activate more than 60 known genes, which are related to cell proliferation, survival, apoptosis, cell mortality, adhesion, erythropoiesis, cytoskeletal structure, pH regulation, epithelial homeostasis, drug resistance, iron, nucleotide, glucose, energy, amino acid, and extracellular-matrix metabolisms, vascular tone, and angiogenesis (Semenza, 2003). HIF1 α is upregulated in many human cancers. Among all the angiogenic factors, VEGF is the most potent one in physiological and pathological angiogenesis.

HIF-1 α expression is regulated by PI3K activation in response to growth factors. Insulin and EGF induced expression of HIF-1 α and VEGF by PI3K signaling pathway (Jiang *et al.*, 2001). Cobalt and hypoxia induced HIF-1 α expression through PI3K-dependent mechanism in airway smooth muscle and pulmonary artery smooth muscle cells (Belaiba *et al.*, 2007; Chachami *et al.*, 2004). HIF-1-dependent gene transcription was blocked by AKT-DN or PI3K, and by wild-type PTEN, whereas transcription was stimulated by constitutively active form of AKT. PI3K inhibitor LY294002 and mTOR inhibitor rapamycin also inhibited growth factor- and mitogen-induced secretion of VEGF, which may provide the connection of PI3K/PTEN/AKT to mTOR, HIF-1, and tumor angiogenesis (Jiang *et al.*, 2001; Zhong *et al.*, 2000). On the other hand, overexpression of PI3K or AKT elevated the mRNA levels of VEGF. LY294002 suppressed VEGF mRNA expression, while this inhibition was restored by overexpression of PI3K or AKT (Jiang *et al.*, 2000). These results indicate that PI3K is sufficient to induce angiogenesis, and the effect may be partially through increasing HIF-1 and VEGF expression. Similarly, VEGF transcriptional activation in ovarian cancer cells was regulated by PI3K/AKT through HIF-1 α expression (Skinner *et al.*, 2004). A number of studies have demonstrated that PI3K/PTEN/AKT signaling regulates HIF-1 and VEGF expression in different types of cancer cells, Ras-transformed cells, airway smooth muscle cells, pulmonary artery smooth muscle cells, osteoblasts, pulmonary vascular endothelial cells, and mast cells (Belaiba *et al.*, 2007; Carver *et al.*, 2007; Chachami *et al.*, 2004; Jiang *et al.*, 2001; Lee *et al.*, 2008; Mazure *et al.*, 1997; Trisciuglio *et al.*, 2005; Yen *et al.*, 2005; Zhong *et al.*, 2000). Mast cells mediated VEGF expression by HIF-1 α activation through PI3K-HIF-1 α pathway in mice with allergic airway disease, resulting in the increase of vascular permeability (Lee *et al.*, 2008). Hypoxia exposure of melanoma cells overexpressing bcl-2 activated phosphorylation of AKT and extracellular signal-regulated kinase (ERK)1/2 proteins, induced VEGF and HIF-1 expression, which can be suppressed by PI3K and MAPK inhibitors, suggesting that bcl-2 synergizes with hypoxia to promote expression of angiogenesis factors in melanoma cells through both PI3K and ERK pathways (Trisciuglio *et al.*, 2005).

Consistent with those results *in vitro*, *in vivo* studies showed that LY294002 significantly decreased the tumor burden of mice and inhibited peritoneal and tumor vascularization, which resulted in numerous leaky, irregular, tortuous vessels in scant, straight, relatively impermeable vessels, demonstrating the role of PI3K in mediating angiogenesis and vascular permeability associated with ovarian carcinoma (Hu *et al.*, 2005). Specific downregulation of p110 α expression in ovarian cancer cells using small interfering RNA (siRNA) showed that p110 α knockdown greatly decreased ovarian tumor growth and angiogenesis, inhibited VEGF expression through decreasing HIF-1 α expression in both ovarian cancer cells and tumor tissues. Moreover, AKT1 is a major downstream mediator for regulating tumor growth, angiogenesis, and VEGF expression, suggesting that p110 α and AKT1 play an important role in tumor growth by inducing angiogenesis and by increasing HIF-1 α and VEGF expression (Xia *et al.*, 2006). Inhibition of PI3K activity by LY294002 decreased cancer cell-induced angiogenesis (Fang *et al.*, 2007). Reconstitution of PTEN or over-expression of AKT dominant negative also inhibited angiogenesis and tumor growth associated with the decrease of HIF-1 α and VEGF expression in the tumor xenographs (Fang *et al.*, 2007). These results suggest that PI3K and AKT may regulate tumorigenesis and angiogenesis through HIF-1 and VEGF expression in cancer cells.

VI. THE DOWNSTREAM SIGNALING MOLECULES MEDIATED BY PI3K/PTEN IN REGULATING TUMOR GROWTH AND ANGIOGENESIS

Overexpression and activation of AKT play an important role in carcinogenesis (Engelman *et al.*, 2006; Hennessy *et al.*, 2005; Jiang and Liu, 2008). The mutations or deletions of PTEN are presented in many kinds of solid tumors. As shown in Fig. 1, upon the stimulation of VEGF and other growth factors, RTKs can activate PI3K which exerts its effect through AKT and other downstream targets (Engelman *et al.*, 2006; Jiang and Liu, 2008). GSK-3 β , the downstream target of AKT, together with the adenomatous polyposis coli (APC) protein and axin, forms a multiprotein complex which phosphorylates β -catenin making it for subsequent ubiquitination and degradation (Liu *et al.*, 2005; Rubinfeld *et al.*, 1996). Thus, the reduced expression of GSK-3 β can cause the increase of β -catenin activity. On the other hand, PI3K may indirectly activate ERK and p38 MAPK signaling pathways through Rho GTPases (Mizukami *et al.*, 2006; Xue *et al.*, 2006). Recent study has demonstrated that in addition to suppress AKT activation, PTEN also controls the activity of Jun N-terminal kinase (JNK) (Vivanco *et al.*, 2007). Both AKT and ERK can activate NF- κ B pathway, performing a complicated network in regulating tumor growth, metastasis, and angiogenesis (Fig. 1). The downstream signaling molecules related to tumorigenesis and angiogenesis are outlined in Fig. 1, and briefly described below.

A. Tumor Growth

PI3K may transmit oncogenic signals to AKT for regulating tumorigenesis through several downstream targets. AKT can directly phosphorylate human double minute 2 (HDM2) and regulate HDM2 through p70S6K1 activation (Fang *et al.*, 2005; Mayo and Donner, 2001; Skinner *et al.*, 2004). HDM2 regulates tumor suppressor p53 by promoting its proteasome-mediated degradation (Fang *et al.*, 2006; Skinner *et al.*, 2004). p53 plays a key role in carcinogenesis and cellular apoptosis. AKT activates NF- κ B pathway by the phosphorylation of I kappaB kinase (IKK) α/β (Hurt *et al.*, 2002; Lu and Wahl, 2005; Ozes *et al.*, 1999; Tanaka *et al.*, 2005). Activated AKT pathway also exhibits the antiapoptotic effect through the activation of nitric oxide synthase (NOS), the inhibition of FOXO-mediated transcription of proapoptotic proteins, and the inactivation of proapoptotic protein BAD by phosphorylation to activate survival signals. In addition, AKT regulates cell proliferation and tumor growth by increasing the cell-cycle progression. AKT blocks FOXO-mediated transcription of cell-cycle inhibitors, and promotes G1 to S phase transition. AKT stabilizes c-Myc and cyclin D1 through

the activation of NF- κ B pathway and GSK-3 β / β -catenin-signaling axis. Cell proliferation, size, and growth are tightly regulated by the activation of mTOR through PI3K/AKT and MAPK pathways. AKT and MAPK can regulate mTOR to control protein synthesis and cell proliferation, which are associated with carcinogenesis. The regulation of cell survival and cell cycle is associated with the increased cell number in tumors.

B. Tumor Metastasis

The basement membrane forms a cellular support for tumors, and is made up of a complex mix of extracellular matrix (ECM) proteins. The proteolytic enzymes including matrix metalloproteinases (MMPs) can degrade ECM (Orlichenko and Radisky, 2008). PI3K activates MMP-2, MMP-9, and Urokinase-type plasminogen activator (uPA), leading to destruction of ECM (Ispanovic and Haas, 2006; Shukla *et al.*, 2007). PI3K activity is shown to be higher in metastatic cells when compared to non-metastatic cancer cells. Increased levels of MMPs are also due to the activation of AKT/IKK/NF- κ B pathway and AKT/GSK-3 β / β -catenin axis (Agarwal *et al.*, 2005; Amiri and Richmond, 2005; Ispanovic and Haas, 2006; Kim *et al.*, 2005). PI3K signaling also regulates chemokine (C-X-C motif) ligand 1 (CXCL-1), cyclooxygenase-2 (COX-2), and interleukin-8 (CXCL-8) that enhance tumor metastasis. PI3K and AKT regulate epithelial-mesenchymal transition (EMT), which is a change thought to herald tissue invasion and prophesize metastatic potential (Cheng *et al.*, 2008; Onoue *et al.*, 2006). NF- κ B plays a key role in EMT by the activation of mesenchymal program (involving genes such as MMP2/9, VCAM-1, ICAM-1, and Cathepsins B and Z) (Huber *et al.*, 2004) and the repression of E-cadherin, a metastasis suppressor protein, by activating bcl-2 and TWIST (Naugler and Karin, 2008). E-cadherin is a key marker of EMT and loss of E-cadherin disrupts not only cell-cell junctions, but also allows for loss of the normal organ architecture. β -Catenin plays an important role in downregulating E-cadherin expression (Brabletz *et al.*, 2005; Lu *et al.*, 2003). PI3K and AKT also increase invasiveness and downregulate E-cadherin expression (Grille *et al.*, 2003; Larue and Bellacosa, 2005; Schramek *et al.*, 2003; Thiery and Sleeman, 2006). Cell motility is a fundamental process during tumor metastasis. PI3K in combination with the small GTPase Rac and Cdc42 regulates cell motility by controlling actin dynamics in motile cells (Engelman *et al.*, 2006). ERK pathway is also involved in regulating the expression of MMPs, cell migration, and EMT (Reddy *et al.*, 2003).

C. Tumor Angiogenesis

First, PI3K and AKT may regulate tumor angiogenesis by several downstream targets such as mTOR/p70S6K1 signaling axis, the inhibition of FOXO, the induction of NOS (Emerling *et al.*, 2008; Engelman *et al.*, 2006; Quintero *et al.*, 2006; Wang *et al.*, 2004), and/or the inhibition of GSK-3 β . These targets commonly increase HIF-1 α expression which induces VEGF transcriptional activation. Inhibition of GSK-3 β by the activation of PI3K/AKT can upregulate HIF-1 α expression, and increases β -catenin activity, which can enhance HIF-1-mediated transcription through the β -catenin-HIF-1 α interaction at the promoter region of HIF-1 target genes (Kaidi *et al.*, 2007; Mottet *et al.*, 2003). In addition, hypoxia is a hallmark of the tumor microenvironment in the fast growth tumor. Hypoxia induces HIF-1 α production through the increase of its stability and the activation of ERK1/2 pathway. In some kinds of cancer cells, hypoxia stimulates multiple K-*ras* effectors and PI3K, which induces VEGF expression in a HIF-1-dependent manner or via PI3K/Rho/ROCK/c-myc pathway (Mizukami *et al.*, 2006; Xue *et al.*, 2006). PI3K can induce VEGF expression through HIF-1, ERK1/2, and NF- κ B activation to induce tumor angiogenesis. NF- κ B can also stimulate tumor necrosis factor (TNF), CXCL-8, IL-1, and IL-6 to induce VEGF (Amiri and Richmond, 2005; Sparmann and Bar-Sagi, 2004). Growing evidence has shown the key roles of PI3K, AKT, mTOR, and their effectors HIF-1 α and VEGF in regulating cancer cell-induced angiogenesis (Fang *et al.*, 2007; Hu *et al.*, 2005; Xia *et al.*, 2006).

Next, the angiogenesis and vasculature are regulated through the change of balance between the collective actions of proangiogenic factors (e.g., VEGF) and angiogenic inhibitors (e.g., TSP-1). PI3K/AKT can increase VEGF expression and suppress TSP-1, the endogenous antiangiogenic molecule, in both cancer cells and endothelial cells (Niu *et al.*, 2004; Wen *et al.*, 2001). Furthermore, *AKT1*^{-/-} mice showed impaired vascular maturation with decreased expression of TSP-1 and TSP-2, while reexpression of TSP-1 and TSP-2 in mice transplanted with wild-type bone marrow is associated with the angiogenic abnormalities in *AKT1*^{-/-} mice (Chen *et al.*, 2005). Thus, PI3K/AKT signaling pathway induces tumor growth through the overexpression of angiogenic factors and the inhibition of antiangiogenic molecules.

Third, tumor angiogenesis is regulated by the tumor microenvironments composed of tumor cells, vascular endothelial cells, and stromal cells. In addition to cancer cells, the microvascular endothelial cells recruited by the tumor are important for cancer development (Carmeliet and Jain, 2000; Stoeltzing *et al.*, 2006). PI3K/AKT pathway also controls tumor microenvironments, including endothelial cells (Phung *et al.*, 2006; Yuan *et al.*, 2007). PI3K can regulate endothelial migration, proliferation, and survival through the effect of its downstream targets such as NOS, p70S6K1, and FOXO to regulate tumor angiogenesis (Fosbrink *et al.*, 2006; Nakao *et al.*, 2007; Zheng *et al.*, 2008). Class IA PI3Ks regulate vessel integrity during development and tumorigenesis (Yuan *et al.*, 2008). Further analysis of p110 isoforms has demonstrated that p110 α is required to control endothelial cell migration and angiogenesis, and p110 α ^{-/-} endothelial cells lead to embryonic lethality with severe defects in angiogenic sprouting and vascular remodeling (Graupera *et al.*, 2008; Suzuki *et al.*, 2007). PTEN^{-/-} endothelial cells cause embryonic lethality due to endothelial cell hyperproliferation and impaired vascular remodeling; PTEN^{+/-} endothelial cells enhance postnatal neovascularization and tumor angiogenesis to increase tumor growth (Suzuki *et al.*, 2007). Transgenic expression of Myr-AKT1 in endothelial cells is sufficient to recapitulate the abnormal structural and functional features of tumor blood vessels in nontumor tissues, likely due to the induction of VEGF-A (Jiang *et al.*, 2000; Phung *et al.*, 2006). Sustained endothelial AKT activation causes enlarged and hyperpermeable blood vessels and its effect can be completely reversed by AKT inhibition or by rapamycin treatment (Phung *et al.*, 2006). Our studies using chimeric tumor model found that overexpression of p70S6K1 in human dermal microvascular endothelial cells (HDMECs) enhanced tumor growth and angiogenesis, while over-expression of p70S6K1-kinase mutant, or of HIF-1 α siRNA significantly inhibited tumor growth and angiogenesis, suggesting that endothelial p70S6K1 controls tumor angiogenesis through HIF-1 α and VEGF expression (Liu *et al.*, 2008b).

The interaction of cancer cells and vascular endothelial cells in the tumor microenvironment affects angiogenesis. In cancer cells, stimuli such as growth factors, insulin, and other hormones activate PI3K/AKT/mTOR/HIF-1 α axis, and induce the production of VEGF, which switches angiogenic response and causes endothelial cell activation and permeability increased by PI3K pathway (Nyberg *et al.*, 2008; Stoeltzing *et al.*, 2006). Thus, inhibition of PI3K/AKT/mTOR pathway is one of the choices in cancer treatment, which is going on under the preclinical and clinical trials. The signaling pathway of PI3K related to tumor growth, metastasis, and angiogenesis is shown in Fig. 1.

VII. INHIBITION OF PI3K SIGNALING PATHWAY FOR CANCER TREATMENT AND PREVENTION

Given the important role of PI3K signaling pathway in regulating tumor growth and angiogenesis, development of therapeutic drugs using PI3K, AKT, and mTOR inhibitors becomes important for cancer treatment. Here, we introduce the inhibitors of PI3K, AKT, and mTOR.

A. PI3K Inhibitors

PI3K inhibitors, wortmannin, and LY294002, are commonly used to inhibit cancer cell proliferation and tumor growth, and sensitize tumor cells to the treatment of chemotherapeutic drugs and radiation (Granville *et al.*, 2006). Wortmannin is a fungal product isolated from *Penicillium wortmanni* in 1957, which exerts its effect by the covalent interaction to the conserved Lys802 of the PI3K α catalytic subunit and Lys833 in PI3K γ (Walker *et al.*, 2000; Wymann *et al.*, 1996). The pan-PI3K inhibitor LY294002 was synthesized in the early nineties. Both wortmannin and LY294002 also cross-react with PI3K-related kinases such as mTOR and DNA-dependent protein kinases (DNA-PKs). These PI3K inhibitors have poor solubility and high toxicity because they target a broad range of PI3K-related enzymes, which limits their clinical application (Marone *et al.*, 2008). To overcome these shortcomings, many derivatives of wortmannin and LY294002 are being developed (Marone *et al.*, 2008). In addition, inositol (1,3,4,5,6) pentakisphosphate [Ins(1,3,4,5,6)P₅], the PI3K/AKT inhibitor, inhibits tumor growth and angiogenesis *in vitro* and *in vivo* (Maffucci *et al.*, 2005). PWT-458, a novel pegylated 17-hydroxywortmannin, is water-soluble and has shown significant improvements in drug stability as well as *in vivo* pharmacokinetic parameters. It inhibits PI3K signaling and suppresses growth of solid tumors in nude mice (Yu *et al.*, 2005). SF1126, a small molecule conjugate containing a pan-PI3K inhibitor, suppresses PI3K class IA isoforms and other key members of the PI3K superfamily including DNA-PK. In preclinical studies, it has been shown to inhibit tumor growth, dissemination, and angiogenesis (Garlich *et al.*, 2008). The other two pan-PI3K inhibitors, PI-103 and ZSTK474 share the arylmorpholine structure of LY294002. PI-103 is a dual PI3K IA/mTOR inhibitor, while ZSTK474 inhibits the activity of all class I PI3Ks. Both of these drugs exhibit antitumor effect on various kinds of cancers (Chaisuparat *et al.*, 2008; Fan *et al.*, 2006; Kong and Yamori, 2007; Yaguchi *et al.*, 2006; Yuan and Cantley, 2008). IC486068, a p110 δ specific inhibitor, enhances radiation-induced tumor vascular destruction (Geng *et al.*, 2004). NVP-BEZ235, an orally administered inhibitor of dual pan-class I PI3K and mTOR kinase, inhibits the growth of breast and prostate cancer cells with active mutations of PI3K, and decreases tumor vasculature (Maira *et al.*, 2008; Schnell *et al.*, 2008; Serra *et al.*, 2008). Recent study has shown that the dual PI3K/PDK-1 inhibitor, BAG956, has inhibitory effect on BCR-ABL- and mutant FLT3-expressing cells both *in vitro* and *in vivo* (Weisberg *et al.*, 2008).

Several PI3K inhibitors are used in clinical trials now. For example, XL147 and XL765, the exelixis compounds, are in phase I trials for the treatment of solid tumors. NVP-BEZ235 and another Novartis compound, BGT226, are in ongoing trials for breast and other solid tumors with some promising results (Yuan and Cantley, 2008).

B. AKT Inhibitors

AKT is a major downstream target of PI3K for regulating tumor growth and angiogenesis. The first developed group of AKT inhibitors were lipid-based inhibitors that include perifosine, phosphatidylinositol ether lipid analogs (PIAs), and D-3-deoxy-phosphatidylmyoinositol-1-[(R)-2-methoxy-3-octadecyloxypropyl hydrogen phosphate] (PX-316), which showed antitumor effects *in vitro* and *in vivo* (Gills *et al.*, 2006; Granville *et al.*, 2006; Jiang and Liu, 2008; Meuillet *et al.*, 2004). Several other AKT antagonists such as 9-methoxy-2-methylellipticinium acetate (API-59-OMe), indazole-pyridine A-443654, and isoform-specific canthine alkaloid analogs have been identified using high-throughput screening of the chemical libraries and shown to inhibit human cancer cell growth and induce apoptosis (Granville *et al.*, 2006; Liu *et al.*, 2008c; Shi *et al.*, 2005). Other kinds of AKT inhibitors being developed include peptide-based inhibitors of AKT (e. g., KP372-1), pseudopeptide substrates of AKT, a single-chain antibody (scFv) against AKT, an inhibitory form of AKT expressed by adenovirus virus system, and siRNA against AKT (Granville *et al.*, 2006; Jiang and Liu, 2008; Litman *et al.*, 2007; Mandal *et al.*, 2006; Xia *et al.*, 2006).

Perifosine is one of the best-characterized AKT inhibitors, which inhibits the translocation of AKT to the cell membrane. Perifosine inhibits tumor growth in several different kinds of solid tumors. It has been used for clinical trials for the treatment of prostate, breast, gastrointestinal stromal tumors, melanoma, and soft tissue sarcoma, but the clinical outcomes were not satisfied (Table II).

C. mTOR Inhibitors

The mTOR inhibitor, rapamycin (sirolimus) and its analogs CCI-779 (temsirolimus), RAD001 (everolimus), and AP-23573 (deforolimus) inhibit mTOR activation by binding to FK506-binding protein-12 (Hennessy *et al.*, 2005). These drugs are currently under the clinical trials for cancer treatment. Preclinical studies with these compounds indicated that these compounds have synergistic effects for inhibiting tumor growth when they are used with conventional chemotherapy agent or radiation treatment. In clinical studies, these compounds have been shown to be effective against many types of cancers (Easton and Houghton, 2006; Faivre *et al.*, 2006). In phase I trials, rapamycin has shown anticancer activity in recurrent glioblastoma and gefitinib plus rapamycin can be safely coadministered on a continuous, daily dosing schedule (Cloughesy *et al.*, 2008; Reardon *et al.*, 2006). In phase II and III clinical studies, CCI-779 has been shown to have effects for treating patients with advanced breast cancer and advanced refractory renal-cell carcinoma (Atkins *et al.*, 2004; Chan *et al.*, 2005). Moreover, CCI-779 increased the effect of interferon alpha, improved overall survival among patients with metastatic renal-cell carcinoma, and a poor prognosis (Hudes *et al.*, 2007; Motzer *et al.*, 2007). RAD001 is administered orally for clinical application. The phase II clinical studies have shown that RAD001 treatment enhances the effect of gefitinib in advanced nonsmall cell lung cancer patients, increased the effect of lerazole in advanced breast cancer patients. It is also shown benefits for treating low- to intermediate- grade neuroendocrine tumor combination with octreotide (Awada *et al.*, 2008; Milton *et al.*, 2007; Yao *et al.*, 2008). A recent study has shown that treatment with RAD001 prolongs progression-free patient survival when compared to placebo treated patients with metastatic renal-cell carcinoma that has progressed on other targeted therapies (Motzer *et al.*, 2008). AP-23573 is a phosphorus-containing derivative of rapamycin, and developed in both intravenous and oral formulations for clinical trials. Recent clinical trials have demonstrated that it was well tolerated and showed encouraging activity across a broad range of malignancies, and antitumor activity was observed in patients with heavily hemotologic malignancies (Mita *et al.*, 2008; Rizzieri *et al.*, 2008). The published results in the clinical trials were summarized in Table II.

VIII. CONCLUDING REMARKS

PI3K/PTEN signaling pathway plays a central role in regulating various kinds of cellular functions in response to growth factors, insulin, and other hormones. The intensive interests are on the study of PI3K and PTEN in tumorigenesis. Recent studies have shown that the active form of PI3K is an oncogene, and that amplifications and mutations of PI3K are commonly found in many kinds of human cancers. PTEN, as the tumor suppressor and antagonist of PI3K, is frequently mutated or lost in a number of human cancers. PI3K/PTEN signaling regulates angiogenesis through the interaction of cancer cells and tumor microenvironments, especially endothelial cells. Angiogenesis inducers such as VEGF and angiopoietins activate PI3K signaling for inducing angiogenesis. Forced expression of PI3K alone is sufficient to increase angiogenesis. Genetic alterations of PI3K lead to dysfunction of vasculature and angiogenesis. Mutations of RTKs regulate tumor growth and angiogenesis through PI3K/PTEN signaling. PI3K in turn regulates tumor growth and angiogenesis through downstream targets AKT, mTOR, and p70S6K1; and through effectors, HIF-1 and VEGF. A growing list of evidence shows that PI3K, PTEN, and their upstream and downstream molecules are commonly altered in human cancers; and play an important role in tumorigenesis and angiogenesis. The inhibitors

to this signaling pathway, including PI3K, AKT, and mTOR inhibitors, are currently in clinical trials with promising outcomes.

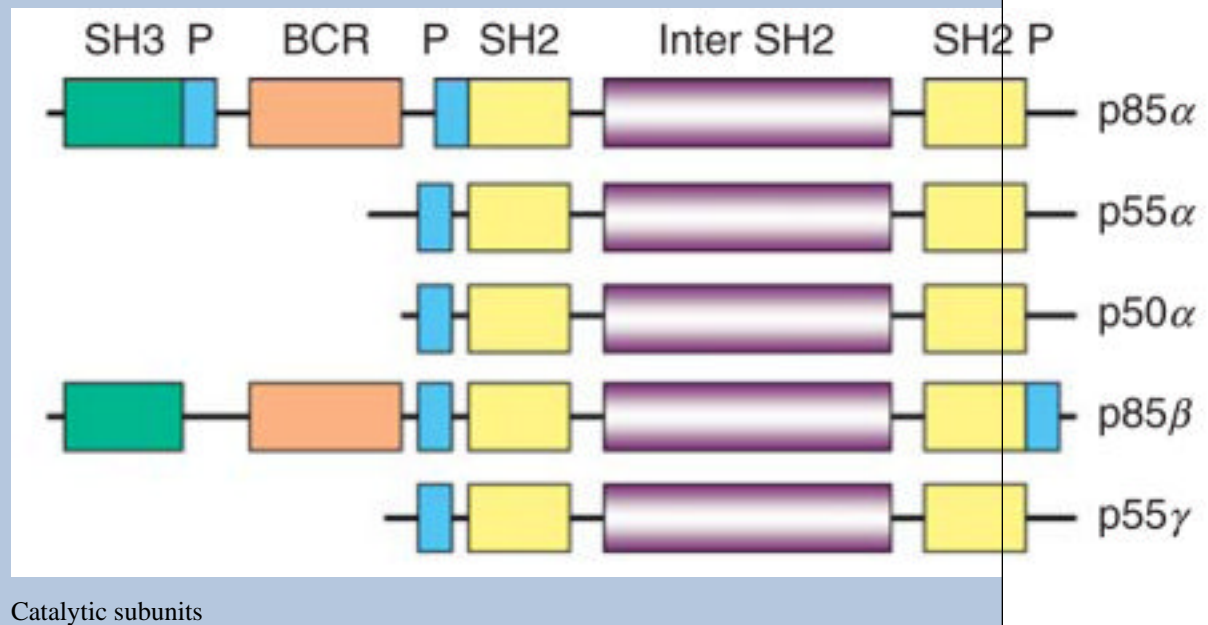
Pan-PI3K inhibitors were initially discovered, and some recently developed versions of pan-PI3K inhibitors broadly target the class IA PI3Ks (p110 α , p110 β , and p110 δ), and the catalytic site of mTOR. Isoform-specific PI3K inhibitors have less toxicity to the cells than those pan-PI3K inhibitors, which could be used to specifically target PI3K activation in certain cancer cells. Clinical data indicates that mTOR inhibitors have stronger effect and more promising results than PI3K and AKT inhibitors. However, there is a feedback loop because p70S6K1 negatively regulates IRS and PDGFR. Rapamycin or its analogs can activate upstream molecules including AKT due to the loss of feedback inhibition. Thus, it is important to exploit the potential benefits of the targeted therapies and optimal treatment with these inhibitors. PI3K pathway inhibitors are likely more effective in patients with active PI3K/AKT pathway, such as *PIK3CA* mutations or *PTEN* mutations. In addition, PI3K/AKT signaling is involved in resistance to both chemotherapeutic and radiotherapeutic treatments. Therefore, it would be beneficial to combine these therapeutic agents with PI3K inhibitors. We anticipate that the therapeutic methods targeting PI3K pathway would represent the promising cancer therapy in the near future.

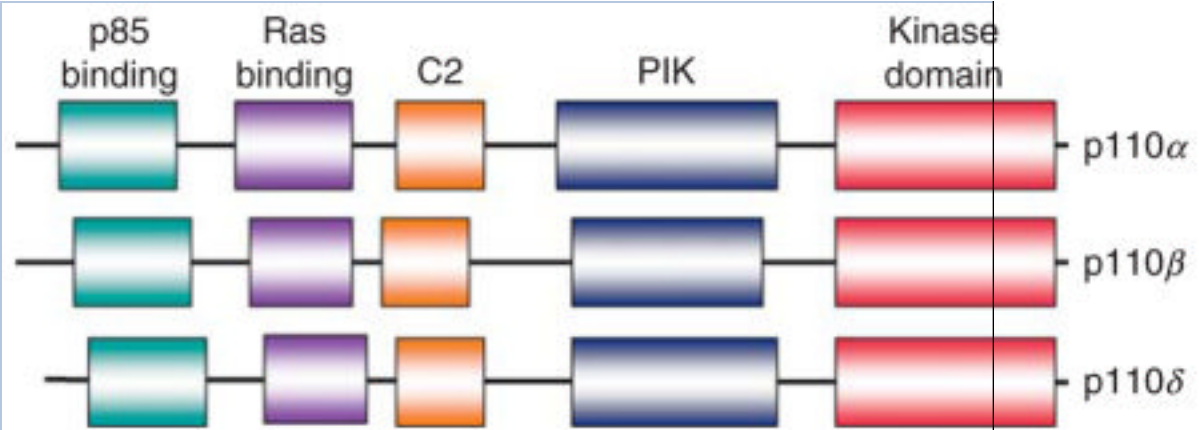
Box 1

PI3K Family and Its Cellular Function

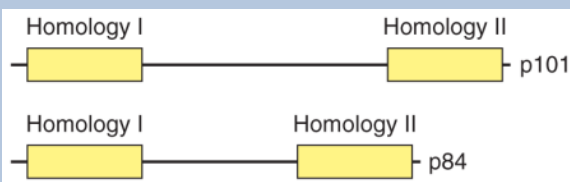
PI3K composes of three classes based on the substrate, structure, distribution, mechanism of activation, and function. The structure of class I, II, and III PI3Ks is shown as below.

Class IA Regulatory subunits

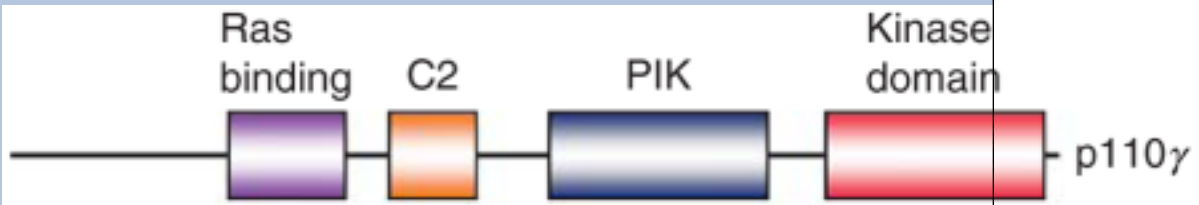




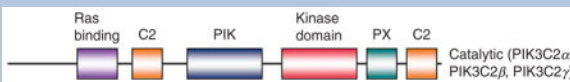
Class IB Regulatory subunits



Catalytic subunits



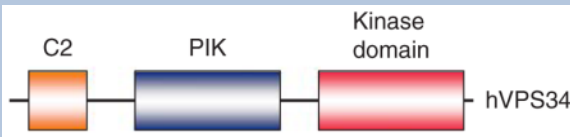
Class II



Class III Regulatory subunits



Catalytic subunits



PI3K exerts various cellular functions through its downstream target AKT.

Cell metabolism

AKT promotes glucose uptake in muscle and fat cells by stimulating the glucose transporter, GLUT4, to cell membrane. AKT increases glycogen synthesis by inhibiting glycogen synthase kinase 3 (GSK-3) (Cohen and Frame, 2001). AKT also regulates fatty acid

synthesis by activating ATP citrate lyase (Berwick *et al.*, 2002). Moreover, AKT inhibits gluconeogenesis by blocking forkhead (FOXO)-mediated transcription of gluconeogenic enzymes and regulates insulin metabolism in the liver (Engelman *et al.*, 2006). Abnormality of AKT is related with diabetes. AKT2-deficient mice exhibit a diabetes-like syndrome with an elevated fasting plasma glucose level, elevated hepatic glucose output, and peripheral insulin resistance (Cho *et al.*, 2001a; Garofalo *et al.*, 2003).

Initiation of translation and protein synthesis

AKT inhibits the GTPase-activating protein (GAP) activity of the tuberous sclerosis complex 1 (TSC1)-TSC2 complex by phosphorylating TSC2 tuberin protein, leading to the accumulation and activation of the mTOR-raptor kinase complex. mTOR mediates the phosphorylation of the ribosomal protein S6 kinases (p70S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) leading to the release of the translation initiation factor eIF4E (Hennessy *et al.*, 2005; Schmelzle and Hall, 2000). However, there are complicated interactions and feedback loops in this signaling pathway since TSC/mTOR/S6K cascade also inhibits PI3K/AKT pathway by down-regulating insulin receptor substrate (IRS) 1/2 and PDGFR (Harrington *et al.*, 2004; Zhang *et al.*, 2003).

Cell survival/inhibition of apoptosis

One of the important downstream targets of AKT is FOXO family of transcription factors. AKT inactivates FOXO proteins by phosphorylation. Some other important targets of AKT are GSK-3, BAD (Bcl2-antagonist of cell death), I κ B kinase (IKK), and MDM2. AKT blocks FOXO-mediated transcription of some proapoptotic proteins such as Fas-ligand (FasL) and Bim, directly phosphorylates the proapoptotic protein BAD, thus repressing the prosurvival molecule Bcl-XL. The phosphorylation of IKK results in phosphorylating I κ B (inhibitor of NF- κ B), leading to its proteasomal degradation and NF- κ B nuclear localization. On the other hand, the phosphorylation of MDM2 leads to the degradation of p53, exhibiting the antiapoptotic effect (Brazil *et al.*, 2002). In addition, eIF4E also has antiapoptotic activity *in vitro* and *in vivo* (Contreras *et al.*, 2008; Yamaguchi *et al.*, 2008).

Cell cycle

AKT promotes G1-S phase transition by blocking FOXO-mediated transcription of cell-cycle inhibitors including p27Kip1 (Chandramohan *et al.*, 2004; Schmidt *et al.*, 2002). AKT also indirectly stabilizes the cell-cycle protein c-Myc and cyclin D1 by inhibiting GSK-3 (Diehl *et al.*, 1998; Engelman *et al.*, 2006; Gregory *et al.*, 2003).

In addition, PI3K plays a role in regulating cell polarity and motility (Engelman *et al.*, 2006).

Acknowledgments

This work was supported in part by Grants CA109460, ES017237, and HL091456 from National Institutes of Health by the National Basic Research Program of China Grant 2007CB947002.

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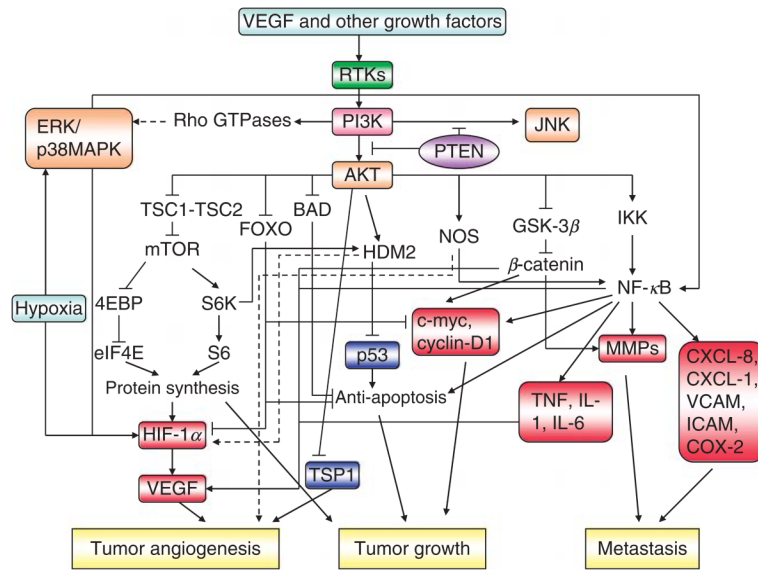


Fig. 1. Targets of PI3K and PTEN in regulating tumor growth, metastasis, and angiogenesis. (See Page 1 in Color Section at the back of the book.)

Table 1
Transgenic Ablation Models of PI3K/PTEN/AKT Signaling Pathway Related to Carcinogenesis, Vasculature, and Angiogenesis

Targeted subunit	Genetic alteration	Comments
p110 α	p110 $\alpha^{-/-}$	Embryonic lethality, multiple vascular defects, lower Tie2 protein levels (Lelievre <i>et al.</i> , 2005)
	Endothelial cell-specific-p110 $\alpha^{-/-}$	Embryonic lethality at mid gestation because of severe defects in angiogenic sprouting and vascular remodeling (Graupera <i>et al.</i> , 2008)
p110 γ	p110 $\gamma^{-/-}$	The significantly diminished vascular permeability in response to both Ras and VEGF (Serban <i>et al.</i> , 2008)
p85 α /p55 α /p50 α (pan-p85 α)	pan-p85 $\alpha^{-/-}$	Embryonic lethality, subepidermal blebs flanking the neural tube and bleeding into the blebs during the turning process (Brachmann <i>et al.</i> , 2005)
p85 subunits	Muscle-specific pan-p85 $\alpha^{-/-}$ p85 $\beta^{-/-}$	Viable, exhibit attenuated AKT signaling in the heart, reduced heart size, and altered cardiac gene expression (Luo <i>et al.</i> , 2005)
PTEN	PTEN $^{-/-}$	Early embryonic lethality
	PTEN $^{+/+}$	Showed neoplasms in multiple organs including prostate, skin and endometrium, liver, colon, gastrointestinal tract, and thymus, spontaneously developed germ cell, gonadostromal, breast, thyroid tumors, and lymphomas (Di Cristofano <i>et al.</i> , 1998; Podyspamina <i>et al.</i> , 1999; Stambolic <i>et al.</i> , 2000; Suzuki <i>et al.</i> , 1998)
	PTEN $^{-/-}$ in smooth muscle cells	Died before 6 weeks, increase in phosphorylated AKT in major vessels, hearts, and lungs, pathological vascular remodeling and vascular recruitment of progenitor cells, widespread smooth muscle cell hyperplasia and abdominal leiomyosarcomas (Hermando <i>et al.</i> , 2007; Nemenoff <i>et al.</i> , 2008)
	Bronchioalveolar epithelium-specific PTEN $^{-/-}$	90% of <i>SOPrep^{flac/lox}</i> (E10–E16) mice died within 2 h of birth, surviving mice developed spontaneous lung adenocarcinomas with hyperplasia of bronchioalveolar epithelial cells and myofibroblast precursors, enlarged alveolar epithelial cells, and impaired production of surfactant proteins. <i>K-ras</i> was frequently mutated in adeno carcinomas (Yanagi <i>et al.</i> , 2007)
	Hepatocyte-specific PTEN $^{-/-}$	Massive hepatomegaly and steatohepatitis with triglyceride accumulation followed by liver fibrosis and hepatocellular carcinoma (Horie <i>et al.</i> , 2004; Watanabe <i>et al.</i> , 2007)
	PTEN $^{-/-}$ in endothelial cells	Embryonic lethality due to endothelial cell hyperproliferation and impaired vascular remodeling (Suzuki <i>et al.</i> , 2007)
	PTEN $^{-/-}$ in endothelial cells	Enhances postnatal neovascularization, including tumor angiogenesis necessary for tumor growth (Suzuki <i>et al.</i> , 2007)
	Urothelium-specific PTEN $^{-/-}$	Exhibited urothelial hyperplasia, 10% of mice spontaneously developed pedicellate papillary transitional cell carcinomas (Tsuruta <i>et al.</i> , 2006)
	Pancreas-specific PTEN $^{-/-}$	Progressive replacement of the acinar pancreas with highly proliferative ductal structures, a fraction of these mice develop ductal malignancy (Stanger <i>et al.</i> , 2005)
	Prostate-targeted PTEN $^{-/-}$	Hyperproliferation and neoplastic changes in prostate (Backman <i>et al.</i> , 2004; Ma <i>et al.</i> , 2005; Trotman <i>et al.</i> , 2003; Wang <i>et al.</i> , 2003, 2006)
	Astrocytes-specific PTEN $^{-/-}$	Hypertrophy and increased proliferation of astrocytes <i>in vivo</i> (Fraser <i>et al.</i> , 2004)
	Skin-specific PTEN $^{-/-}$	Hyperproliferation and spontaneous tumorigenesis of the skin keratinocytes (Komazawa <i>et al.</i> , 2004)
	PTEN $^{+/+}$ in primordial germ cells	Testicular teratoma and enhanced embryonic germ cell production (Kimura <i>et al.</i> , 2003)
	Mammary-specific PTEN $^{-/-}$	Precocious development and neoplasia in the mammary gland (Li <i>et al.</i> , 2002)
AKT1	AKT1 $^{-/-}$	Smaller litter sizes, reduced fetal weight, and a higher fetal mortality due to the impaired extraembryonic vascularization and placental hypotrophy (Chen <i>et al.</i> , 2001; Cho <i>et al.</i> , 2001b; 2001c; Yang <i>et al.</i> , 2003)

Targeted subunit	Genetic alteration	Comments
AKT2	AKT2 ^{-/-}	<p>Impairment of blood vessel maturation and increased vascular permeability, reduced activation of eNOS, and reduced expression of thrombospondins 1 (TSP-1) and TSP-2 (Chen <i>et al.</i>, 2005)</p> <p>Defective ischemia- and VEGF-induced angiogenesis and severe peripheral vascular disease (Ackah <i>et al.</i>, 2005)</p> <p>Abrogated polarity, migratory directionality, and breast cancer onset of mammary epithelial cells with ErbB2 overexpression (Ju <i>et al.</i>, 2007)</p> <p>Resistant to tumors and skin carcinogenesis induced MMTV-v-H-Ras- induced (Skeen <i>et al.</i>, 2006)</p> <p>Displayed normal cardiac growth in responses to provocative stimulation, and sensitized to cardiomyocyte apoptosis in response to ischemic injury (DeBosch <i>et al.</i>, 2006)</p>

Table II

Clinical Trials of PI3K/AKT/mTOR Pathway Inhibitors for Cancer Therapy

Targets	Drug name	Phase	Tumor types	Comments and references
AKT	Perifosine	I	Incurable solid malignancies	In order to get suitable dose, pharmacokinetic data, and side effects (Crul <i>et al.</i> , 2002; Van Ummersen <i>et al.</i> , 2004)
		I	Advanced solid tumors	Pharmacokinetic study showed that perifosine can be safely combined with fractionated radiotherapy (Vink <i>et al.</i> , 2006)
		I/II	Gastrointestinal stromal tumor in combination with imatinib	Ocular toxicity and ulcerative keratitis were associated with Perifosine (Shome <i>et al.</i> , 2008)
		II	Advanced breast cancer	No objective responses were seen in this group of pretreated metastatic breast cancer patients (Leigh <i>et al.</i> , 2008)
		II	Androgen independent prostate cancer	No significant clinical activity against prostate cancer was observed in this population (Posadas <i>et al.</i> , 2005)
		II	Advanced soft tissue sarcoma	Optimism remains for this agent in STS patients (Bailey <i>et al.</i> , 2006)
		II	Recurrent, hormone-sensitive prostate cancer	No significant response was seen (Knowing <i>et al.</i> , 2006)
		II	Pancreatic adenocarcinoma	Modest single-agent clinical activity (Chee <i>et al.</i> , 2007)
		II	Recurrent or metastatic head and neck cancer (SCCHN)	Perifosine did not appear to be worthy of further study in this group of patients (Marsh <i>et al.</i> , 2007)
mTOR	Rapamycin (sirolimus)	I	Hepatocellular and cholangiocellular cancer	Perifosine in the doses and schedule used lacked single-agent activity in SCCHN (Argiris <i>et al.</i> , 2006)
		I	Chronic myeloid leukaemia (CML)	A temporary disease-control rate was identified and the toxicity was acceptable (Rizell <i>et al.</i> , 2008)
		I	Nonsmall cell lung cancer (NSCLC)	Rapamycin showed antileukemic effects in imatinib-resistant CML (Sillaber <i>et al.</i> , 2008)
		I	Recurrent PTEN-deficient glioblastoma	Combination therapy with sirolimus, radiation, and cisplatin was well tolerated in patients (Sarkaria <i>et al.</i> , 2007)
		I	Recurrent malignant glioma	Rapamycin had anticancer activity in PTEN-deficient glioblastoma and warrants further clinical study alone or in combination with PI3K pathway inhibitors (Cloughesy <i>et al.</i> , 2008)
		I	Solid tumor, recurrent malignant glioma, advanced renal cancer	Gefitinib plus sirolimus was safely coadministered on a continuous, daily dosing schedule (Reardon <i>et al.</i> , 2006)
	CCI-779 (temsirolimus)	I	Solid tumors or lymphomas	To establish the safety, tolerability, and pharmacokinetic parameters of CCI-779 (Kuhn <i>et al.</i> , 2007; Peralba <i>et al.</i> , 2003; Raymond <i>et al.</i> , 2004)
		I	Advanced solid tumors	Antitumor efficacy was observed and CCI-779 was generally well tolerated on this intermittent schedule (Hidalgo <i>et al.</i> , 2006)
		I	Recurrent malignant glioma	The administration of CCI-779 and 5-FU/LV at these doses and schedule resulted in unacceptable toxicity and therefore it is not recommended (Punt <i>et al.</i> , 2003)
		I/II	Advanced renal-cell carcinoma	The recommended dose of CCI-779 for patients on enzyme-inducing antiepileptic drugs was 250 mg IV weekly (Chang <i>et al.</i> , 2004)
		II	Advanced breast cancer	The combination of CCI-779 and IFN had an acceptable safety profile and displays antitumor activity in patients with advanced RCC (Morzer <i>et al.</i> , 2007)
		II	Advanced breast cancer	CCI-779 showed antitumor activity and a generally tolerable safety profile (Chan <i>et al.</i> , 2005)

Targets	Drug name	Phase	Tumor types	Comments and references
		II	Recurrent glioblastoma multiforme	CCI-779 was well tolerated in recurrent GBM patients. No response or radiographic improvement was observed in 36% of CCI-779 treated patients (Chang <i>et al.</i> , 2005; Galanis <i>et al.</i> , 2005)
		II	Advanced neuroendocrine carcinomas	CCI-779 appeared to have little activity and does not warrant further single-agent evaluation in advanced NEC (Duran <i>et al.</i> , 2006)
		II	Extensive-stage small-cell lung cancer	CCI-779 seemed not to increase the progression-free survival in this patient population (Pandya <i>et al.</i> , 2007)
		II	Metastatic melanoma	CCI-779 was not sufficiently active in this patient population (Margolin <i>et al.</i> , 2005)
		II	Advanced refractory renal-cell carcinoma	In patients with advanced RCC, CCI-779 showed antitumor activity and encouraging survival (Atkins <i>et al.</i> , 2004)
		III	Advanced renal-cell carcinoma	CCI-779 increased the effect of interferon alpha, improved overall survival among patients with metastatic renal-cell carcinoma and a poor prognosis (Hudes <i>et al.</i> , 2007)
	RAD001 (everolimus)	I	Refractory solid tumors in children	Continuous, orally administered RAD001 was well tolerated in children with recurrent or refractory solid tumors and significantly inhibited the mTOR signaling pathway (Fouladi <i>et al.</i> , 2007)
		I	Advanced solid tumors	RAD001 was satisfactorily tolerated at dosages up to 70 mg/week and 10 mg/day, a dosage of 10 mg/day or 50 mg/week was recommended for further development (O'Donnell <i>et al.</i> , 2008; Tabernero <i>et al.</i> , 2008)
		I	Advanced NSCLC	A dose of 5 mg daily in combination with daily gefitinib 250 mg was recommended. The two patients with radiographic responses identified were encouraging (Milton <i>et al.</i> , 2007)
		I	Advanced breast cancer	Daily therapy with RAD001 plus letrozole was promising and a daily dose of RAD001 10 mg was recommended for further trials (Awada <i>et al.</i> , 2008)
		I/II	Relapsed or refractory hematologic malignancies	RAD001 was well tolerated at a daily dose of 10 mg daily and was effective in patients with myelodysplastic syndrome (Yee <i>et al.</i> , 2006)
		II	Relapsed chronic lymphocytic leukemia	Although the patient initially responded to therapy, the patient subsequently developed a rapidly fatal Epstein-Barr-virus-associated lymphoproliferative disorder (Gotze <i>et al.</i> , 2007)
		II	Low- to intermediate-grade neuroendocrine tumors	RAD001 at 5 or 10 mg/d was well tolerated in combination with octreotide with promising antitumor activity (Yao <i>et al.</i> , 2008)
		III	Advanced renal-cell carcinoma	Treatment with everolimus prolonged progression-free survival relative to placebo in patients with metastatic renal-cell carcinoma that had progressed on other targeted therapies (Motzer <i>et al.</i> , 2008)
	AP23573 (deforolimus)	I	Advanced malignancies	Deforolimus was well tolerated with encouraging antitumor activity across a broad range of malignancies (Mita <i>et al.</i> , 2008)
		II	Relapsed or refractory hematologic malignancies	Deforolimus was well tolerated in patients with heavily pretreated hematologic malignancies, and antitumor activity was observed (Rizzieri <i>et al.</i> , 2008)