Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1784 (2008) 150–158
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbapap

Review

PI3K/PTEN signaling in tumorigenesis and angiogenesis

Bing-Hua Jiang [a,*], Ling-Zhi Liu [a,b]

[a] Cancer Center, Department of Pathology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu, China
[b] Mary Babb Randolph Cancer Center, Department of Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV 26506, USA

Received 22 June 2007; received in revised form 22 August 2007; accepted 21 September 2007
Available online 29 September 2007

Abstract

The phosphatidyl inositol 3-kinase (PI3K) can be activated by a variety of extracellular signals and involved in a number of cellular processes including cell proliferation, survival, protein synthesis, and tumor growth. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is an antagonist of PI3K. The alterations of PI3K pathway such as activation of oncogenes, gene amplification, and inactivation of tumor suppressors, commonly occur in many human cancers. Angiogenesis is required for tumor growth and metastasis when the tumor reaches more than 1 mm in diameter. Recent studies have shown that PI3K and Akt play an important role in regulating tumor growth and angiogenesis through VEGF and HIF-1 expression. PI3K regulates the expression of these two proteins through HDM2 and p70S6K1 in human cancer cells. The frequent dysregulation of the PI3K/PTEN pathway in human cancer demonstrates that this pathway is an appropriate target for cancer therapeutics. In this review, we describe the recent advances in understanding the PI3K/PTEN pathway, the role and mechanism of PI3K in regulating tumor growth and angiogenesis, and the potential therapeutic opportunities for targeting this pathway for cancer treatment.

Keywords: PI3K; PTEN; Akt; mTOR; p70S6K1; VEGF; HIF-1; HDM2; Angiogenesis; Tumorigenesis

1. Introduction: PI3K

Phosphatidylinositol 3-kinases (PI3Ks) have been classified into three major subfamilies according to their structure and the substrate specificity [1]. Class I PI3Ks are activated by cell surface receptors and consist of two subfamilies, Class IA and Class IB, based on the associated adaptors. Class IA PI3Ks are composed of heterodimers of a p110 catalytic subunit and a p85 regulatory subunit [1,2]. There are three different isoforms of p110 catalytic subunit: p110α, p110β and p110δ, that are encoded by *PIK3CA*, *PIK3CB* and *PIK3CD*, respectively. The p85 regulatory subunit also has three major isoforms: p85α, p85β and p55γ that are encoded by *PIK3R1, PIK3R2* and *PIK3R3,* respectively. The *PIK3R1* gene codes for two shorter isoforms, p55α and p50α through alternative splicing. Class IB PI3Ks are composed of heterodimers of a p101 regulatory subunit and a p110γ catalytic subunit. There are two other p101 homologues, p84 and p87PIKAP (PI3Kγ adaptor protein of 87 kDa) [3]. Class IA PI3Ks can be activated by receptor tyrosine kinases (RTKs), while Class IB PI3Ks by G-protein-coupled receptors (GPCRs). Phosphatidylinositol 3,4-bisphosphate [PI(3,4)P2] and phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3] are the major products of class I PI3Ks generated from phosphatidylinositol-4,5-bisphosphate [PI-(4,5)-P2] [3,4].

Abbreviations: PI3K, phosphatidylinositol 3-kinase; RTK, receptor tyrosine kinase; mTOR, mammalian target of rapamycin; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PH domain, pleckstrin-homology domain; PDK, phosphoinositide-dependent kinase; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; FOXO, forkhead; G6Pase, glucose-6-phosphatase; GSK3, glycogen synthase kinase 3; HDM2, human double minute 2; Cdk4, cyclin-dependent kinase 4; MAPK, mitogen-activated protein kinase; Rb, retinoblastoma protein; cdc25A, cell division cycle 25A; PTEN, phosphatase and tensin homolog deleted on chromosome 10; CDDP, cisplatin; CAM, chicken chorioallantoic membrane; HIF-1, hypoxia-inducible factor 1; PX-316, phosphatidylmyoinositol-1-[(*R*)-2-methoxy-3-octadecyloxypropyl hydrogen phosphate]; API-59-Ome, 9-methoxy-2-methylellipticinium acetate; VQD-002, triciribine

* Corresponding author.
E-mail address: bhjiang@njmu.edu.cn (B.-H. Jiang).

1570-9639/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2007.09.008

Class II PI3Ks consist of the ubiquitously expressed PIK3C2α and PIK3C2β, and liver-specific isoforms PIK3C2γ that are characterized by the presence of a C2 domain at the C-terminus. Class II PI3Ks lack adaptor subunits and preferentially use PtdIns and PtdIns(4)P as substrates. They bind clathrin and localize to coated pits, suggesting a regulatory function in membrane trafficking and receptor internalization [1]. Class II PI3Ks can also be activated by RTKs, cytokine receptors and integrins; but the specific functions in response to these activators are not understood [5]. Class III PI3Ks are heterodimeric enzymes of catalytic (Vps34, 100 kDa) and adaptor (p150) subunits. The class III PI3Ks use only phosphatidylinositol as a substrate (e.g. mammalian PI3K and yeast Vps34p). Class III PI3Ks are implicated in the regulation of mammalian target of rapamycin (mTOR) activity in response to amino-acid availability and the regulation of autophagy in response to cellular stress, indicating the importance of Class III PI3K in controlling cell growth and survival [1]. The most extensively investigated PI3Ks are class I PI3Ks, especially class IA PI3Ks. Class I PI3Ks are involved in a number of cellular functions including cell growth, proliferation, and survival. In this review, we will focus on the roles of Class IA PI3Ks in regulating tumor growth and angiogenesis.

2. Activation of PI3K by growth factors and oncogenes

In response to growth factors, PI3K can be activated by receptor protein tyrosine kinases (RTKs), and nonreceptor protein tyrosine kinases. RTKs include epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), insulin-like growth factor 1 receptor (IGF-1R), interleukin receptors, vascular endothelial growth factor receptor (VEGFR), interferon receptors, and integrin receptors. RTKs interact with the p85 regulatory subunit of PI3K, while Ras protein directly interacts with the p110 catalytic subunit of PI3K in a GTP-dependent manner. In response to growth factors, activated receptors interact with p85 Src homology 2 (SH2) domains, and localize PI3K to the plasma membrane. In addition to RTKs, intracellular proteins such as protein kinase C (PKC), SHP1, Rac, Rho, and Src can also activate PI3K in the cells [4]. Upon activation, PI3K phosphorylates the D3 hydroxyl of PI(4,5)P2 to produce PI(3,4,5)P3 as a second messenger. PI(3,4,5)P3 binds to a subset of proteins containing pleckstrin-homology (PH), FYVE, Phox (PX), C1, and C2 domains; and activates these downstream targets with lipid-binding domains at the membrane. PH-domain-containing targets include Akt (protein kinase B, PKB), Tec family tyrosine kinases, guanine nucleotide exchange factors (GEF) for Rac, adenosine diphosphate (ADP)-ribosylating factor 6 (ARF6), and GTPase-activating proteins (GAPs) [2–4]. Activation of Rac (and perhaps ARF6) by compartmental production of PI(3,4,5)P3 plays an important role in the remodeling of actin cytoskeleton and in regulation of cytosolic calcium concentrations and gene expression [1]. In addition, the mutations of PI3K upstream elements such as the epidermal growth factor (EGFR), ErbB2, and IGF-1R, as shown in Table 1, can also increase PI3K activity in various cancer cell types [6–10]. PI3K regulates a number of cellular functions through the activation of Akt [11].

Table 1
Alterations of PI3K/PTEN/Akt pathway and its upstream molecules in cancer

Gene alteration	Type of carcinoma
PIK3CA mutation	colorectal, glioblastomas, gastric, breast, lung, ovarian, hepatocellular, thyroid, endometrial cancers, nasopharyngeal carcinoma, acute leukemia and other nervous system malignancies [22,28–32]
PIK3CA amplification	ovarian, uterine cervical, breast, gastric, thyroid, lung cancers, oral, head and neck squamous cell carcinoma [22–26]
PIK3R1 mutant	glioblastoma, ovarian, colon cancer [33,34]
PTEN mutation and loss	head and neck squamous cell carcinoma, glioblastoma, gastric, breast, prostate, ovarian, lung, kidney, cancers, uterine endometrioid, cervical carcinomas, astrocytoma, melanoma [23,24,35–38]
Akt1 amplification	glioblastoma, gliosarcoma, gastric cancer [53–55]
Akt2 amplification	head and neck squamous cell carcinoma, pancreatic, ovarian, breast cancers [24,56–58]
Increased *Akt3* mRNA	breast, prostate cancers [59]
Alterations of EGFR, ErbB2, IGF-1R, and Ras	glioblastoma, gliosarcoma, lung, ovarian, prostate, cervical, endometrial, liver, breast cancers, esophageal and Barrett's adenocarcinomas [6–10]

3. PI3K activates Akt for regulating cellular functions

Akt, also known as protein kinase B, is one of the major downstream targets of PI3K, and the cellular homologue of AKT8 retroviral oncogene (PKB) [12]. When the pleckstrin homology (PH) domain of Akt binds to PI(3,4,5)P3, Akt is recruited to the cellular membrane, whereby it is phosphorylated by phosphoinositide-dependent kinase 1 (PDK1) at Thr 308 (Akt1) [3,13]. Additional phosphorylation of Akt in the hydrophobic C-terminal domain (Ser 473 in Akt1) by PDK2 is required for its full activation [4,14]. Upon activation, Akt moves to the cytoplasm and nucleus, and phosphorylates a number of downstream targets for regulating various cellular functions (Fig. 1). The activation and biological functions of PI3K/Akt pathway in cancer occurrence and development are outlined in Fig. 1.

Akt can increase glycogen synthesis and cell metabolism through the inactivation of forkhead (FOXO) family of transcription factors and glycogen synthase kinase 3 (GSK3) [15,16]. Akt is well known to enhance cell survival through the inhibition of pro-apoptotic proteins such as FOXO and Bcl2-antagonist of cell death (BAD). FOXO regulates transcription of pro-apoptotic proteins such as Fas-ligand (FasL) and Bim. BAD inhibits the function of pro-survival molecule BclXL for inducing apoptosis. Akt can also block the apoptosis through the induction of survival proteins such as Bcl2, IkappaB kinase (IKK), and human double minute 2 (HDM2) [3,17].

The PI3K/Akt signaling pathway has been demonstrated to play a key role in regulating cell cycle progression and proliferation. Akt promotes the G_1-S phase transition by blocking FOXO-mediated transcription of cell-cycle inhibitors, including

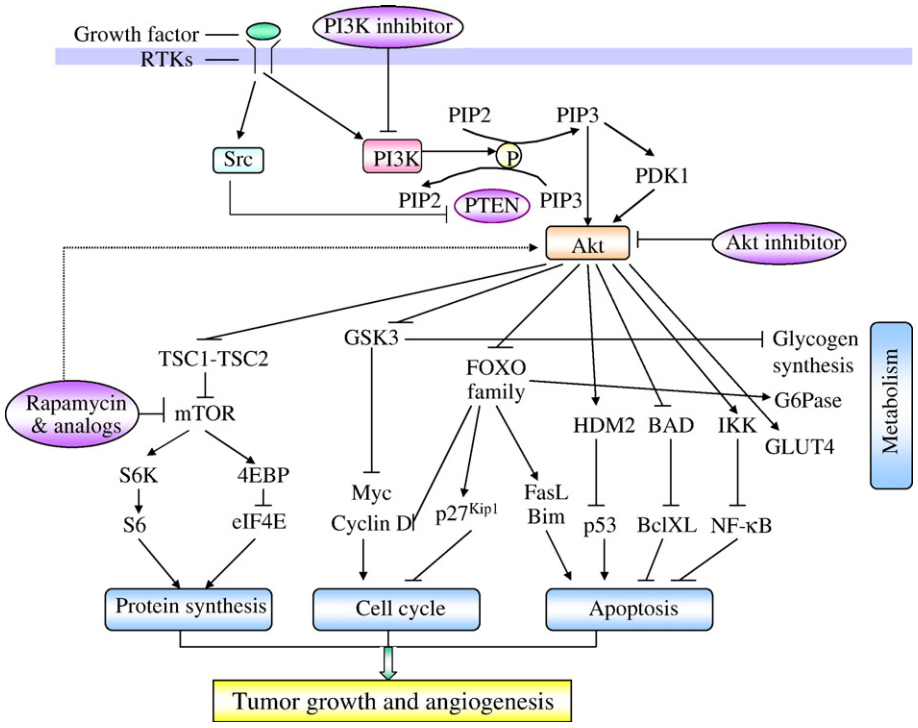

Fig. 1. Schematic representation of PI3K/PTEN/Akt pathway. The PI3K pathway is important in regulating carcinogenesis, tumor growth and angiogenesis. Activation of receptor tyrosine kinases (RTKs) initiates receptor dimerization, and activates the intracellular pathways to increase PI3K activity. PI3K produces phosphatidylinositol-3,4-diphosphate (PIP2) and phosphatidylinositol-3,4,5-triphosphate (PIP3), which activates PDK1 and Akt. Akt regulates multiple cellular functions including metabolism, protein synthesis, cell cycle progression, anti-apoptosis, tumor growth, and angiogenesis through different downstream targets. mTOR, mammalian target of rapamycin; GSK3, glycogen synthase kinase 3; NF-κB, nuclear factor kappa B; PDK1, phosphoinositide-dependent kinase 1; PTEN, phosphatase and tensin homolog deleted on chromosome 10; TSC, tuberous sclerosis complex; BAD, Bcl2-antagonist of cell death; IKK, IkappaB kinase; FOXO, forkhead; G6Pase, glucose-6-phosphatase; GLUT4, glucose transporter 4; FasL, Fas-ligand.

p27Kip1, or directly phosphorylates and inactivates p27Kip1. Akt induces cell proliferation by phosphorylating the tuberous sclerosis complex 2 (TSC2) protein tuberin, and by inhibiting the GAP activity of the TSC1-TSC2 complex. These processes lead to the accumulation and activation of the mTOR-raptor kinase complex, which in turn mediates p70S6K1 activation and the phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) to regulate protein synthesis [3]. Akt can also indirectly stabilize the cell-cycle protein c-Myc and cyclin D1 by inhibiting GSK3 [3]. The control of cell growth by the PI3K/Akt pathway via regulating cell survival and cell cycle progression implicates a crucial role of this pathway in carcinogenesis and cancer development. We have demonstrated that the PI3K inhibition by LY294002 inhibited ovarian cancer cell proliferation and induced the G_1 cell cycle arrest [18]. This effect was accompanied by decreasing expression of G_1-associated proteins, including cyclin D1, cyclin-dependent kinase 4 (Cdk4), cdc25A, and Rb phosphorylation at Ser780, Ser795, and Ser807/811 [19]. The expression of Cdk6 and β-actin was not affected by LY294002. The level of the cyclin kinase inhibitor, $p16^{INK4a}$, was induced by the PI3K inhibitor, while the level of $p21^{CIP1/WAF1}$ was decreased in the same experiment. The inhibition of PI3K also inhibited the activation of Akt and p70S6K1, but did not affect mitogen-activated protein kinase (MAPK). PI3K transmits the mitogenic signal through Akt, the mammalian target of rapamycin (mTOR) to p70S6K1. The mTOR inhibitor rapamycin has similar inhibitory effects on G_1 cell cycle progression and expression of cyclin D1, Cdk4, cell division cycle 25A (cdc25A), and retinoblastoma protein (Rb) phosphorylation [18,19]. These results suggest that PI3K mediates G_1 progression and cyclin expression through activation of Akt/ mTOR/p70S6K1 signaling pathway, and inhibits the expression of $p16^{INK4a}$ in ovarian cancer cells.

4. PI3K mutations in human cancer

The gain function of PI3K mutations has been frequently observed in many human cancers. The subunit mutations of PI3K have been implicated in a number of human cancers, such as ovarian, breast, gastric, and hepatocellular carcinoma [20]. For example, an increased copy number of *PIK3CA*, the gene encoding the p110α catalytic subunit of PI3K, was observed in approximately 40% of ovarian cancer occurrences [21]. *PIK3CA* is also found to be amplified and overexpressed in several other types of cancers including cervical, gastric, ovarian, and breast cancers through a large-scale mutational analysis [22–26]. In addition, somatic mutations of *PIK3CA* were found in 25% to 32% of colorectal cancers, 27% of glioblastomas, 25% of gastric cancers, 3% to 8% of breast, and 4% of lung cancer [27]. The *PIK3CA* mutations were also found in ovarian, hepatocellular, thyroid, endometrial cancers, and acute leukemia, as well as in malignancies of the central nervous

system [28–32]. These mutations of *PIK3CA* constitutively increased PI3K activities in the cells. PI3K regulatory subunit p85 dimerizes with catalytic subunit p110, and inhibits PI3K activity in normal cells. The deletion and somatic mutations of p85α regulatory subunit (*PIK3R1*) were found in primary human glioblastoma, colon and ovarian cancers [33,34]. The deletion of p85 protein that lacks the inhibitory domain, and loss of the autophosphorylation site at the p85 inhibitory domain, commonly increase PI3K activity [33,34]. The mutations of *PIK3CA* and *PIK3R1* in human cancers are summarized in Table 1.

5. PTEN mutations and Akt activation in human cancer

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is the antagonist of PI3K, which removes the 3′ phosphate of PIP3 and attenuates signaling downstream of activated PI3K. The tumor suppressor PTEN is frequently mutated or lost in primary glioblastomas, breast cancer, kidney and uterine endometrioid carcinomas, lung cancer, and melanoma biopsies [23,35–38]. In addition, the decreasing levels of PTEN expression are correlated with the progressive outcome of solid cancers, including ovarian, prostate, and cervical cancers [36]. Mice with PTEN deletion and mutation are highly susceptible to tumor induction [39]. Conditional knockout of PTEN leads to neoplasia in multiple organs such as the mammary gland, skin, and prostate [40,41], demonstrating the key role of PTEN in cancer development.

Akt regulates cell survival, cell cycle progression, migration, proliferation, metabolism, tumor growth, and angiogenesis [42,43]. Human Akt has three isoforms: Akt1, Akt2, and Akt3 (also known as PKBα, PKBβ, and PKBγ). Akt can be activated by cytokines and growth factors, including vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), and angiopoietin [44–49]. Oncogenes such as Ras and Src, amplification or activated mutations of PI3K, and the somatic mutations or deletions of tumor suppressor PTEN can also activate Akt pathway [50–52]. Akt gene amplification has been observed in a number of human cancers (Table 1), including a single gastric carcinoma, glioblastoma and gliosarcoma [53–55]. *Akt2* amplification was found in head and neck squamous cell carcinoma, pancreatic, ovarian, and breast cancers [24,56–58]. Increased Akt3 mRNA is correlated to breast and prostate cancers [59]. In addition to amplification, recent studies have indicated that elevated Akt activities are detected in different kinds of cancers, and are associated with a poor prognosis in human cancers [60,61].

Furthermore, Akt activation is found to correlate with the resistance to chemotherapy and radiation therapy. The combination treatment of Akt inhibitor with antitumor drugs renders cancer cells sensitive to the drug treatment [62,63]. Expression of the multi-drug resistance-associated protein 1 (MRP-1), which is a transmembrane pump for drug transport, has been shown to depend on PI3K activity in advanced prostate cancer and in human acute myelogenous leukemia blasts [64,65]. Recent studies have shown that a low level of PTEN expression is strongly associated with amplification of *PIK3CA* and with the increase of PI3K and Akt activities in OVCAR-3 cells, contributing to cisplatin (CDDP) resistance in ovarian cancer [66]. Our recent study indicates that Akt1 amplification and overexpression are associated with CDDP resistance acquired by human lung cancer cells [67]. Akt1 activity is required and sufficient for regulating CDDP resistance in lung cancer cells. The results further demonstrate that Akt activation is highly related to the CDDP chemosensitivity in human cancer tissues and that Akt1 induces the lung cancer cells resistant to CDDP treatment through the mTOR signaling pathway [67]. These studies indicate that Akt plays an important role in regulating CDDP resistance.

6. PI3K and Akt in regulating tumor angiogenesis

Angiogenesis is essential for embryo development, female reproduction, tissue repair, inflammatory diseases, and tumor growth and metastasis. The angiogenesis process includes dissolution of the basement membrane of the vessel, migration and proliferation of endothelial cells, formation of a new vessel lumen and vessel branches, maturation of the new vessels by the recruitment of pericytes, and the formation of the basement membrane [68]. Tumor growth and metastasis require angiogenesis when the tumor reaches 1–2 mm in diameter [69]. Tumor angiogenesis occurs via the sprouting of new vessels from preexisting blood vessels or via the insertion of interstitial tissue columns into the lumen of pre-existing vessels (i.e., intussusception) [70]. Tumor angiogenesis can be triggered by extracellular signals such as growth factors, by genetic alterations such as activation of oncogenes, and by mutations of tumor suppressor genes such as PTEN and p53.

The first direct evidence of PI3K and Akt involvement in regulating angiogenesis *in vivo* was observed by the forced expression of PI3K and Akt in chicken chorioallantoic membrane (CAM) by the RCAS retroviral vector [43]. In addition, it has been found that PI3K/Akt regulated VEGF and hypoxia-inducible factor 1 (HIF-1) expression through HDM2 and p70S6K1 activation (Fig. 2). VEGF and HIF-1 are the mediators that transmit PI3K-induced oncogenic signals for tumor growth and angiogenesis [71,72]. VEGF is a potent inducer of angiogenesis, and HIF-1 is the major regulator of VEGF transcriptional activation through the binding to the hypoxia response element (HRE) of VEGF promoter. HIF-1 is a heterodimeric transcription factor composed of two subunits: HIF-1α and HIF-1β [73,74]. HIF-1α can be induced by hypoxia, growth factors, and oncogenes; whereas HIF-1β protein is constitutively expressed in human cells. Various studies show that PI3K/AKT signaling is important for regulating HIF-1α and VEGF expression [43,71]. We have demonstrated that the expression of PI3K dominant negative mutants or expression of PTEN inhibits tumor growth [75]. Overexpression of PTEN mutants lacking phosphatase activity abrogates the inhibitory effect of PTEN on tumor growth, suggesting that the expression of PTEN requires its phosphotase activity to inhibit tumor growth *in vivo*. Overexpression of

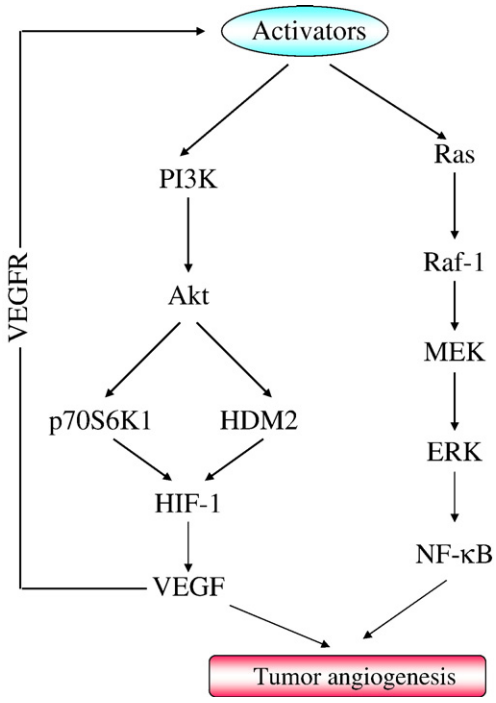

Fig. 2. PI3K and Ras in inducing tumor angiogenesis. PI3K and Ras are activated by upstream activators such as growth factors, activation of receptor tyrosine kinases, and oncogenes. PI3K induces Akt activation, which increases the expression of hypoxia-inducible factor 1 (HIF-1) through p70S6K1 and HDM2. HIF-1 increases VEGF transcriptional expression. Ras/Raf-1 is a parallel signaling pathway for regulating NF-κB expression. VEGF and NF-κB have synergistic effects in inducing tumor angiogenesis. VEGF and VEGFR can form an autocrine loop to regulate tumor angiogenesis.

active forms of PI3K induced angiogenesis *in vivo*, characterized by extensive sprouting of new blood vessels and enlargement of preexisting vessels [43]. Akt is an essential downstream target of PI3K for mediating the angiogenic signal. Overexpression of the tumor suppressor PTEN and of dominant-negative constructs of PI3K by adenovirus in prostate cancer cells significantly inhibited angiogenesis, suggesting that PI3K/Akt signaling is required for tumor angiogenesis. The potential downstream targets of PI3K/PTEN in regulating tumor growth and angiogenesis are outlined in Fig. 1. Akt is the major target of PI3K for transmitting the oncogenic and angiogenic signals. PI3K/Akt signaling is also demonstrated to be involved in growth factor- and hormone-induced tumor growth and angiogenesis, and in tumor-induced angiogenesis through reactive oxygen species [76,77]. The Ras/MEK signaling pathway is another major pathway for regulating angiogenesis, and MEK induces NF-κB activation for regulating angiogenesis (Fig. 2). A schematic representation of the possible mechanism of tumor angiogenesis induced by PI3K and Ras is shown in Fig. 2.

7. PI3K inhibition for cancer treatment

Recent study shows that inhibition of PI3K suppresses angiogenesis and tumor growth [72,78]. 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. But the poor solubility and high toxicity of these inhibitors limit the clinical application. To overcome these shortcomings, derivatives of LY294002 and wortmannin are being developed. It has been reported that PX-866, a wortmannin derivative has more potent and less toxic effects than wortmannin. It potentiates the antitumor activity of the EGFR inhibitor gefitinib against tumor xenografts induced by A549 non-small cell lung cancer in the early stages of treatment [79]. In addition, LY294002 derivatives such as TGX115 and TGX126, and PI3K isoform selective inhibitors such as halenaquinone and deguelin, have been developed and shown to have greater water solubility, lower toxicity, improved pharmacodynamics, and more specific PI3K selectivity [2,80]. SF1126 is a small molecule conjugate containing a pan-PI3K inhibitor that inhibits all isoforms of PI3K class IA and other key members of the PI3K superfamily, including DNA-PK. In preclinical studies, SF1126 has been shown to be a key inhibitor of many biological processes involved in tumor growth, dissemination, and angiogenesis. BEZ235 is an orally administered inhibitor of PI3K. Both SF1126 and BEZ235 have been identified to be effective for tumor treatment in a variety of preclinical mouse tumor models, including prostate, breast, ovarian, lung, multiple myeloma, brain, and other cancers. With the development of RNA interfering technologies, RNA interference, especially small interfering RNA (siRNA), has been demonstrated to be effective *in vivo*, providing the possibility for selectively targeting p110 or p85 isoforms using siRNA technology [4].

8. Blocking of Akt and mTOR activities for cancer treatment

Akt is a major downstream of PI3K for regulating tumor growth and angiogenesis. In animal study, inhibition of Akt by siRNA technique is effective to decrease ovarian tumor growth and angiogenesis [72]. Lipid-based inhibitors of Akt were the first group of inhibitors to be developed. These Akt inhibitors include perifosine, phosphatidylinositol ether lipid analogues, and D-3-deoxy-phosphatidylmyoinositol-1-[(*R*)-2-methoxy-3-octadecyloxyropyl hydrogen phosphate] (PX-316). Perifosine is the best-characterized Akt inhibitor, which inhibits the translocation of Akt to the cell membrane, and inhibits the growth of several different solid tumors [63]. The perifosine treatment overcomes the cancer cell resistance to the treatment with chemotherapeutic drugs and radiation. A set of structurally modified phosphatidylinositol ether lipid analogues (PIAs) shows the inhibitory effect on the Akt PH domain *in vitro* [81]. PX-316 has been demonstrated to inhibit the xenograft growth induced by breast cancer and colon cancer cells in mice [82]. PX-316 inhibits Akt activation through its PH domain.

Several other Akt antagonists such as 9-methoxy-2-methylellipticinium acetate (API-59-OMe), the indazole-pyridine A-443654, and isoform-specific canthine alkaloid analogues have been identified using high-throughput screening of the chemical libraries [80]. API-59-OMe is a small molecule inhibitor. It inhibits cell growth and induces apoptosis of human prostate, endometrial, breast, and ovarian cancer cells

[83,84]. A-443654 is a selective indazole-pyridine based Akt inhibitor, which was shown to increase the effect of paclitaxel treatment both *in vitro* and *in vivo* [85]. To identify the inhibitors specifically against Akt1 or Akt2, isoform-specific Akt inhibitors have been produced and selected by an iterative analog library synthesis approach [86]. Recently, two isoform-specific Akt inhibitors were identified and demonstrated to be effective to inhibit Akt1 or Akt2 *in vitro* [87,88]. But their effects *in vivo* remain to be investigated. Triciribine (VQD-002) is a known anti-cancer agent that was used in a number of clinical trials since the 1980s [89]. Recent study showed that triciribine is a specific inhibitor of Akt [90]. NCI-60 cell lines with *PIK3CA* mutation are more sensitive to triciribine treatment than those without the mutations [91]. Several other Akt inhibitors being developed include peptide-based inhibitors of Akt, 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 [80].

mTOR is a serine/threonine kinase downstream of Akt, which controls protein synthesis, cell cycle progression, cell proliferation, and tumor growth. Rapamycin and its analogues CCI-779, RAD001, and AP-23573 inhibit mTOR activation by binding to FK506-binding protein-12 [4]. CCI-779, RAD001, and AP-23573 are currently under the clinical trials for cancer treatment. Preclinical studies with these compounds indicate that these compounds have cytostatic activity as a single agent in animal models, and have synergistic effects for inhibiting tumor growth when they are used with conventional chemotherapy agent tamoxifen, or with radiation treatment. In clinical studies, these compounds have been shown to be effective against many types of solid cancers [80]. In Phase II clinical studies, CCI-779 has been shown to have effects for treating patients with renal cell carcinoma and glioblastoma, who were previously treated with the standard therapy methods [92,93]. The combination treatment of CCI-779 and the aromatase inhibitor letrozole is currently used for metastatic breast cancer in a Phase III study. RAD001 is administered orally for clinical application. RAD001 treatment enhances chemotherapy effect for treating relapsed non-small cell lung cancer and refractory gastrointestinal stromal tumor [80]. AP-23573 is a phosphorus-containing derivative of rapamycin, and developed in both intravenous and oral formulations for clinical trials. However, the optimal dose, schedule, patient selection, and combination strategies using these mTOR inhibitors remain to be elucidated.

9. Concluding remarks

A huge body of information shows that deregulation of PI3K and PTEN is associated with many cancers. The activation of PI3K signaling pathway may be due to multiple mechanisms, including the amplification and mutations of PI3K, lost function of PTEN, and activation of RTKs. Akt is an essential downstream of PI3K in regulating tumor growth and angiogenesis. The amplification and mutations of AKT are also observed in different cancers. The PI3K/PTEN/Akt signaling pathway exerts a key role in tumor growth and angiogenesis. Inhibition of signaling molecules in this pathway has been shown to have very strong effects on inhibiting tumor growth and angiogenesis in animal models. Akt activates downstream molecules mTOR and p70S6K1 for regulating tumor growth. Thus, the inhibitors of PI3K, Akt, mTOR, and p70S6K1 would have strong effects to inhibit the activation of PI3K/Akt in the human cancers. In addition, PI3K and mTOR inhibitors combined with other therapeutic drugs may have synergistic effects to inhibit tumor development. Although PI3K/Akt/mTOR signaling pathway is also required for normal cell proliferation and survival, low levels of these kinase activities may be sufficient for the normal cell survival. PI3K and mTOR inhibitors preferentially induced the apoptosis of cancer cells with the activation of PI3K and Akt, but not the normal cells. There are strong interests to develop the inhibitors of PI3K, Akt, or mTOR for the cancer therapy. The future challenges are to develop highly specific inhibitors of these kinases, and to determine the clinical efficacy and maximum tolerated doses of these inhibitors for clinical cancer treatment.

Acknowledgements

This work was supported by National Natural Science Foundation of China grants 30470361, 30570962, and 30200118.

References

[1] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655–1657.
[2] S.G. Ward, P. Finan, Isoform-specific phosphoinositide 3 (2003) 426–434.
[3] J.A. Engelman, J. Luo, L.C. Cantley, The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism, Nat. Rev., Genet. 7 (2006) 606–619.
[4] B.T. Hennessy, D.L. Smith, P.T. Ram, Y. Lu, G.B. Mills, Exploiting the PI3K/AKT pathway for cancer drug discovery, Nat. Rev., Drug Discov. 4 (2005) 988–1004.
[5] B. Urso, R.A. Brown, S. O'Rahilly, P.R. Shepherd, K. Siddle, The alpha-isoform of class II phosphoinositide 3-kinase is more effectively activated by insulin receptors than IGF receptors, and activation requires receptor NPEY motifs, FEBS Lett. 460 (1999) 423–426.
[6] B. Actor, J.M. Cobbers, R. Buschges, M. Wolter, C.B. Knobbe, P. Lichter, G. Reifenberger, R.G. Weber, Comprehensive analysis of genomic alterations in gliosarcoma and its two tissue components, Genes Chromosomes Cancer 34 (2002) 416–427.
[7] A. Hollestelle, F. Elstrodt, J.H. Nagel, W.W. Kallemeijn, M. Schutte, Phosphatidylinositol-3-OH kinase or RAS pathway mutations in human breast cancer cell lines, Mol. Cancer Res. 5 (2007) 195–201.
[8] P.A. van Dam, I.B. Vergote, D.G. Lowe, J.V. Watson, P. van Damme, J.C. van der Auwera, J.H. Shepherd, Expression of c-erbB-2, c-*myc*, and c-ras oncoproteins, insulin-like growth factor receptor I, and epidermal growth factor receptor in ovarian carcinoma, J. Clin. Pathol. 47 (1994) 914–919.
[9] D.A. Douglas, H. Zhong, J.Y. Ro, C. Oddoux, A.D. Berger, M.R. Pincus, J.M. Satagopan, W.L. Gerald, H.I. Scher, P. Lee, I. Osman, Novel mutations of epidermal growth factor receptor in localized prostate cancer, Front. Biosci. 11 (2006) 2518–2525.
[10] C.A. Livasy, F.C. Reading, D.T. Moore, J.F. Boggess, R.A. Lininger, EGFR expression and HER2/neu overexpression/amplification in endometrial carcinosarcoma, Gynecol. Oncol. 100 (2006) 101–106.
[11] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-kinase AKT pathway in human cancer, Nat. Rev., Cancer 2 (2002) 489–501.
[12] S.R. Datta, A. Brunet, M.E. Greenberg, Cellular survival: a play in three Akts, Genes Dev. 13 (1999) 2905–2927.
[13] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation

and regulation of Akt/PKB by the rictor-mTOR complex, Science 307 (2005) 1098–1101.
[14] P. Blume-Jensen, T. Hunter, Oncogenic kinase signalling, Nature 411 (2001) 355–365.
[15] S.A. Summers, A.W. Kao, A.D. Kohn, G.S. Backus, R.A. Roth, J.E. Pessin, M.J. Birnbaum, The role of glycogen synthase kinase 3beta in insulin-stimulated glucose metabolism, J. Biol. Chem. 274 (1999) 17934–17940.
[16] L.N. Cong, H. Chen, Y. Li, L. Zhou, M.A. McGibbon, S.I. Taylor, M.J. Quon, Physiological role of Akt in insulin-stimulated translocation of GLUT4 in transfected rat adipose cells, Mol. Endocrinol. 11 (1997) 1881–1890.
[17] L. Stephens, R. Williams, P. Hawkins, Phosphoinositide 3-kinases as drug targets in cancer, Curr. Opin. Pharmacol. 5 (2005) 357–365.
[18] D.C. N.Gao, Z. Flynn, X.S. Zhang, V. Zhong, K.J. Walker, X. Liu, B.H. Shi, G1 cell cycle progression and the expression of G1 cyclins are regulated by PI3K/AKT/mTOR/p70S6K1 signaling in human ovarian cancer cells, Am. J. Physiol., Cell Physiol. 287 (2004) C281–C291.
[19] N. Gao, Z. Zhang, B.H. Jiang, X. Shi, Role of PI3K/AKT/mTOR signaling in the cell cycle progression of human prostate cancer, Biochem. Biophys. Res. Commun. 310 (2003) 1124–1132.
[20] J.W. Lee, Y.H. Soung, S.Y. Kim, H.W. Lee, W.S. Park, S.W. Nam, S.H. Kim, J.Y. Lee, N.J. Yoo, S.H. Lee, PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas, Oncogene 24 (2005) 1477–1480.
[21] L. Shayesteh, Y. Lu, W.L. Kuo, R. Baldocchi, T. Godfrey, C. Collins, D. Pinkel, B. Powell, G.B. Mills, J.W. Gray, PIK3CA is implicated as an oncogene in ovarian cancer, Nat. Genet. 21 (1999) 99–102.
[22] B.I. Bertelsen, S.J. Steine, R. Sandvei, A. Molven, O.D. Laerum, Molecular analysis of the PI3K-AKT pathway in uterine cervical neoplasia: frequent PIK3CA amplification and AKT phosphorylation, Int. J. Cancer 118 (2006) 1877–1883.
[23] D.S. Byun, K. Cho, B.K. Ryu, M.G. Lee, J.I. Park, K.S. Chae, H.J. Kim, S.G. Chi, Frequent monoallelic deletion of PTEN and its reciprocal association with PIK3CA amplification in gastric carcinoma, Int. J. Cancer 104 (2003) 318–327.
[24] J.M. Pedrero, D.G. Carracedo, C.M. Pinto, A.H. Zapatero, J.P. Rodrigo, C.S. Nieto, M.V. Gonzalez, Frequent genetic and biochemical alterations of the PI 3-K/AKT/PTEN pathway in head and neck squamous cell carcinoma, Int. J. Cancer 114 (2005) 242–248.
[25] J. Willner, K. Wurz, K.H. Allison, V. Galic, R.L. Garcia, B.A. Goff, E.M. Swisher, Alternate molecular genetic pathways in ovarian carcinomas of common histological types, Hum. Pathol. 38 (2007) 607–613.
[26] G. Wu, M. Xing, E. Mambo, X. Huang, J. Liu, Z. Guo, A. Chatterjee, D. Goldenberg, S.M. Gollin, S. Sukumar, B. Trink, D. Sidransky, Somatic mutation and gain of copy number of PIK3CA in human breast cancer, Breast Cancer Res. 7 (2005) R609–R616.
[27] Y. Samuels, V.E. Velculescu, Oncogenic mutations of PIK3CA in human cancers, Cell Cycle 3 (2004) 1221–1224.
[28] I.G. Campbell, S.E. Russell, D.Y. Choong, K.G. Montgomery, M.L. Ciavarella, C.S. Hooi, B.E. Cristiano, R.B. Pearson, W.A. Phillips, Mutation of the PIK3CA gene in ovarian and breast cancer, Cancer Res. 64 (2004) 7678–7681.
[29] G.L. Gallia, V. Rand, I.M. Siu, C.G. Eberhart, C.D. James, S.K. Marie, S.M. Oba-Shinjo, C.G. Carlotti, O.L. Caballero, A.J. Simpson, M.V. Brock, P.P. Massion, B.S. Carson Sr., G.J. Riggins, PIK3CA gene mutations in pediatric and adult glioblastoma multiforme, Mol. Cancer Res. 4 (2006) 709–714.
[30] K. Oda, D. Stokoe, Y. Taketani, F. McCormick, High frequency of coexistent mutations of PIK3CA and PTEN genes in endometrial carcinoma, Cancer Res. 65 (2005) 10669–10673.
[31] M. Ollikainen, A. Gylling, M. Puputti, N.N. Nupponen, W.M. Abdel-Rahman, R. Butzow, P. Peltomaki, Patterns of PIK3CA alterations in familial colorectal and endometrial carcinoma, Int. J. Cancer 121 (2007) 915–920.
[32] Y. Samuels, Z. Wang, A. Bardelli, N. Silliman, J. Ptak, S. Szabo, H. Yan, A. Gazdar, S.M. Powell, G.J. Riggins, J.K. Willson, S. Markowitz, K.W. Kinzler, B. Vogelstein, V.E. Velculescu, High frequency of mutations of the PIK3CA gene in human cancers, Science 304 (2004) 554.
[33] M. Mizoguchi, C.L. Nutt, G. Mohapatra, D.N. Louis, Genetic alterations of phosphoinositide 3-kinase subunit genes in human glioblastomas, Brain Pathol. 14 (2004) 372–377.
[34] A.J. Philp, I.G. Campbell, C. Leet, E. Vincan, S.P. Rockman, R.H. Whitehead, R.J. Thomas, W.A. Phillips, The phosphatidylinositol 3′-kinase p85alpha gene is an oncogene in human ovarian and colon tumors, Cancer Res. 61 (2001) 7426–7429.
[35] A. Birck, V. Ahrenkiel, J. Zeuthen, K. Hou-Jensen, P. Guldberg, Mutation and allelic loss of the PTEN/MMAC1 gene in primary and metastatic melanoma biopsies, J. Invest. Dermatol. 114 (2000) 277–280.
[36] Y. Harima, S. Sawada, K. Nagata, M. Sougawa, V. Ostapenko, T. Ohnishi, Mutation of the PTEN gene in advanced cervical cancer correlated with tumor progression and poor outcome after radiotherapy, Int. J. Oncol. 18 (2001) 493–497.
[37] T. Kohno, M. Takahashi, R. Manda, J. Yokota, Inactivation of the PTEN/MMAC1/TEP1 gene in human lung cancers, Genes Chromosomes Cancer 22 (1998) 152–156.
[38] P.A. Steck, M.A. Pershouse, S.A. Jasser, W.K. Yung, H. Lin, A.H. Ligon, L.A. Langford, M.L. Baumgard, T. Hattier, T. Davis, C. Frye, R. Hu, B. Swedlund, D.H. Teng, S.V. Tavtigian, Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers, Nat. Genet. 15 (1997) 356–362.
[39] A. Suzuki, J.L. de la Pompa, V. Stambolic, A.J. Elia, T. Sasaki, B.B. del, A.I. Ho, A. Wakeham, A. Itie, W. Khoo, M. Fukumoto, T.W. Mak, High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice, Curr. Biol. 8 (1998) 1169–1178.
[40] S.A. Backman, D. Ghazarian, K. So, O. Sanchez, K.U. Wagner, L. Hennighausen, A. Suzuki, M.S. Tsao, W.B. Chapman, V. Stambolic, T.W. Mak, Early onset of neoplasia in the prostate and skin of mice with tissue-specific deletion of Pten, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 1725–1730.
[41] G. Li, G.W. Robinson, R. Lesche, H. Martinez-Diaz, Z. Jiang, N. Rozengurt, K.U. Wagner, D.C. Wu, T.F. Lane, X. Liu, L. Hennighausen, H. Wu, Conditional loss of PTEN leads to precocious development and neoplasia in the mammary gland, Development 129 (2002) 4159–4170.
[42] V. Duronio, M.P. Scheid, S. Ettinger, Downstream signalling events regulated by phosphatidylinositol 3-kinase activity, Cell. Signal. 10 (1998) 233–239.
[43] B.H. Jiang, J.Z. Zheng, M. Aoki, P.K. Vogt, Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 1749–1753.
[44] P. Carmeliet, M.G. Lampugnani, L. Moons, F. Breviario, V. Compernolle, F. Bono, G. Balconi, R. Spagnuolo, B. Oostuyse, M. Dewerchin, A. Zanetti, A. Angellilo, V. Mattot, D. Nuyens, E. Lutgens, F. Clotman, M.C. de Ruiter, A. Gittenberger-de Groot, R. Poelmann, F. Lupu, J.M. Herbert, D. Collen, E. Dejana, Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis, Cell 98 (1999) 147–157.
[45] A. Zubilewicz, C. Hecquet, J.C. Jeanny, G. Soubrane, Y. Courtois, F. Mascarelli, Two distinct signalling pathways are involved in FGF2-stimulated proliferation of choriocapillary endothelial cells: a comparative study with VEGF, Oncogene 20 (2001) 1403–1413.
[46] B.M. Burgering, P.J. Coffer, Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction, Nature 376 (1995) 599–602.
[47] A. Khwaja, K. Lehmann, B.M. Marte, J. Downward, Phosphoinositide 3-kinase induces scattering and tubulogenesis in epithelial cells through a novel pathway, J. Biol. Chem. 273 (1998) 18793–18801.
[48] J.S. Oh, J.E. Kucab, P.R. Bushel, K. Martin, L. Bennett, J. Collins, R.P. DiAugustine, J.C. Barrett, C.A. Afshari, S.E. Dunn, Insulin-like growth factor-1 inscribes a gene expression profile for angiogenic factors and cancer progression in breast epithelial cells, Neoplasia 4 (2002) 204–217.
[49] A. Papapetropoulos, D. Fulton, K. Mahboubi, R.G. Kalb, D.S. O'Connor, F. Li, D.C. Altieri, W.C. Sessa, Angiopoietin-1 inhibits endothelial cell apoptosis via the Akt/survivin pathway, J. Biol. Chem. 275 (2000) 9102–9105.
[50] B.T. Hennessy, D.L. Smith, P.T. Ram, Y. Lu, G.B. Mills, Exploiting the PI3K/AKT pathway for cancer drug discovery, Nat. Rev., Drug Discov. 4 (2005) 988–1004.

[51] L. Shayesteh, Y. Lu, W.L. Kuo, R. Baldocchi, T. Godfrey, C. Collins, D. Pinkel, B. Powell, G.B. Mills, J.W. Gray, PIK3CA is implicated as an oncogene in ovarian cancer, Nat. Genet. 21 (1999) 99–102.
[52] L.C. Cantley, B.G. Neel, New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4240–4245.
[53] C.B. Knobbe, G. Reifenberger, Genetic alterations and aberrant expression of genes related to the phosphatidyl-inositol-3′-kinase/protein kinase B (Akt) signal transduction pathway in glioblastomas, Brain Pathol. 13 (2003) 507–518.
[54] B. Actor, J.M. Cobbers, R. Buschges, M. Wolter, C.B. Knobbe, P. Lichter, G. Reifenberger, R.G. Weber, Comprehensive analysis of genomic alterations in gliosarcoma and its two tissue components, Genes Chromosomes, Cancer 34 (2002) 416–427.
[55] S.P. Staal, 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. 84 (1987) 5034–5037.
[56] J.Q. Cheng, B. Ruggeri, W.M. Klein, G. Sonoda, D.A. Altomare, D.K. Watson, J.R. Testa, Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 3636–3641.
[57] K. Nakayama, N. Nakayama, R.J. Kurman, L. Cope, G. Pohl, Y. Samuels, V.E. Velculescu, T.L. Wang, I. Shih, Sequence mutations and amplification of PIK3CA and AKT2 genes in purified ovarian serous neoplasms, Cancer Biol. Ther. 5 (2006) 779–785.
[58] A. Bellacosa, D. de Feo, A.K. Godwin, D.W. Bell, J.Q. Cheng, D.A. Altomare, M. Wan, L. Dubeau, G. Scambia, V. Masciullo, G. Ferrandina, P.P. Benedetti, S. Mancuso, G. Neri, J.R. Testa, Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas, Int. J. Cancer 64 (1995) 280–285.
[59] K. Nakatani, D.A. Thompson, A. Barthel, H. Sakaue, W. Liu, R.J. Weigel, R.A. Roth, Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines, J. Biol. Chem. 274 (1999) 21528–21532.
[60] R.P. Ermoian, C.S. Furniss, K.R. Lamborn, D. Basila, M.S. Berger, A.R. Gottschalk, M.K. Nicholas, D. Stokoe, D.A. Haas-Kogan, Dysregulation of PTEN and protein kinase B is associated with glioma histology and patient survival, Clin. Cancer Res. 8 (2002) 1100–1106.
[61] S. Yamamoto, Y. Tomita, Y. Hoshida, T. Morooka, H. Nagano, K. Dono, K. Umeshita, M. Sakon, O. Ishikawa, H. Ohigashi, S. Nakamori, M. Monden, K. Aozasa, Prognostic significance of activated Akt expression in pancreatic ductal adenocarcinoma, Clin. Cancer Res. 10 (2004) 2846–2850.
[62] J. Brognard, A.S. Clark, Y. Ni P.A., Dennis Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation, Cancer Res. 61 (2001) 3986–3997.
[63] A.M. Martelli, P.L. Tazzari, G. Tabellini, R. Bortul, A.M. Billi, L. Manzoli, A. Ruggeri, R. Conte, L. Cocco, A new selective AKT pharmacological inhibitor reduces resistance to chemotherapeutic drugs, TRAIL, all-trans-retinoic acid, and ionizing radiation of human leukemia cells, Leukemia 17 (2003) 1794–1805.
[64] J.T. Lee Jr., L.S. Steelman, J.A. McCubrey, Phosphatidylinositol 3′-kinase activation leads to multidrug resistance protein-1 expression and subsequent chemoresistance in advanced prostate cancer cells, Cancer Res. 64 (2004) 8397–8404.
[65] P.L. Tazzari, A. Cappellini, F. Ricci, C. Evangelisti, V. Papa, T. Grafone, G. Martinelli, R. Conte, L. Cocco, J.A. McCubrey, A.M. Martelli, Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase/Akt signal transduction network in human acute myelogenous leukemia blasts, Leukemia 21 (2007) 427–438.
[66] S. Lee, E.J. Choi, C. Jin, D.H. Kim, Activation of PI3K/Akt pathway by PTEN reduction and PIK3CA mRNA amplification contributes to cisplatin resistance in an ovarian cancer cell line, Gynecol. Oncol. 97 (2005) 26–34.
[67] L.Z. Liu, X. Zhou, G. Qian, X. Shi, J. Fang, B.H. Jiang, AKT1 amplification regulates cisplatin resistance in human lung cancer cells through mTOR/p70S6K1 pathway, Cancer Res. 67 (2007) 6325–6332.
[68] W. Risau, Mechanisms of angiogenesis, Nature 386 (1997) 671–674.
[69] J. Folkman, Angiogenesis, Annu. Rev. Med. 57 (2006) 1–18.
[70] P. Carmeliet, R.K. Jain, Angiogenesis in cancer and other diseases, Nature 407 (2000) 249–257.
[71] H.D. Skinner, J.Z. Zheng, J. Fang, F. Agani, B.H. Jiang, Vascular endothelial growth factor transcriptional activation is mediated by hypoxia-inducible factor 1alpha, HDM2, and p70S6K1 in response to phosphatidylinositol 3-kinase/AKT signaling, J. Biol. Chem. 279 (2004) 45643–45651.
[72] C. Xia, Q. Meng, Z. Cao, X. Shi, B.H. Jiang, Regulation of angiogenesis and tumor growth by p110 alpha and AKT1 via VEGF expression, J. Cell. Physiol. 209 (2006) 56–66.
[73] B.H. Jiang, E. Rue, G.L. Wang, R. Roe, G.L. Semenza, Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1, J. Biol. Chem. 271 (1996) 17771–17778.
[74] G.L. Wang, B.H. Jiang, E.A. Rue, G.L. Semenza, Hypoxia-inducible factor 1 is a basic-helix–loop–helix–PAS heterodimer regulated by cellular O2 tension, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 5510–5514.
[75] J. Fang, M. Ding, L. Yang, L.Z. Liu, B.H. Jiang, PI3K/PTEN/AKT Signaling Regulates Prostate Tumor Angiogenesis, Cell. Signal. 19 (2007) 2487–2497.
[76] L.Z. Liu, X.W. Hu, C. Xia, J. He, Q. Zhou, X. Shi, J. Fang, B.H. Jiang, Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1alpha expression through activation of AKT and P70S6K1 in human ovarian cancer cells, Free Radic. Biol. Med. 41 (2006) 1521–1533.
[77] S. Carnesecchi, J.L. Carpentier, M. Foti, I. Szanto, Insulin-induced vascular endothelial growth factor expression is mediated by the NADPH oxidase NOX3, Exp. Cell Res. 312 (2006) 3413–3424.
[78] J.L. Arbiser, T. Kau, M. Konar, K. Narra, R. Ramchandran, S.A. Summers, C.J. Vlahos, K. Ye, B.N. Perry, W. Matter, A. Fischl, J. Cook, P.A. Silver, J. Bain, P. Cohen, D. Whitmire, S. Furness, B. Govindarajan, J.P. Bowen, Solenopsin, the alkaloidal component of the fire ant (*Solenopsis invicta*), is a naturally occurring inhibitor of phosphatidylinositol-3-kinase signaling and angiogenesis, Blood 109 (2007) 560–565.
[79] N.T. Ihle, G. Paine-Murrieta, M.I. Berggren, A. Baker, W.R. Tate, P. Wipf, R.T. Abraham, D.L. Kirkpatrick, G. Powis, The phosphatidylinositol-3-kinase inhibitor PX-866 overcomes resistance to the epidermal growth factor receptor inhibitor gefitinib in A-549 human non-small cell lung cancer xenografts, Mol. Cancer Ther. 4 (2005) 1349–1357.
[80] C.A. Granville, R.M. Memmott, J.J. Gills, P.A. Dennis, Handicapping the race to develop inhibitors of the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway, Clin. Cancer Res. 12 (2006) 679–689.
[81] S.S. Castillo, J. Brognard, P.A. Petukhov, C. Zhang, J. Tsurutani, C.A. Granville, M. Li, M. Jung, K.A. West, J.G. Gills, A.P. Kozikowski, P.A. Dennis, Preferential inhibition of Akt and killing of Akt-dependent cancer cells by rationally designed phosphatidylinositol ether lipid analogues, Cancer Res. 64 (2004) 2782–2792.
[82] E.J. Meuillet, N. Ihle, A.F. Baker, J.M. Gard, C. Stamper, R. Williams, A. Coon, D. Mahadevan, B.L. George, L. Kirkpatrick, G. Powis, In vivo molecular pharmacology and antitumor activity of the targeted Akt inhibitor PX-316, Oncol. Res. 14 (2004) 513–527.
[83] X. Jin, D.R. Gossett, S. Wang, D. Yang, Y. Cao, J. Chen, R. Guo, R.K. Reynolds, J. Lin, Inhibition of AKT survival pathway by a small molecule inhibitor in human endometrial cancer cells, Br. J. Cancer 91 (2004) 1808–1812.
[84] H.J. Tang, X. Jin, S. Wang, D. Yang, Y. Cao, J. Chen, D.R. Gossett, J. Lin, A small molecule compound inhibits AKT pathway in ovarian cancer cell lines, Gynecol. Oncol. 100 (2006) 308–317.
[85] E.K. Han, J.D. Leverson, T. McGonigal, O.J. Shah, K.W. Woods, T. Hunter, V.L. Giranda, Y. Luo, Akt inhibitor A-443654 induces rapid Akt Ser-473 phosphorylation independent of mTORC1 inhibition, Oncogene 26 (2007) 5655–5661.
[86] C.W. Lindsley, Z. Zhao, W.H. Leister, R.G. Robinson, S.F. Barnett, D. Defeo-Jones, R.E. Jones, G.D. Hartman, J.R. Huff, H.E. Huber, M.E. Duggan, Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 761–764.
[87] D. Defeo-Jones, S.F. Barnett, S. Fu, P.J. Hancock, K.M. Haskell, K.R.

Leander, E. McAvoy, R.G. Robinson, M.E. Duggan, C.W. Lindsley, Z. Zhao, H.E. Huber, R.E. Jones, Tumor cell sensitization to apoptotic stimuli by selective inhibition of specific Akt/PKB family members, Mol. Cancer Ther. 4 (2005) 271–279.

[88] S.F. Barnett, D. Defeo-Jones, S. Fu, P.J. Hancock, K.M. Haskell, R.E. Jones, J.A. Kahana, A.M. Kral, K. Leander, L.L. Lee, J. Malinowski, E.M. McAvoy, D.D. Nahas, R.G. Robinson, H.E. Huber, Identification and characterization of pleckstrin-homology-domain-dependent and isoenzyme-specific Akt inhibitors, Biochem. J. 385 (2005) 399–408.

[89] L.G. Feun, J.A. Blessing, R.J. Barrett, P. Hanjani, A phase II trial of tricyclic nucleoside phosphate in patients with advanced squamous cell carcinoma of the cervix. A Gynecologic Oncology Group Study, Am. J. Clin. Oncol. 16 (1993) 506–508.

[90] L. Yang, H.C. Dan, M. Sun, Q. Liu, X.M. Sun, R.I. Feldman, A.D. Hamilton, M. Polokoff, S.V. Nicosia, M. Herlyn, S.M. Sebti, J.Q. Cheng, Akt/protein kinase B signaling inhibitor-2, a selective small molecule inhibitor of Akt signaling with antitumor activity in cancer cells overexpressing Akt, Cancer Res. 64 (2004) 4394–4399.

[91] D.B. Whyte, S.L. Holbeck, Correlation of PIK3Ca mutations with gene expression and drug sensitivity in NCI-60 cell lines, Biochem. Biophys. Res. Commun. 340 (2006) 469–475.

[92] E. Galanis, J.C. Buckner, M.J. Maurer, J.I. Kreisberg, K. Ballman, J. Boni, J.M. Peralba, R.B. Jenkins, S.R. Dakhil, R.F. Morton, K.A. Jaeckle, B.W. Scheithauer, J. Dancey, M. Hidalgo, D.J. Walsh, Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study, J. Clin. Oncol. 23 (2005) 5294–5304.

[93] S. Chan, M.E. Scheulen, S. Johnston, K. Mross, F. Cardoso, C. Dittrich, W. Eiermann, D. Hess, R. Morant, V. Semiglazov, M. Borner, M. Salzberg, V. Ostapenko, H.J. Illiger, D. Behringer, N. Bardy-Bouxin, J. Boni, S. Kong, M. Cincotta, L. Moore, Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer, J. Clin. Oncol. 23 (2005) 5314–5322.