

## Roles of protein kinase B/Akt in lung cancer

Cheng-Xiong Xu<sup>1</sup>, Hua Jin<sup>2</sup>, Ji-Young Shin<sup>3</sup>, Ji-Eun Kim<sup>3,4</sup>, Choong-Man Hong<sup>5</sup>, Myung-Haing Cho<sup>3,4</sup>

<sup>1</sup>Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia 30322, USA, <sup>2</sup>Center for Developmental and Therapeutics, Seattle Children's Research Institute, Seattle, Washington 98101, USA, <sup>3</sup>Laboratory of Toxicology, College of Veterinary Medicine, <sup>4</sup>Department of Nano Fusion Technology, Graduate School of Convergence Science and Technology Seoul National University, Seoul 151-742, Korea, <sup>5</sup>National Institute of Food and Drug Safety, Seoul 122-704, Korea

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Role of Akt signaling in lung tumorigenesis
  - 3.1. Hyperactivation of Akt in lung cancer
  - 3.2. Akt activated via mutation or upstream signals in lung cancer
  - 3.3. Activated Akt stimulates lung tumorigenesis through regulation of many cellular processes
    - 3.3.1. Activated Akt stimulates lung cancer cell growth and proliferation
    - 3.3.2. Activated Akt attenuates apoptosis in lung cancer
    - 3.3.3. Activated Akt increases angiogenesis in lung cancer
    - 3.3.4. Activated Akt stimulates lung cancer metastasis
4. Role of Akt pathway in chemo- and radiotherapy resistance of lung cancer
5. Akt pathway as a therapeutic target of lung cancer
6. Summary
7. Acknowledgements
8. References

## 1. ABSTRACT

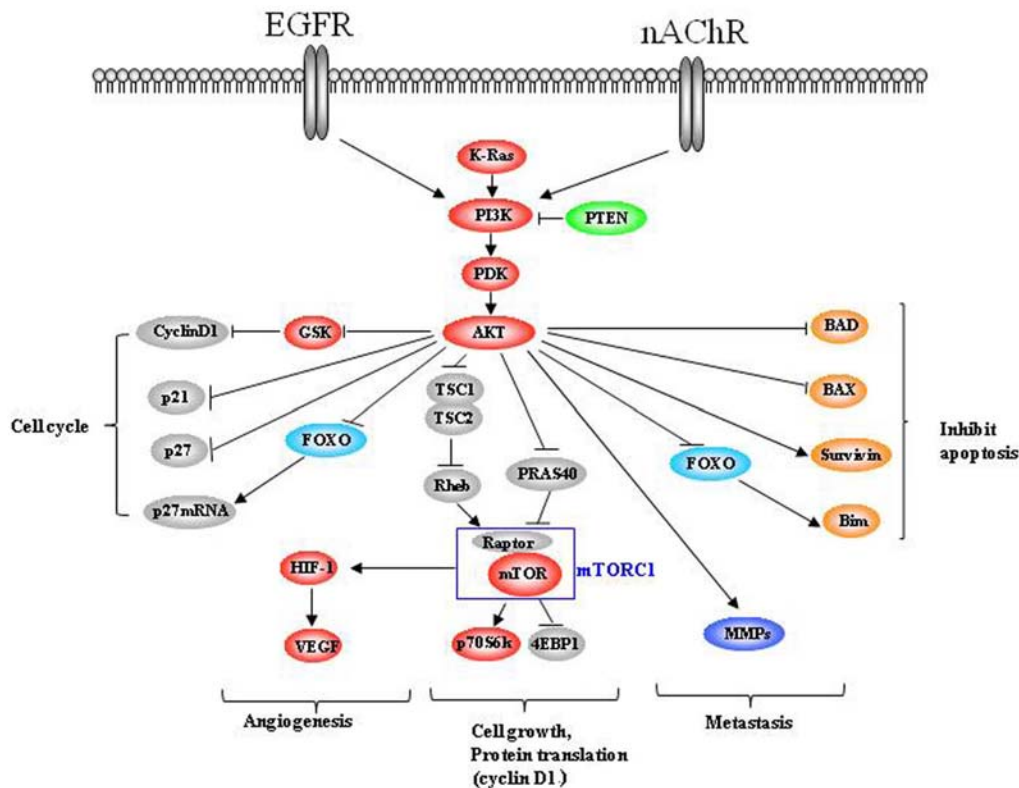
Lung cancer is the leading cause of cancer-related death worldwide and has frequently been associated with over-activated protein kinase B (PKB)/Akt. Akt is a serine/threonine protein kinase that plays an important role in cell growth, proliferation, and survival. Many lines of evidence point to the contribution of deregulated Akt in development or progression of lung cancer. In addition, recent studies have demonstrated that cancer cells defend themselves from therapeutic treatment through activation of pro-survival signals, including the Akt pathway. In this review, we described the way in which the Akt pathway is activated in development and progression of lung cancer, and the way in which deregulated Akt plays a significant role in lung tumorigenesis and resistance to chemo- or radiotherapy. In this review, we also discussed the potential of the Akt pathway as a target of lung cancer therapy.

## 2. INTRODUCTION

Lung cancer is currently the most frequently diagnosed solid tumor and is the most common cause of cancer mortality worldwide. In fact, lung cancer was the leading cause of cancer death in 2009, with 159,390 estimated deaths in the United States (1, 2). Lung cancer can be divided into two major forms, non-small-cell lung cancer (NSCLC) (85% of all lung cancer) and small-cell lung cancer (SCLC) (15% of all lung cancer) (3). Despite advances in early detection and standard treatment, NSCLC is often diagnosed at an advanced stage and has a poor prognosis (3). In NSCLC patients, the 5-year survival rate is only 15% (4), and in SCLC patients the 5-year survival rate is less than 5% (5).

PKB, also known as Akt, is a serine/threonine protein kinase. Akt as a central effector plays a crucial role

## Deregulated PKB stimulates lung tumorigenesis



**Figure 1.** Overview of upstream activators and downstream mediators of the Akt pathway in lung cancer. Protein kinase B (PKB/Akt) was activated by PI3K through phosphoinositide-dependent kinase-1 (PDK1), and PI3K was activated by upstream activators, such as epithelial growth factor receptor (EGFR), nicotinic acetylcholine receptors (nAChR), and K-Ras. The tumor suppressor PTEN opposes activity of PI3K. Activated Akt increases cell survival through phosphorylation and inactivation of the pro-apoptotic proteins BAD and BAX, and increases expression of the anti-apoptotic protein survivin. Activated Akt increases protein translation, cell cycle activity, and angiogenesis through regulation of downstream mediators, such as mammalian target of rapamycin (mTOR), glycogen synthase kinase (GSK), and forkhead box O (FOXO). Activated Akt also increases cancer metastasis related protein matrix metalloproteinases (MMPs).

in diverse cellular processes, including modulation of cell growth, proliferation, metabolism, neo-vascularization, and survival (6, 7). Activated Akt stimulates protein translation through activation of its downstream protein, mammalian target of rapamycin (mTOR, 8), and modification of protein translation is known to affect an immense number of biological processes, including cell size and growth (9). Activated Akt also stimulates cell cycle processing through reduction of cell cycle inhibitors, and increased cell cycle activity (10-12). In addition, activated Akt attenuates apoptosis through suppression of pro-apoptotic proteins (13) and inactivates the cell death protease known as caspase-9 (14). Recent studies have shown that Akt is one of the most frequently hyperactivated kinases in human lung cancer and its involvement in oncogenesis has been demonstrated (15, 16). In this review, we discuss the way in which the Akt pathway is activated and the way in which the hyperactivated Akt pathway contributes to lung cancer development and maintenance. In addition, we have summarized the mechanisms of therapeutic resistance to activated Akt pathway-induced lung cancer, and discussed the potential of the Akt pathway as a therapeutic target in lung cancer.

### 3. ROLE OF AKT SIGNALING IN LUNG TUMORIGENESIS

#### 3.1. Hyperactivation of Akt in lung cancer

Akt is known for its central node in a signaling pathway consisting of many components that implicate transformation, survival, proliferation, angiogenesis, and metastasis of cancer including lung cancer (Figure 1) (16-18). Akt encodes a serine/threonine kinase that has an amino-terminal pleckstrin-homology (PH) domain, a central catalytic domain and a short carboxyl-terminal regulatory domain (16). Akt up-stream protein phosphatidylinositol 3-kinase (PI3K) activation recruits Akt by direct interaction with its PH domain, and then another PH domain-containing serine/threonine kinase, 3-phosphoinositide-dependent protein kinase (PDK) phosphorylates Akt on Threonine 308 and Serine 473, thereby, causes full activation of Akt (16). Increased phosphorylation of Akt was detected in pre-malignant human bronchial epithelial cells, but not in normal bronchial cells (19). Tobacco specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) modulated the phenotype of normal human airway epithelial cells through rapid activation of Akt (20). Activated Akt was also detected in preneoplastic bronchial

## Deregulated PKB stimulates lung tumorigenesis

lesions, and these patients were reported to exhibit an increased propensity for development of lung cancer (21). Immunohistochemical study demonstrated activation of Akt in bronchial dysplasia, and Akt activation is important in genesis of a subset of NSCLCs (22, 23).

Hyperactivation of Akt was also detected in most NSCLC cell lines (24-26), and in 30-75% NSCLCs (15). In addition, studies have demonstrated that activation of Akt is associated with poor prognosis in patients with early-stage NSCLCs (27). High levels (70%) of phosphor-Akt have also been detected in tumor tissues from SCLC patients by immunohistochemical analysis, and have implicated the activated Akt pathway in cancer progression (28).

### 3.2. Akt activated via mutation or upstream signals in lung cancer

Recent studies discovered Akt1 somatic mutation (E17K) in the PH domain, and these studies also demonstrated that somatic mutation of Akt1 caused constitutive activation of Akt1 in human cancer patients including lung cancer (29, 30). Akt1 is one isoform of Akt1, 2, 3 and plays important role in the lung cancer progression (31). Malanga *et al.* found Akt1 E17 mutation in squamous cell carcinoma of the lung and they reported E17K point mutation induced hyperactivation of Akt1 and such mutation of Akt1 may contribute to the development of these tumors (29).

Eighty-five to ninety percent of lung cancer cases are associated with tobacco use (32). Tobacco components promote lung tumorigenesis through genotoxic effects and biochemical modulation of signaling pathways, including the Akt pathway (33). PI3K is an important upstream protein of Akt that contributes to Akt activation in the lung cancer (34), and tobacco carcinogen induces PI3K-dependent activation of Akt in lung epithelial cells (20, 35). PIK3CA encodes the PI3K catalytic subunit and its mutation has been observed in human cancers including lung cancer, and such changes in PIK3CA are associated with increased PI3K activity and p-Akt activation (36). Many lines of evidence show that tobacco components activate the PI3K/Akt pathway via activated multiple upstream signals of PI3K, including growth factor tyrosine kinase receptor, Ras, and phosphatase tensin homologue deleted on chromosome ten (PTEN). PI3K is composed of a regulatory subunit (p85) and a catalytic subunit (p110) that contains Src-homology 2 domains (37). Interaction of these domains with phosphotyrosine residues occurs on growth factor tyrosine kinase receptors, such as ErbB (33). Epidermal growth factor receptor (EGFR) is a member of the ErbB family and the ErbB signal is a major upstream signal of PI3K. Overexpressed EGFR was detected in bronchial epithelial cells of smokers (38) and *in vitro* studies have shown that tobacco-specific carcinogen NNK induces transformation of bronchial epithelial cells via increased EGFR expression (39). Overexpressed EGFR has also been detected in approximately 40-80% of NSCLC patients (40). EGFR mutations also caused constant activation of EGFR (41) and higher activation of Akt was detected in NSCLC patients with EGFR mutations (42).

These mutations of EGFR included extracellular and intracytoplasmic domain mutation. Extracellular mutation of EGFR is deletion mutation, and EGFR mutation gene product does not have ligand binding site, however, such EGFR can be constantly activated without any ligand binding, thus, stimulates cell proliferation in SLCs (43, 44). Intracytoplasmic domain mutation can be divided into 4 major types: point mutation in exon 18, insertion in exon 20, deletion in exon 19, and point mutation in exon 21. The last two mutations are the most frequent mutations of EGFR (45). These two intracytoplasmic domain mutations of EGFR cause conformational change of the ATP-binding domain, which results in constant activation of EGFR without ligand binding (45). The PI3K/Akt pathway was also activated by direct interaction with Ras (33, 46). Activating mutations of *K-ras* were detected in 25% of smoking-associated human lung adenocarcinomas (47). In addition, tobacco-specific carcinogen NNK induced *K-ras* mutation (48) and *K-ras* gene mutation enhanced motility of lung adenocarcinoma cells via Akt activation (46). Nicotine is another tobacco-specific carcinogen that also activates the Akt pathway, and such activated Akt pathways were blocked by inhibitors of nicotinic acetylcholine receptors (nAChR) (20). Although nAChR has the ability to activate Akt, it is dependent on PI3K (33), meaning that activation of PI3K/Akt via stimulation of nAChR is also one of the mechanisms of tobacco component activation of the PI3K/Akt pathway. Lipid phosphatase PTEN is another PI3K upstream protein that negatively regulates the PI3K/Akt pathway through dephosphorylation of PIP3 at the plasma membrane, while mutated PTEN does not (37). Studies have shown that loss of PTEN occurs in ~70% of NSCLC through inactivating mutations (49), and such inactivation of PTEN causes constitutively activated PI3K/Akt signaling, which contributes to lung carcinogenesis (50). Overexpressed microRNAs also downregulate PTEN expression in lung cancer. microRNAs represent a class of small RNAs frequently deregulated in human malignancies, and some miRNAs are overexpressed in NSCLC like miR-21, miR-221 and miR-222 (51, 52). Moreover, such overexpressed miRNAs downregulate PTEN gene expression, thereby, promote NSCLC invasion (51, 52). The homogenous deletion of PTEN gene and methylation of PTEN promoter are other important mechanism of lack of PTEN activity in lung cancer (53). Noro *et al.* analyzed PTEN levels in 25 lung cancer cell lines and demonstrated that 6 out of 25 cell lines displayed low expression of PTEN protein (54). In addition, they demonstrated that genomic analysis of 2 of the 6 cell lines revealed homozygous deletions of the PTEN gene and another 2 of the 6 cell lines showed hypermethylation of PTEN gene promoter. Taken together, hyperactivation of Akt in lung cancer was caused by somatic mutation of Akt or deregulation of several upstream signals, including EGFR activation, Ras activation, PI3K activation, and PTEN inactivation (15).

### 3.3. Activated Akt stimulates lung tumorigenesis through regulation of many cellular processes

Deregulated Akt stimulates lung tumorigenesis through enhancement of cancer cell growth, survival, and proliferation. Such effects of Akt in lung tumorigenesis

## Deregulated PKB stimulates lung tumorigenesis

were presented via regulation of multiple downstream signaling pathways (Figure 1), such as mTOR, forkhead box class O (FOXO), and glycogen synthase kinase 3 (GSK3) (15).

### 3.3. 1. Activated Akt stimulates lung cancer cell growth and proliferation

mTOR is one of the most important downstream proteins of Akt, and the activated Akt/mTOR pathway contributes to development and maintenance of lung cancer (35, 55). Like Akt, mTOR drives tumorigenesis through regulation of cell growth, proliferation, protein synthesis, and metabolism (56). In fact, frequent Akt activation and mTOR phosphorylation were found in 51% of NSCLC patient samples and in 74% of NSCLC cell lines (21). mTOR is present in two distinct complexes, mTOR complex 1 (mTORC1, mTOR/Raptor) and mTOR complex 2 (mTORC2, mTOR/Rictor) (57). mTORC1 increases protein synthesis by activation of p70 ribosomal protein S6 kinase (S6K1) and inactivation of the eIF4E binding protein (4E-BP1), which increases the level of many proteins needed for cell cycle progression, proliferation, angiogenesis, and survival pathways (57). Akt activates mTORC1 through inhibition of the mTORC1 inhibitor PRAS40 (58, 59) and phosphorylation of tuberous sclerosis complex 2 (TSC2) because phosphorylated TSC2 can inhibit Rheb of the mTORC1 activator (60-62). Studies have shown that tobacco components stimulate NSCLC growth and proliferation through activation of mTORC1 and increase phosphorylation of S6K and 4E-BP1; such events were inhibited by treatment with Akt siRNA or the mTOR inhibitor (63, 64).

Akt activation stimulates cell cycle progression through increases of cell cycle promoter cyclin D1 and inactivation of cell cycle inhibitors p21 and p27 (15). Cyclin D1 is one of the G1 cyclins, which control cell cycle progression by allowing transition of G1 to S. Previous studies have demonstrated that cyclin D1 was overexpressed in lung cancer, and that cyclin D1 overexpression is involved in tumorigenesis of NSCLCs from the early stage, which could be a molecular marker for poorer outcome of cancer (65, 66). Activated Akt can increase cyclin D1 through two different mechanisms, which include control of synthesis and stability of cyclin D1 (67, 68). GSK3beta, an Akt substrate as well as a negative regulator of cyclin D1, can degrade cyclin D1 (69), however, activated Akt can phosphorylate and inactivate GSK3beta to prevent cyclin D1 degradation in lung adenocarcinoma (70). Activated Akt can also directly increase cyclin D1 expression through activation of Akt/mTOR-dependent protein translation signals (71). In addition, Akt also controls other important cell cycle regulators, such as p21 and p27 (69). Previous studies have demonstrated that p27 was decreased in cancerous lung tissues compared to non-neoplastic lung tissue (72), and low levels of p21, p27 are significantly correlated with survival in NSCLC patients (73-75). Activated Akt directly antagonizes the action of p21 and p27 by phosphorylation of a site located near the nuclear localization signal to induce cytoplasmic retention of these cell cycle inhibitors (15, 76). Recent studies have suggested that the PI3K/Akt pathway regulates p27 protein stability through upregulation of S-phase kinase-associated protein-2 (SKP2)

(69). SKP2 is a key component of the SCF<sup>SKP2</sup> ubiquitin ligase complex that mediates degradation of p27 (77). Akt regulates abundance of p27 mRNA by phosphorylation and inactivation of the FOXO transcription factors (78). Recent lung cancer studies using animal models showed that PI3K inhibitor treatment rapidly decreased phosphorylated Akt and phosphorylated p27, concomitant with an increase in nuclear p27; such events inhibited tumor growth (79).

### 3.3.2. Activated Akt attenuates apoptosis in lung cancer

Apoptosis is a highly regulated natural process, and maintains the health of organisms through removal of unwanted, redundant, or damaged cells (80, 81); therefore, dysregulation of apoptosis often results in development of human disease, including cancer (82). Cancer is often characterized by too little apoptosis; such defects of apoptosis are known to be caused by several deregulated pathways and by tumorigenesis (83). During development of lung cancer, the activation of Akt pathway leads to survival of cancer cells through inhibition of pro-apoptotic protein and increases anti-apoptotic proteins. Increased survivin by activated Akt is one of the anti-apoptotic mechanisms. Survivin is one anti-apoptotic protein, and overexpressed survivin has been detected in lung cancer (84, 85). Furthermore, survivin has been identified as a negative prognostic factor in NSCLCs (86). In SCLCs, constitutively active Akt can attenuate apoptosis through increased survivin expression, whereas negative modulation of Akt decreased survivin expression (87). BAD is a pro-apoptotic protein, and is suppressed by activation of the PI3K/Akt pathway in response to nicotine exposure, leading to a cell growth advantage (40). Nicotine-dependent Akt activation also effectively leads to increased phosphorylation of Bax, another member of the Bcl-2 protein family, thereby, abrogating its pro-apoptotic function (88).

Akt also controls apoptosis through regulation of the major substrate FOXO transcription factors. FOXO protein promotes apoptosis by translocating to the nucleus and upregulation of several pro-apoptotic target genes including Fas-L, TRADD and Bim (89, 90). However, such effect of FOXO in apoptosis can be blocked by Akt activation. Activated Akt phosphorylates FOXO and such phosphorylated FOXO proteins are relocalized to cytoplasm from nucleus, so sequestering them from their gene targets (91). In addition, activated Akt induces the degradation of FOXO through phosphorylation of FOXO (92, 93). Skp2 induces ubiquitin-dependent proteasome degradation of FOXO1 and this effect of Skp2 requires Akt-specific phosphorylation of FOXO1 at Ser256 (94). Moreover, Akt activation upregulates Skp2 (95). Several lines of researches showed that phosphorylated Akt and FOXO proteins were increased in lung tumors (79) and inhibition of Akt using RNA interference led to FOXO1 translocation to the nucleus and initiation of apoptosis in NSCLC cells (96).

### 3.3.3. Activated Akt increases angiogenesis in lung cancer

Angiogenesis is required for tumor growth and metastasis, and vascular endothelial growth factor (VEGF)

## Deregulated PKB stimulates lung tumorigenesis

is crucial in cancer induced endothelial cell proliferation and vascular permeability, leading to neo-angiogenesis (97). VEGF levels in bronchial epithelial cells of smokers were increased in association with progression of bronchial dysplasia (98), and high vascularization was detected in SCLCs (99). In addition, high levels of VEGF in plasma of SCLC patients were associated with poor prognosis (100, 101). Hypoxia, an important phenomenon in solid tumors (102), increases hypoxia-inducible factor-1 (HIF-1) through Akt pathway (103-106). HIF-1, a key transcription factor, increases VEGF expression (103-105). These findings suggest a close association between Akt activation and VEGF-induced angiogenesis in lung cancer. In fact, natural dietary flavonoid apigenin inhibits tumor angiogenesis through decreasing VEGF and HIF-1 expression via PI3K/Akt/p70S6K1 pathway in many cancers including lung cancer (107, 108).

### 3.3.4. Activated Akt stimulates lung cancer metastasis

Cancer metastasis is the primary cause of morbidity and mortality for patients with cancer (109), and matrix metalloproteinases (MMPs) play an important role in cancer metastasis (110). In lung cancer patients with clinically evident metastasis, serum levels of MMP-2 were significantly elevated compared to those without metastasis (111). Compared to healthy volunteers, MMP-9 was also increased in patients with lung cancer (112). Tumors with lymph node metastasis showed a tendency toward higher levels of expression of MMP-7 mRNA compared to those without lymph node metastasis (113). Studies of Akt pathway activation in lung cancer have demonstrated partial regulation of MMPs gene expression by the PI3K/Akt pathway (114, 115). These results suggest that Akt may play an important role in lung cancer metastasis through control of MMP expression. In fact, findings from several studies have demonstrated that inhibition of MMP-2, MMP-9, and MMP-7 via down-regulation the PI3K/Akt signaling pathway can suppress lung cancer invasion and migration (116-118).

## 4. ROLE OF AKT PATHWAY IN CHEMO- AND RADIOTHERAPY RESISTANCE OF LUNG CANCER

Therapeutic resistance is a major obstacle to successful cancer therapy, and the Akt/mTOR pathway may play an important role in therapeutic resistance of cancer cells. Radiation induces activation of multiple intracellular signaling pathways and in general, this radiation-induced signaling will lead to radioprotective signals, including the Akt pathway (37, 119). In addition, the activated Akt pathway was also closely associated with chemotherapy resistance in lung cancer (120).

Cancer cells can acquire resistance to apoptosis through various mechanisms that interfere at different levels of apoptosis signaling (121). Anti-apoptotic protein overexpression or pro-apoptotic protein decrease is one of the therapeutic resistance mechanisms. Activated Akt has been detected in chemotherapy resistant lung cancers and Akt is known to regulate cancer cell survival through control of anti- and pro-apoptotic proteins. In fact,

transfection of constitutively active Akt into NSCLC cells with low Akt activity increased Akt activity and attenuated chemotherapy and radiation-induced apoptosis (25). In addition, inhibition of Akt activity in these cell lines using a pharmacological or genetic approach resulted in enhanced cellular responsiveness to chemo- or irradiation therapy (25). Findings from these studies suggest that resistance to the apoptosis mechanism in lung cancer is related to the activated Akt pathway. Mcl-1 and cellular FLICE-like inhibitory protein (c-FLIP) are anti-apoptotic proteins, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a potential anticancer agent. Recent studies have shown that lung cancer cells can acquire resistance to potential anticancer agents, TRAIL-induced cytotoxicity through Akt-mediated eminent expression of c-FLIP and Mcl-1 (122). Findings from this study demonstrated increased expression of c-FLIP, and Mcl-1 expression was dramatically decreased by Akt siRNA treatment. In addition, Chen *et al.* (101) demonstrated that increased Mcl-1 was dependent on the Akt-COX-2 pathway in this TRAIL-resistant lung cancer. BAD is an important pro-apoptotic protein associated with chemo- and radiation therapy resistance. In SCLC cells, constitutively activated Akt increased chemo- and radio-resistance through phosphorylation and inactivation of BAD (123). Cisplatin is an anticancer agent that stimulates cancer cell apoptosis. However, cisplatin treatment increased Akt pathway-dependent pro-survival protein survivin expression in SCLC, so that partially protected cancer cells were produced from drug-induced apoptosis (87).

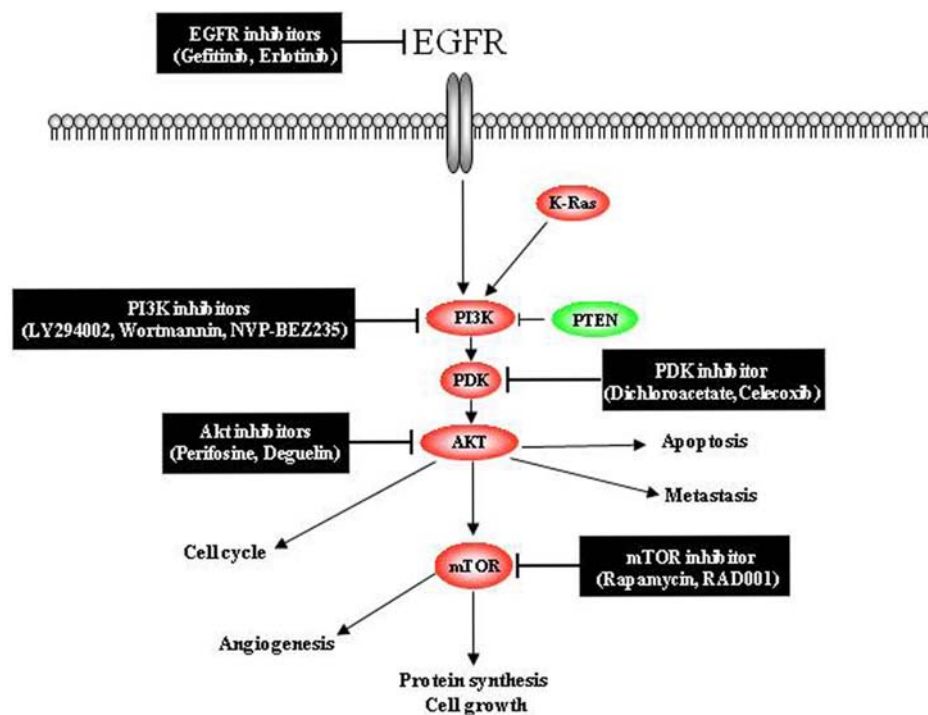
mTOR is a major target downstream of Akt, therefore, many inhibitors of mTOR, such as rapamycin and RAD001, have been developed as antitumor drugs. In fact, several rapamycin analogues are now in clinical trials in oncology. However, clinical studies have shown that some cancers including lung cancer occur due to resistance to inhibitors of mTOR through rapamycin feedback activation of Akt (124).

Autophagy is a lysosome-dependent degradative pathway that is frequently activated in cancer cells treated with chemo- or radiotherapy (125). Autophagy was negatively regulated by mTOR (126), so that inhibition of the Akt/mTOR pathway results in increased autophagy in lung cancer (120). Autophagy has recently been demonstrated as important for conferring resistance to chemotherapy, radiation therapy, and immunotherapy (127) because autophagy constitutes a stress adaptation for avoidance of cell death and suppression of apoptosis (128). In fact, combination treatment of the Akt inhibitor perifosine with autophagy inhibitors enhanced apoptosis and tumor growth in lung cancer (129).

## 5. AKT PATHWAY AS A THERAPEUTIC TARGET OF LUNG CANCER

As described above, activation of Akt pathway is closely associated with lung tumorigenesis. Overactivation of Akt pathway strongly contributes cell proliferation, survival as well as angiogenesis, which are

## Deregulated PKB stimulates lung tumorigenesis



**Figure 2.** Targeting of EGFR/PI3K/Akt/mTOR signal transduction pathways in lung cancer therapy. Potential sites of action of small molecular weight inhibitors are indicated. EGFR: epidermal growth factor receptor; mTOR: mammalian target of rapamycin; PDK: phosphoinositide-dependent kinase; PI3K: phosphoinositide 3-kinases; PTEN: phosphatase and tensin homolog.

responsible for the important aspects of lung tumorigenesis. Therefore, Akt pathway can be an important therapeutic target for treatment of lung cancer. In fact, many clinical studies have demonstrated that inhibition of Akt pathway by a pharmacological or genetic approach can significantly reduce lung cancer progression (31, 63, 129). Therefore, many research groups are actively developing Akt inhibitors as an anti-cancer drug.

Perifosine is a lipid-based phosphatidylinositol analogue that inhibits Akt activation through preventing Akt with  $\text{PtdIns}(3,4,5)\text{P}_3$  and undergoing membrane translocation (130). Studies have shown that perifosine presents anti-cancer effects through inhibiting Akt/mTOR signaling and inducing apoptosis in human lung cancer cells (129, 131). Natural plant product deguelin is also an Akt inhibitor and recent studies have demonstrated that deguelin has chemopreventive effects on tobacco-induced lung tumorigenesis (132). Deguelin also exhibits therapeutic activities through inducing apoptosis in premalignant and malignant human bronchial epithelial cells (133).

As we have discussed above, Akt was activated by several upstream receptor tyrosine kinases and plays a significant role through control of several downstream signals during development and progression of lung cancer. Therefore, selective inhibition of these upstream or downstream signals of Akt may also be an important strategy in lung cancer therapy. In fact, many inhibitors of

the Akt pathway have now been developed as anti-cancer drugs (Figure 2), and studies have shown that use of these inhibitors for inactivation of EGFR (gefitinib and erlotinib), PI3K (LY294002, wortmannin, and NVP-BE235), PDK (dichloroacetate and celecoxib), and mTOR (rapamycin and RAD001) or delivery of wild PTEN to increase PTEN expression can significantly inhibit lung tumor progression through Akt pathway-dependent increase of apoptosis or inhibition of proliferation and growth (134-137). Recently, a new orally available dual PI3K/mTOR inhibitor, NVP-BE235 was developed as an anti-cancer drug which exhibited more anti-proliferative effect than mTOR inhibitor treatment (138).

However, prolonged treatment with a single inhibitor of the PI3K/Akt pathway induces resistance through reactivation of the PI3K/Akt pathway, so that multi-target approaches may be a good strategy for better efficacy in lung cancer therapeutics and for reduced risk for development of secondary resistance. For example, treatment with LY294002, a PI3K/Akt inhibitor, did not induce apoptosis in lung adenocarcinoma cells, however, simultaneous inhibition of the PI3K/Akt pathway by LY294002 and Bcl-xL function by Bcl-xL siRNA greatly enhanced the apoptotic response (139). Sun *et al.* (115) also reported that rapamycin induced Akt activation attenuates rapamycin's growth-inhibitory effects and combined treatment of rapamycin with PI3K inhibitor can induce enhanced inhibitory effects on the growth of lung cancer.

## Deregulated PKB stimulates lung tumorigenesis

In lung cancer progression, the Akt pathway can be often activated by multiple upstream activators, so that inactivation of one upstream activator of Akt may not result in significant therapeutic efficacy. Even though EGFR is an upstream activator of the Akt pathway, some lung cancer resistancy may be associated with EGFR inhibitor treatment. In fact, the Akt pathway can be activated EGFR-independent signals such as Ras activation or PTEN loss (37, 140). In this case, inhibition of the Akt pathway with PI3K/Akt inhibitor led to sensitization of lung cancer to EGFR inhibitor chemotherapy (141). Akt is not the sole gene activated in lung cancer. Rather, many other activated oncogene pathways cooperate with the Akt pathway in the promotion of lung cancer cell proliferation and growth. Therefore, inactivation of these pathways with the Akt pathway together may enhance Akt pathway targeted lung cancer therapeutic efficacy. In fact, Lee *et al.* (118) have reported that the PI3K/Akt and MAPK pathways cooperate in the promotion of NSCLC cell proliferation through maintenance of cell survival, and concurrent inhibition of both pathways have showed enhanced anti-proliferative effects by increasing apoptosis.

As described above, radiotherapy resistance is also partly associated with the reactivated Akt pathway in lung cancer, so that combination treatment that includes radiation along with Akt pathway targeted therapy can enhance the efficacy of the radiation therapy effect in lung cancer development and progression. Konstantinidou *et al.* (119) reported that PI3K/mTOR inhibitor treatment can increase radiation-induced apoptosis in NSCLC. Park *et al.* (120) also reported on combination of PTEN and radiation enhanced cell death and G2/M arrest through inactivation of Akt activity and p21 induction in NSCLC cells.

### 6. SUMMARY

The Akt pathway plays a significant role in cell growth, proliferation, and survival. During development and progression of lung cancer, the Akt pathway is often activated by carcinogens or various genetic mutations of upstream regulators; such a deregulated Akt pathway is clearly a central pathway in critical aspects of malignant transformation. Consequently, this pathway plays a key role in radio- and chemotherapy resistance in patients with lung cancer. Therefore, combination treatment of Akt pathway targeted inhibitors with other chemo- or radiotherapy can enhance therapeutic efficacy in treatment of lung cancer by reducing the risk for development of secondary resistance.

### 7. ACKNOWLEDGEMENTS

This work was supported by a grant from the NRL (#20090078641) of the Ministry of Education, Science and Technology in Korea. Part of this work is also supported by Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Korea.

### 8. REFERENCES

1. A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu and M. J. Thun: Cancer statistics, 2009. *CA Cancer J Clin*, 59(4), 225-49 (2009)

2. P. Nana-Sinkam, H. Golpon, R. L. Keith, R. J. Oyer, S. Sotto-Santiago, M. D. Moore, W. Franklin, R. A. Nemenoff and M. W. Geraci: Prostacyclin in human non-small cell lung cancers. *Chest*, 125(5 Suppl), 141S (2004)

3. R. S. Herbst, J. V. Heymach and S. M. Lippman: Lung cancer. *N Engl J Med*, 359(13), 1367-80 (2008)

4. J. R. Molina, A. A. Adjei and J. R. Jett: Advances in chemotherapy of non-small cell lung cancer. *Chest*, 130(4), 1211-9 (2006)

5. R. Govindan, N. Page, D. Morgensztern, W. Read, R. Tierney, A. Vlahiotis, E. L. Spitznagel and J. Piccirillo: Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. *J Clin Oncol*, 24(28), 4539-44 (2006)

6. E. Gonzalez and T. E. McGraw: The Akt kinases: isoform specificity in metabolism and cancer. *Cell Cycle*, 8(16), 2502-8 (2009)

7. K. M. Nicholson and N. G. Anderson: The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal*, 14(5), 381-95 (2002)

8. D. Ruggero and N. Sonenberg: The Akt of translational control. *Oncogene*, 24(50), 7426-34 (2005)

9. J. D. Richter and N. Sonenberg: Regulation of cap-dependent translation by eIF4E inhibitory proteins. *Nature*, 433(7025), 477-80 (2005)

10. V. Serra, B. Markman, M. Scaltriti, P. J. Eichhorn, V. Valero, M. Guzman, M. L. Botero, E. Llonch, F. Atzori, S. Di Cosimo, M. Maira, C. Garcia-Echeverria, J. L. Parra, J. Arribas and J. Baselga: NVP-BE225, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res*, 68(19), 8022-30 (2008)

11. B. P. Zhou, Y. Liao, W. Xia, B. Spohn, M. H. Lee and M. C. Hung: Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. *Nat Cell Biol*, 3(3), 245-52 (2001)

12. P. Blume-Jensen and T. Hunter: Oncogenic kinase signalling. *Nature*, 411(6835), 355-65 (2001)

13. S. R. Datta, H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh and M. E. Greenberg: Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*, 91(2), 231-41 (1997)

14. M. H. Cardone, N. Roy, H. R. Stennicke, G. S. Salvesen, T. F. Franke, E. Stanbridge, S. Frisch and J. C. Reed: Regulation of cell death protease caspase-9 by phosphorylation. *Science*, 282(5392), 1318-21 (1998)

## Deregulated PKB stimulates lung tumorigenesis

15. D. A. Altomare and J. R. Testa: Perturbations of the AKT signaling pathway in human cancer. *Oncogene*, 24(50), 7455-64 (2005)
16. I. Vivanco and C. L. Sawyers: The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*, 2(7), 489-501 (2002)
17. S. G. Kennedy, E. S. Kandel, T. K. Cross and N. Hay: Akt/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. *Mol Cell Biol*, 19(8), 5800-10 (1999)
18. P. Brodt, A. Samani and R. Navab: Inhibition of the type I insulin-like growth factor receptor expression and signaling: novel strategies for antimetastatic therapy. *Biochem Pharmacol*, 60(8), 1101-7 (2000)
19. K. H. Chun, J. W. Kosmider, 2nd, S. Sun, J. M. Pezzuto, R. Lotan, W. K. Hong and H. Y. Lee: Effects of deguelin on the phosphatidylinositol 3-kinase/Akt pathway and apoptosis in premalignant human bronchial epithelial cells. *J Natl Cancer Inst*, 95(4), 291-302 (2003)
20. K. A. West, J. Brognard, A. S. Clark, I. R. Linnoila, X. Yang, S. M. Swain, C. Harris, S. Belinsky and P. A. Dennis: Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *J Clin Invest*, 111(1), 81-90 (2003)
21. B. R. Balsara, J. Pei, Y. Mitsuchi, R. Page, A. Klein-Szanto, H. Wang, M. Unger and J. R. Testa: Frequent activation of AKT in non-small cell lung carcinomas and preneoplastic bronchial lesions. *Carcinogenesis*, 25(11), 2053-9 (2004)
22. A. S. Tsao, T. McDonnell, S. Lam, J. B. Putnam, N. Bekele, W. K. Hong and J. M. Kurie: Increased phospho-AKT (Ser473) expression in bronchial dysplasia: implications for lung cancer prevention studies. *Cancer Epidemiol Biomarkers Prev*, 12(7), 660-4 (2003)
23. J. W. Tichelaar, Y. Zhang, J. C. leRiche, P. W. Biddinger, S. Lam and M. W. Anderson: Increased staining for phospho-Akt, p65/RELA and cIAP-2 in pre-neoplastic human bronchial biopsies. *BMC Cancer*, 5, 155 (2005)
24. P. P. Massion, W. L. Kuo, D. Stokoe, A. B. Olshen, P. A. Treseler, K. Chin, C. Chen, D. Polikoff, A. N. Jain, D. Pinkel, D. G. Albertson, D. M. Jablons and J. W. Gray: Genomic copy number analysis of non-small cell lung cancer using array comparative genomic hybridization: implications of the phosphatidylinositol 3-kinase pathway. *Cancer Res*, 62(13), 3636-40 (2002)
25. J. Brognard, A. S. Clark, Y. Ni and 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(10), 3986-97 (2001)
26. J. M. Kurie: Role of protein kinase B-dependent signaling in lung tumorigenesis. *Chest*, 125(5 Suppl), 141S-4S (2004)
27. J. Tsurutani, J. Fukuoka, H. Tsurutani, J. H. Shih, S. M. Hewitt, W. D. Travis, J. Jen and P. A. Dennis: Evaluation of two phosphorylation sites improves the prognostic significance of Akt activation in non-small-cell lung cancer tumors. *J Clin Oncol*, 24(2), 306-14 (2006)
28. F. H. Blackhall, M. Pintilie, M. Michael, N. Leighl, R. Feld, M. S. Tsao and F. A. Shepherd: Expression and prognostic significance of kit, protein kinase B, and mitogen-activated protein kinase in patients with small cell lung cancer. *Clin Cancer Res*, 9(6), 2241-7 (2003)
29. D. Malanga, M. Scrima, C. De Marco, F. Fabiani, N. De Rosa, S. De Gisi, N. Malara, R. Savino, G. Rocco, G. Chiappetta, R. Franco, V. Tirino, G. Pirozzi and G. Vigiutto: Activating E17K mutation in the gene encoding the protein kinase AKT1 in a subset of squamous cell carcinoma of the lung. *Cell Cycle*, 7(5), 665-9 (2008)
30. J. D. Carpten, A. L. Faber, C. Horn, G. P. Donoho, S. L. Briggs, C. M. Robbins, G. Hostetter, S. Boguslawski, T. Y. Moses, S. Savage, M. Uhlik, A. Lin, J. Du, Y. W. Qian, D. J. Zeckner, G. Tucker-Kellogg, J. Touchman, K. Patel, S. Mousses, M. Bittner, R. Schevitz, M. H. Lai, K. L. Blanchard and J. E. Thomas: A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature*, 448(7152), 439-44 (2007)
31. C. X. Xu, D. Jere, H. Jin, S. H. Chang, Y. S. Chung, J. Y. Shin, J. E. Kim, S. J. Park, Y. H. Lee, C. H. Chae, K. H. Lee, G. R. Beck, Jr., C. S. Cho and M. H. Cho: Poly(ester amine)-mediated, aerosol-delivered Akt1 small interfering RNA suppresses lung tumorigenesis. *Am J Respir Crit Care Med*, 178(1), 60-73 (2008)
32. C. Gridelli, P. Maione and A. Rossi: The potential role of mTOR inhibitors in non-small cell lung cancer. *Oncologist*, 13(2), 139-47 (2008)
33. R. M. Memmott and P. A. Dennis: The role of the Akt/mTOR pathway in tobacco carcinogen-induced lung tumorigenesis. *Clin Cancer Res*, 16(1), 4-10 (2010)
34. A. Arcaro, U. K. Khanzada, B. Vanhaesebroeck, T. D. Tetley, M. D. Waterfield and M. J. Seckl: Two distinct phosphoinositide 3-kinases mediate polypeptide growth factor-stimulated PKB activation. *EMBO J*, 21(19), 5097-108 (2002)
35. K. A. West, I. R. Linnoila, S. A. Belinsky, C. C. Harris and P. A. Dennis: Tobacco carcinogen-induced cellular transformation increases activation of the phosphatidylinositol 3'-kinase/Akt pathway *in vitro* and *in vivo*. *Cancer Res*, 64(2), 446-51 (2004)
36. H. Yamamoto, H. Shigematsu, M. Nomura, W. W. Lockwood, M. Sato, N. Okumura, J. Soh, M. Suzuki, Wistuba, II, K. M. Fong, H. Lee, S. Toyooka, H. Date, W.



## Deregulated PKB stimulates lung tumorigenesis

- L. Lam, J. D. Minna and A. F. Gazdar: PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res*, 68(17), 6913-21 (2008)
37. O. C. Schuurbiens, J. H. Kaanders, H. F. van der Heijden, R. P. Dekhuijzen, W. J. Oyen and J. Bussink: The PI3-K/AKT-pathway and radiation resistance mechanisms in non-small cell lung cancer. *J Thorac Oncol*, 4(6), 761-7 (2009)
38. R. A. O'Donnell, A. Richter, J. Ward, G. Angco, A. Mehta, K. Rousseau, D. M. Swallow, S. T. Holgate, R. Djukanovic, D. E. Davies and S. J. Wilson: Expression of ErbB receptors and mucins in the airways of long term current smokers. *Thorax*, 59(12), 1032-40 (2004)
39. F. Lonardo, K. H. Dragnev, S. J. Freemantle, Y. Ma, N. Memoli, D. Sekula, E. A. Knauth, J. S. Beebe and E. Dmitrovsky: Evidence for the epidermal growth factor receptor as a target for lung cancer prevention. *Clin Cancer Res*, 8(1), 54-60 (2002)
40. M. Marinov, B. Fischer and A. Arcaro: Targeting mTOR signaling in lung cancer. *Crit Rev Oncol Hematol*, 63(2), 172-82 (2007)
41. T. J. Lynch, D. W. Bell, R. Sordella, S. Gurubhagavatula, R. A. Okimoto, B. W. Brannigan, P. L. Harris, S. M. Haserlat, J. G. Supko, F. G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman and D. A. Haber: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*, 350(21), 2129-39 (2004)
42. S. Zimmer, P. Kahl, T. M. Buhl, S. Steiner, E. Wardelmann, S. Merkelbach-Bruse, R. Buettner and L. C. Heukamp: Epidermal growth factor receptor mutations in non-small cell lung cancer influence downstream Akt, MAPK and Stat3 signaling. *J Cancer Res Clin Oncol*, 135(5), 723-30 (2009)
43. S. K. Batra, S. Castelino-Prabhu, C. J. Wikstrand, X. Zhu, P. A. Humphrey, H. S. Friedman and D. D. Bigner: Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant EGFRvIII gene. *Cell Growth Differ*, 6(10), 1251-9 (1995)
44. M. W. Pedersen, M. Meltorn, L. Damstrup and H. S. Poulsen: The type III epidermal growth factor receptor mutation. Biological significance and potential target for anti-cancer therapy. *Ann Oncol*, 12(6), 745-60 (2001)
45. K. Inamura, H. Ninomiya, Y. Ishikawa and O. Matsubara: Is the epidermal growth factor receptor status in lung cancers reflected in clinicopathologic features? *Arch Pathol Lab Med*, 134(1), 66-72 (2010)
46. K. Okudela, H. Hayashi, T. Ito, T. Yazawa, T. Suzuki, Y. Nakane, H. Sato, H. Ishi, X. KeQin, A. Masuda, T. Takahashi and H. Kitamura: K-ras gene mutation enhances motility of immortalized airway cells and lung adenocarcinoma cells via Akt activation: possible contribution to non-invasive expansion of lung adenocarcinoma. *Am J Pathol*, 164(1), 91-100 (2004)
47. W. H. Westra, R. J. Slebos, G. J. Offerhaus, S. N. Goodman, S. G. Evers, T. W. Kensler, F. B. Askin, S. Rodenhuis and R. H. Hruban: K-ras oncogene activation in lung adenocarcinomas from former smokers. Evidence that K-ras mutations are an early and irreversible event in the development of adenocarcinoma of the lung. *Cancer*, 72(2), 432-8 (1993)
48. S. A. Matzinger, K. A. Crist, G. D. Stoner, M. W. Anderson, M. A. Pereira, V. E. Steele, G. J. Kelloff, R. A. Lubet and M. You: K-ras mutations in lung tumors from A/J and A/J x TSG-p53 F1 mice treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and phenethyl isothiocyanate. *Carcinogenesis*, 16(10), 2487-92 (1995)
49. C. J. Marsit, S. Zheng, K. Aldape, P. W. Hinds, H. H. Nelson, J. K. Wiencke and K. T. Kelsey: PTEN expression in non-small-cell lung cancer: evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. *Hum Pathol*, 36(7), 768-76 (2005)
50. J. M. Tang, Q. Y. He, R. X. Guo and X. J. Chang: Phosphorylated Akt overexpression and loss of PTEN expression in non-small cell lung cancer confers poor prognosis. *Lung Cancer*, 51(2), 181-91 (2006)
51. J. G. Zhang, J. J. Wang, F. Zhao, Q. Liu, K. Jiang and G. H. Yang: MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). *Clin Chim Acta* (2010)
52. R. J. Mayoral, M. E. Pipkin, M. Pachkov, E. van Nimwegen, A. Rao and S. Monticelli: MicroRNA-221-222 regulate the cell cycle in mast cells. *J Immunol*, 182(1), 433-45 (2009)
53. J. C. Soria, H. Y. Lee, J. I. Lee, L. Wang, J. P. Issa, B. L. Kemp, D. D. Liu, J. M. Kurie, L. Mao and F. R. Khuri: Lack of PTEN expression in non-small cell lung cancer could be related to promoter methylation. *Clin Cancer Res*, 8(5), 1178-84 (2002)
54. R. Noro, A. Gemma, A. Miyana, S. Kosai, Y. Minegishi, M. Nara, Y. Kokubo, M. Seike, K. Kataoka, K. Matsuda, T. Okano, A. Yoshimura and S. Kudoh: PTEN inactivation in lung cancer cells and the effect of its recovery on treatment with epidermal growth factor receptor tyrosine kinase inhibitors. *Int J Oncol*, 31(5), 1157-63 (2007)
55. E. Pisick, S. Jagadeesh and R. Salgia: Receptor tyrosine kinases and inhibitors in lung cancer. *ScientificWorldJournal*, 4, 589-604 (2004)
56. D. A. Guertin and D. M. Sabatini: An expanding role for mTOR in cancer. *Trends Mol Med*, 11(8), 353-61 (2005)

## Deregulated PKB stimulates lung tumorigenesis

57. J. J. Gibbons, R. T. Abraham and K. Yu: Mammalian target of rapamycin: discovery of rapamycin reveals a signaling pathway important for normal and cancer cell growth. *Semin Oncol*, 36 Suppl 3, S3-S17 (2009)
58. Y. Sancak, C. C. Thoreen, T. R. Peterson, R. A. Lindquist, S. A. Kang, E. Spooner, S. A. Carr and D. M. Sabatini: PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell*, 25(6), 903-15 (2007)
59. E. Vander Haar, S. I. Lee, S. Bandhakavi, T. J. Griffin and D. H. Kim: Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol*, 9(3), 316-23 (2007)
60. K. Inoki, Y. Li, T. Zhu, J. Wu and K. L. Guan: TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol*, 4(9), 648-57 (2002)
61. B. D. Manning, A. R. Tee, M. N. Logsdon, J. Blenis and L. C. Cantley: Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell*, 10(1), 151-62 (2002)
62. A. Garami, F. J. Zwartkruis, T. Nobukuni, M. Joaquin, M. Rocco, H. Stocker, S. C. Kozma, E. Hafen, J. L. Bos and G. Thomas: Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. *Mol Cell*, 11(6), 1457-66 (2003)
63. S. Han, F. R. Khuri and J. Roman: Fibronectin stimulates non-small cell lung carcinoma cell growth through activation of Akt/mammalian target of rapamycin/S6 kinase and inactivation of LKB1/AMP-activated protein kinase signal pathways. *Cancer Res*, 66(1), 315-23 (2006)
64. J. Tsurutani, S. S. Castillo, J. Brognard, C. A. Granville, C. Zhang, J. J. Gills, J. Sayyah and P. A. Dennis: Tobacco components stimulate Akt-dependent proliferation and NFkappaB-dependent survival in lung cancer cells. *Carcinogenesis*, 26(7), 1182-95 (2005)
65. J. S. Keum, G. Kong, S. C. Yang, D. H. Shin, S. S. Park, J. H. Lee and J. D. Lee: Cyclin D1 overexpression is an indicator of poor prognosis in resectable non-small cell lung cancer. *Br J Cancer*, 81(1), 127-32 (1999)
66. M. Ikehara, F. Oshita, H. Ito, N. Ohgane, R. Suzuki, H. Saito, K. Yamada, K. Noda, A. Mitsuda and Y. Kameda: Expression of cyclin D1 but not of cyclin E is an indicator of poor prognosis in small adenocarcinomas of the lung. *Oncol Rep*, 10(1), 137-9 (2003)
67. J. A. Diehl, F. Zindy and C. J. Sherr: Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev*, 11(8), 957-72 (1997)
68. J. A. Diehl, M. Cheng, M. F. Roussel and C. J. Sherr: Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev*, 12(22), 3499-511 (1998)
69. J. Liang and J. M. Slingerland: Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. *Cell Cycle*, 2(4), 339-45 (2003)
70. G. Sithanandam, G. T. Smith, A. Masuda, T. Takahashi, L. M. Anderson and L. W. Fornwald: Cell cycle activation in lung adenocarcinoma cells by the ErbB3/phosphatidylinositol 3-kinase/Akt pathway. *Carcinogenesis*, 24(10), 1581-92 (2003)
71. M. A. Bjornsti and P. J. Houghton: Lost in translation: dysregulation of cap-dependent translation and cancer. *Cancer Cell*, 5(6), 519-23 (2004)
72. H. Kawana, J. Tamaru, T. Tanaka, A. Hirai, Y. Saito, M. Kitagawa, A. Mikata, K. Harigaya and T. Kuriyama: Role of p27Kip1 and cyclin-dependent kinase 2 in the proliferation of non-small cell lung cancer. *Am J Pathol*, 153(2), 505-13 (1998)
73. S. Tsukamoto, K. Sugio, T. Sakada, C. Ushijima, K. Yamazaki and K. Sugimachi: Reduced expression of cell-cycle regulator p27(Kip1) correlates with a shortened survival in non-small cell lung cancer. *Lung Cancer*, 34(1), 83-90 (2001)
74. S. Mohamed, K. Yasufuku, K. Hiroshima, T. Nakajima, S. Yoshida, M. Suzuki, Y. Sekine, K. Shibuya, T. Iizasa, A. Farouk and T. Fujisawa: Prognostic implications of cell cycle-related proteins in primary resectable pathologic N2 nonsmall cell lung cancer. *Cancer*, 109(12), 2506-14 (2007)
75. S. Ishihara, K. Minato, H. Hoshino, R. Saito, F. Hara, T. Nakajima and M. Mori: The cyclin-dependent kinase inhibitor p27 as a prognostic factor in advanced non-small cell lung cancer: its immunohistochemical evaluation using biopsy specimens. *Lung Cancer*, 26(3), 187-94 (1999)
76. A. Bellacosa, C. C. Kumar, A. Di Cristofano and J. R. Testa: Activation of AKT kinases in cancer: implications for therapeutic targeting. *Adv Cancer Res*, 94, 29-86 (2005)
77. R. Mamillapalli, N. Gavrilova, V. T. Mihaylova, L. M. Tsvetkov, H. Wu, H. Zhang and H. Sun: PTEN regulates the ubiquitin-dependent degradation of the CDK inhibitor p27(KIP1) through the ubiquitin E3 ligase SCF(SKP2). *Curr Biol*, 11(4), 263-7 (2001)
78. L. Hengst and S. I. Reed: Translational control of p27Kip1 accumulation during the cell cycle. *Science*, 271(5257), 1861-4 (1996)
79. K. S. Kelly-Spratt, J. Philipp-Staheli, K. E. Gurley, K. Hoon-Kim, S. Knoblaugh and C. J. Kemp: Inhibition of PI-3K restores nuclear p27Kip1 expression in a mouse model of Kras-driven lung cancer. *Oncogene*, 28(41), 3652-62 (2009)
80. Y. Sun and Z. L. Peng: Programmed cell death and cancer. *Postgrad Med J*, 85(1001), 134-40 (2009)

## Deregulated PKB stimulates lung tumorigenesis

81. J. A. Call, S. G. Eckhardt and D. R. Camidge: Targeted manipulation of apoptosis in cancer treatment. *Lancet Oncol*, 9(10), 1002-11 (2008)
82. C. X. Xu, H. Jin and M. H. Cho: Apoptosis and Apoptosis-Based Therapy in Lung Cancer. *Anticancer Agents Med Chem* (2009)
83. H. Schulze-Bergkamen and P. H. Kramer: Apoptosis in cancer--implications for therapy. *Semin Oncol*, 31(1), 90-119 (2004)
84. D. C. Altieri: Validating survivin as a cancer therapeutic target. *Nat Rev Cancer*, 3(1), 46-54 (2003)
85. U. Zangemeister-Wittke and H. U. Simon: An IAP in action: the multiple roles of survivin in differentiation, immunity and malignancy. *Cell Cycle*, 3(9), 1121-3 (2004)
86. M. Monzo, R. Rosell, E. Felip, J. Astudillo, J. J. Sanchez, J. Maestre, C. Martin, A. Font, A. Barnadas and A. Abad: A novel anti-apoptosis gene: Re-expression of survivin messenger RNA as a prognosis marker in non-small-cell lung cancers. *J Clin Oncol*, 17(7), 2100-4 (1999)
87. L. L. Belyanskaya, S. Hopkins-Donaldson, S. Kurtz, A. P. Simoes-Wust, S. Yousefi, H. U. Simon, R. Stahel and U. Zangemeister-Wittke: Cisplatin activates Akt in small cell lung cancer cells and attenuates apoptosis by survivin upregulation. *Int J Cancer*, 117(5), 755-63 (2005)
88. M. Xin and X. Deng: Nicotine inactivation of the proapoptotic function of Bax through phosphorylation. *J Biol Chem*, 280(11), 10781-9 (2005)
89. S. Rokudai, N. Fujita, O. Kitahara, Y. Nakamura and T. Tsuruo: Involvement of FKHR-dependent TRADD expression in chemotherapeutic drug-induced apoptosis. *Mol Cell Biol*, 22(24), 8695-708 (2002)
90. P. F. Dijkers, R. H. Medema, J. W. Lammers, L. Koenderman and P. J. Coffey: Expression of the proapoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. *Curr Biol*, 10(19), 1201-4 (2000)
91. E. L. Greer and A. Brunet: FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene*, 24(50), 7410-25 (2005)
92. H. Matsuzaki, H. Daitoku, M. Hatta, K. Tanaka and A. Fukamizu: Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. *Proc Natl Acad Sci U S A*, 100(20), 11285-90 (2003)
93. D. R. Plas and C. B. Thompson: Akt activation promotes degradation of tuberin and FOXO3a via the proteasome. *J Biol Chem*, 278(14), 12361-6 (2003)
94. H. Huang, K. M. Regan, F. Wang, D. Wang, D. I. Smith, J. M. van Deursen and D. J. Tindall: Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. *Proc Natl Acad Sci U S A*, 102(5), 1649-54 (2005)
95. D. Gao, H. Inuzuka, A. Tseng and W. Wei: Akt finds its new path to regulate cell cycle through modulating Skp2 activity and its destruction by APC/Cdh1. *Cell Div*, 4, 11 (2009)
96. T. Maekawa, Y. Maniwa, T. Doi, W. Nishio, M. Yoshimura, C. Ohbayashi, Y. Hayashi and Y. Okita: Expression and localization of FOXO1 in non-small cell lung cancer. *Oncol Rep*, 22(1), 57-64 (2009)
97. B. H. Jiang and L. Z. Liu: PI3K/Pten signaling in angiogenesis and tumorigenesis. *Adv Cancer Res*, 102, 19-65 (2009)
98. D. T. Merrick, J. Haney, S. Petrunich, M. Sugita, Y. E. Miller, R. L. Keith, T. C. Kennedy and W. A. Franklin: Overexpression of vascular endothelial growth factor and its receptors in bronchial dysplasia demonstrated by quantitative RT-PCR analysis. *Lung Cancer*, 48(1), 31-45 (2005)
99. D. Stefanou, A. Batistatou, E. Arkoumani, E. Ntzani and N. J. Agnantis: Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in small-cell and non-small-cell lung carcinomas. *Histol Histopathol*, 19(1), 37-42 (2004)
100. P. Salven, T. Ruotsalainen, K. Mattson and H. Joensuu: High pre-treatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. *Int J Cancer*, 79(2), 144-6 (1998)
101. G. Fontanini, P. Faviana, M. Lucchi, L. Boldrini, A. Mussi, T. Camacci, M. A. Mariani, C. A. Angeletti, F. Basolo and R. Pingitore: A high vascular count and overexpression of vascular endothelial growth factor are associated with unfavourable prognosis in operated small cell lung carcinoma. *Br J Cancer*, 86(4), 558-63 (2002)
102. N. Pore, A. K. Gupta, G. J. Cerniglia, Z. Jiang, E. J. Bernhard, S. M. Evans, C. J. Koch, S. M. Hahn and A. Maity: Nelfinavir down-regulates hypoxia-inducible factor 1alpha and VEGF expression and increases tumor oxygenation: implications for radiotherapy. *Cancer Res*, 66(18), 9252-9 (2006)
103. G. L. Semenza: Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*, 3(10), 721-32 (2003)
104. C. C. Hudson, M. Liu, G. G. Chiang, D. M. Otterness, D. C. Loomis, F. Kaper, A. J. Giaccia and R. T. Abraham: Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol Cell Biol*, 22(20), 7004-14 (2002)
105. C. Chetty, S. S. Lakka, P. Bhoopathi and J. S. Rao: MMP-2 alters VEGF expression via alphaVbeta3 integrin-mediated PI3K/AKT signaling in A549 lung cancer cells. *Int J Cancer* (2009)

## Deregulated PKB stimulates lung tumorigenesis

106. G. L. Semenza: HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol*, 88(4), 1474-80 (2000)
107. J. Fang, Q. Zhou, L. Z. Liu, C. Xia, X. Hu, X. Shi and B. H. Jiang: Apigenin inhibits tumor angiogenesis through decreasing HIF-1 $\alpha$  and VEGF expression. *Carcinogenesis*, 28(4), 858-64 (2007)
108. L. Z. Liu, J. Fang, Q. Zhou, X. Hu, X. Shi and B. H. Jiang: Apigenin inhibits expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells: implication of chemoprevention of lung cancer. *Mol Pharmacol*, 68(3), 635-43 (2005)
109. L. C. van Kempen and L. M. Coussens: MMP9 potentiates pulmonary metastasis formation. *Cancer Cell*, 2(4), 251-2 (2002)
110. X. Li and J. F. Wu: Recent Developments in Patent Anti-Cancer Agents Targeting the Matrix Metalloproteinases (MMPs). *Recent Pat Anticancer Drug Discov*, 5(2), 109-41 (2009)
111. S. Garbisa, G. Scagliotti, L. Masiero, C. Di Francesco, C. Caenazzo, M. Onisto, M. Micela, W. G. Stetler-Stevenson and L. A. Liotta: Correlation of serum metalloproteinase levels with lung cancer metastasis and response to therapy. *Cancer Res*, 52(16), 4548-9 (1992)
112. E. Hrabec, M. Streck, D. Nowak and Z. Hrabec: Elevated level of circulating matrix metalloproteinase-9 in patients with lung cancer. *Respir Med*, 95(1), 1-4 (2001)
113. H. Sasaki, H. Yukiue, S. Moiriyama, Y. Kobayashi, Y. Nakashima, M. Kaji, M. Kiriyama, I. Fukai, Y. Yamakawa and Y. Fujii: Clinical significance of matrix metalloproteinase-7 and Ets-1 gene expression in patients with lung cancer. *J Surg Res*, 101(2), 242-7 (2001)
114. A. A. Thant, A. Nawa, F. Kikkawa, Y. Ichigotani, Y. Zhang, T. T. Sein, A. R. Amin and M. Hamaguchi: Fibronectin activates matrix metalloproteinase-9 secretion via the MEK1-MAPK and the PI3K-Akt pathways in ovarian cancer cells. *Clin Exp Metastasis*, 18(5), 423-8 (2000)
115. S. Han, J. D. Ritzenthaler, S. V. Sitaraman and J. Roman: Fibronectin increases matrix metalloproteinase 9 expression through activation of c-Fos via extracellular-regulated kinase and phosphatidylinositol 3-kinase pathways in human lung carcinoma cells. *J Biol Chem*, 281(40), 29614-24 (2006)
116. Y. C. Lee, H. H. Lin, C. H. Hsu, C. J. Wang, T. A. Chiang and J. H. Chen: Inhibitory effects of andrographolide on migration and invasion in human non-small cell lung cancer A549 cells via down-regulation of PI3K/Akt signaling pathway. *Eur J Pharmacol*, 632 (1-3), 23-32 (2010)
117. K. Jiang, J. Sun, J. Cheng, J. Y. Djeu, S. Wei and S. Sebti: Akt mediates Ras downregulation of RhoB, a suppressor of transformation, invasion, and metastasis. *Mol Cell Biol*, 24(12), 5565-76 (2004)
118. Y. W. Shih, P. S. Chen, C. H. Wu, Y. F. Jeng and C. J. Wang: Alpha-chaconine-reduced metastasis involves a PI3K/Akt signaling pathway with downregulation of NF-kappaB in human lung adenocarcinoma A549 cells. *J Agric Food Chem*, 55(26), 11035-43 (2007)
119. P. Dent, A. Yacoub, J. Contessa, R. Caron, G. Amorino, K. Valerie, M. P. Hagan, S. Grant and R. Schmidt-Ullrich: Stress and radiation-induced activation of multiple intracellular signaling pathways. *Radiat Res*, 159(3), 283-300 (2003)
120. J. Y. Hung, Y. L. Hsu, C. T. Li, Y. C. Ko, W. C. Ni, M. S. Huang and P. L. Kuo: 6-Shogaol, an Active Constituent of Dietary Ginger, Induces Autophagy by Inhibiting the AKT/mTOR Pathway in Human Non-Small Cell Lung Cancer A549 Cells. *J Agric Food Chem* (2009)
121. F. H. Igney and P. H. Krammer: Death and anti-death: tumour resistance to apoptosis. *Nat Rev Cancer*, 2(4), 277-88 (2002)
122. X. Wang, W. Chen, W. Zeng, L. Bai, Y. Tesfaigzi, S. A. Belinsky and Y. Lin: Akt-mediated eminent expression of c-FLIP and Mcl-1 confers acquired resistance to TRAIL-induced cytotoxicity to lung cancer cells. *Mol Cancer Ther*, 7(5), 1156-63 (2008)
123. A. C. Kraus, I. Ferber, S. O. Bachmann, H. Specht, A. Wimmel, M. W. Gross, J. Schlegel, G. Suske and M. Schuermann: *In vitro* chemo- and radio-resistance in small cell lung cancer correlates with cell adhesion and constitutive activation of AKT and MAP kinase pathways. *Oncogene*, 21(57), 8683-95 (2002)
124. D. Guo, I. J. Hildebrandt, R. M. Prins, H. Soto, M. M. Mazzotta, J. Dang, J. Czernin, J. Y. Shyy, A. D. Watson, M. Phelps, C. G. Radu, T. F. Cloughesy and P. S. Mischel: The AMPK agonist AICAR inhibits the growth of EGFRvIII-expressing glioblastomas by inhibiting lipogenesis. *Proc Natl Acad Sci U S A*, 106(31), 12932-7 (2009)
125. R. K. Amaravadi, D. Yu, J. J. Lum, T. Bui, M. A. Christophorou, G. I. Evan, A. Thomas-Tikhonenko and C. B. Thompson: Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J Clin Invest*, 117(2), 326-36 (2007)
126. S. Pattingre, L. Espert, M. Biard-Piechaczyk and P. Codogno: Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie*, 90(2), 313-23 (2008)
127. K. M. Livesey, D. Tang, H. J. Zeh and M. T. Lotze: Autophagy inhibition in combination cancer treatment. *Curr Opin Investig Drugs*, 10(12), 1269-79 (2009)
128. M. C. Maiuri, E. Zalckvar, A. Kimchi and G. Kroemer: Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol*, 8(9), 741-52 (2007)

## Deregulated PKB stimulates lung tumorigenesis

129. L. Fu, Y. A. Kim, X. Wang, X. Wu, P. Yue, S. Lonial, F. R. Khuri and S. Y. Sun: Perifosine inhibits mammalian target of rapamycin signaling through facilitating degradation of major components in the mTOR axis and induces autophagy. *Cancer Res*, 69(23), 8967-76 (2009)
130. P. Hilgard, T. Klenner, J. Stekar, G. Nossner, B. Kutscher and J. Engel: D-21266, a new heterocyclic alkylphospholipid with antitumour activity. *Eur J Cancer*, 33(3), 442-6 (1997)
131. H. A. Elrod, Y. D. Lin, P. Yue, X. Wang, S. Lonial, F. R. Khuri and S. Y. Sun: The alkylphospholipid perifosine induces apoptosis of human lung cancer cells requiring inhibition of Akt and activation of the extrinsic apoptotic pathway. *Mol Cancer Ther*, 6(7), 2029-38 (2007)
132. H. Y. Lee, S. H. Oh, J. K. Woo, W. Y. Kim, C. S. Van Pelt, R. E. Price, D. Cody, H. Tran, J. M. Pezzuto, R. M. Moriarty and W. K. Hong: Chemopreventive effects of deguelin, a novel Akt inhibitor, on tobacco-induced lung tumorigenesis. *J Natl Cancer Inst*, 97(22), 1695-9 (2005)
133. H. Y. Lee, Y. A. Suh, J. W. Kosmeder, J. M. Pezzuto, W. K. Hong and J. M. Kurie: Deguelin-induced inhibition of cyclooxygenase-2 expression in human bronchial epithelial cells. *Clin Cancer Res*, 10(3), 1074-9 (2004)
134. H. Jin, C. X. Xu, H. W. Kim, Y. S. Chung, J. Y. Shin, S. H. Chang, S. J. Park, E. S. Lee, S. K. Hwang, J. T. Kwon, A. Minai-Tehrani, M. Woo, M. S. Noh, H. J. Youn, D. Y. Kim, B. I. Yoon, K. H. Lee, T. H. Kim, C. S. Cho and M. H. Cho: Urocanic acid-modified chitosan-mediated PTEN delivery via aerosol suppressed lung tumorigenesis in K-ras(LA1) mice. *Cancer Gene Ther*, 15(5), 275-83 (2008)
135. K. Yu, J. Lucas, T. Zhu, A. Zask, C. Gaydos, L. Toral-Barza, J. Gu, F. Li, I. Chaudhary, P. Cai, J. Lotvin, R. Petersen, M. Ruppen, M. Fawzi, S. Ayril-Kaloustian, J. Skotnicki, T. Mansour, P. Frost and J. Gibbons: PWT-458, a novel pegylated-17-hydroxywortmannin, inhibits phosphatidylinositol 3-kinase signaling and suppresses growth of solid tumors. *Cancer Biol Ther*, 4(5), 538-45 (2005)
136. R. I. Feldman, J. M. Wu, M. A. Polokoff, M. J. Kochanny, H. Dinter, D. Zhu, S. L. Biroc, B. Alicke, J. Bryant, S. Yuan, B. O. Buckman, D. Lentz, M. Ferrer, M. Whitlow, M. Adler, S. Finster, Z. Chang and D. O. Arnaiz: Novel small molecule inhibitors of 3-phosphoinositide-dependent kinase-1. *J Biol Chem*, 280(20), 19867-74 (2005)
137. D. J. Boffa, F. Luan, D. Thomas, H. Yang, V. K. Sharma, M. Lagman and M. Suthanthiran: Rapamycin inhibits the growth and metastatic progression of non-small cell lung cancer. *Clin Cancer Res*, 10(1 Pt 1), 293-300 (2004)
138. G. Konstantinidou, E. A. Bey, A. Rabellino, K. Schuster, M. S. Maira, A. F. Gazdar, A. Amici, D. A. Boothman and P. P. Scaglioni: Dual phosphoinositide 3-kinase/mammalian target of rapamycin blockade is an effective radiosensitizing strategy for the treatment of non-small cell lung cancer harboring K-RAS mutations. *Cancer Res*, 69(19), 7644-52 (2009)
139. J. Qian, Y. Zou, J. S. Rahman, B. Lu and P. P. Massion: Synergy between phosphatidylinositol 3-kinase/Akt pathway and Bcl-xL in the control of apoptosis in adenocarcinoma cells of the lung. *Mol Cancer Ther*, 8(1), 101-9 (2009)
140. M. L. Sos, M. Koker, B. A. Weir, S. Heynck, R. Rabinovsky, T. Zander, J. M. Seeger, J. Weiss, F. Fischer, P. Frommolt, K. Michel, M. Peifer, C. Mermel, L. Girard, M. Peyton, A. F. Gazdar, J. D. Minna, L. A. Garraway, H. Kashkar, W. Pao, M. Meyerson and R. K. Thomas: PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Cancer Res*, 69(8), 3256-61 (2009)
141. N. T. Ihle, G. Paine-Murrieta, M. I. Berggren, A. Baker, W. R. Tate, P. Wipf, R. T. Abraham, D. L. Kirkpatrick and 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(9), 1349-57 (2005)

**Abbreviations:** EGFR: epidermal growth factor receptor; 4E-BP1: eIF4E-binding protein; FOXO: forkhead box O; GSK3: glycogen synthase kinase 3; MAPK: mitogen-activated protein kinase; MMP: matrix metalloproteinases; mTOR: mammalian target of rapamycin; mTORC: mTOR complex; nAChR: nicotinic acetylcholine receptors; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NSCLC: non-small-cell lung cancer; PDK: phosphoinositide-dependent kinase; PI3K: phosphoinositide 3-kinases; PKB: protein kinase B; PTEN: phosphatase and tensin homolog; SCLC: small-cell lung cancer; SKP2: S-phase kinase-associated protein-2; S6K1: p70 ribosomal protein S6 kinase; TRAIL: tumor necrosis-related apoptosis-inducing ligand; TSC2: tuberous sclerosis complex 2; VEGF: vascular endothelial growth factor.

**Key Words:** Akt, Protein kinase B, Lung tumorigenesis, Therapeutic resistance.

**Send correspondence to:** Myung-Haing Cho, Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea, Tel: 82-2-880-1276, Fax: 82-2-873-1268, E-mail: mchotox@snu.ac.kr

<http://www.bioscience.org/current/vol2E.htm>