

Class IA Phosphatidylinositol 3-Kinase Signaling in Non-small Cell Lung Cancer

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Class IA phosphatidylinositol-3 kinases (PI3Ks) together with AKT and mammalian target of rapamycin (mTOR) comprise the central axis of a complex, interconnected signaling network that integrates signals from growth factors, insulin, nutrients and oxygen to play a critical role in controlling cell growth, proliferation, metabolism, survival, and tumor angiogenesis. De-regulation of these processes is a required hallmark of cancer¹ and aberrant activation of the class IA PI3K signaling occurs frequently in many malignancies including non-small cell lung cancer (NSCLC).

The PI3K Family and Downstream Signaling

PI3Ks are lipid kinases that phosphorylate the 3'-hydroxyl group in phosphatidylinositol and phosphoinositides.² They are grouped into three classes based on structure and substrate specificity: Class I PI3Ks convert phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) to phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃). Class I PI3Ks are subdivided into class IA PI3Ks which are predominantly activated by growth factor receptor tyrosine kinases and class IB PI3Ks which are activated by G protein coupled receptors. Many studies have established a link between aberrant activation of class IA PI3Ks and oncogenesis.^{3–7} Class II PI3Ks catalyze the production of PtdIns(3)P and PtdIns(3,4)P₂ and are important for clathrin mediated membrane trafficking.⁸ Class III consists of a single member, hVPS34 which produces PtdIns(3)P and is involved in the regulation of vesicle trafficking, activation of mTOR by amino acids and autophagy.^{9,10}

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Class IA PI3K, Subunits and Activation

Class IA PI3Ks are heterodimeric proteins that consist of a p110 catalytic subunit and a p85 regulatory subunit. There are three isoforms of the p110 catalytic subunit (p110 α , p110 β , and p110 δ) encoded by three genes *PIK3CA*, *PIK3CB*, and *PIK3CD*. p110 α and p110 β are ubiquitously expressed in normal and tumor tissue, while expression of p110 δ is restricted to leukocytes. p110 α is important for growth factor and insulin signaling as well as for vascular development and integrity.^{11–13} To date, only mutations in *PIK3CA* have been commonly described in cancer.^{14,15} p110 β (which may also be activated by G-protein coupled receptors) is important for glucose metabolism, cell proliferation and drives oncogenic transformation in the context of PTEN loss.^{16,17} The p85 regulatory subunit (present in several isoforms: p85 α , p85 β , p55 α , p55 γ , and p50 α) is crucial for mediating the activation of class IA PI3Ks by receptor tyrosine kinases. It binds phosphorylated receptor tyrosine kinases directly or via adapter proteins (such as IRS-1 or Gab1) recruiting PI3K to its site of action at the cell membrane and allowing activation of the p110 catalytic subunit and rapid synthesis of PtdIns(3,4,5)P₃. Activating mutations in the gene encoding p85, *PIK3R1*, have been reported in colon cancer and glioblastoma.^{18–20} Class IA PI3Ks bind and may be directly activated by RAS.^{21,22}

Inactivation of PI3K IA Signaling

The reaction catalyzed by class IA PI3Ks is directly antagonized by the lipid phosphatase PTEN which dephosphorylates the 3' position of PtdIns(3,4,5)P₃ to produce PtdIns(4,5)P₂ or by the Src-homology-2 containing phosphatases SHIP1 and SHIP2 which dephosphorylate PtdIns(3,4,5)P₃ to produce PtdIns(3,4)P₂.²³

The PI3K/AKT/mTOR Pathway

Generation of PtdIns(3,4,5)P₃ within the cell membrane by class IA PI3Ks initiates a signaling cascade (Figure 1) that activates AKT and mTOR (the latter of which is present in two multiprotein complexes mTORC1 and mTORC2, described below). PtdIns(3,4,5)P₃ binds the pleckstrin homology domain of the AKT (a family of highly homologous serine/threonine kinases, AKT1, AKT2, and AKT3) localizing AKT to the cell membrane. AKT1 is then phosphorylated at Thr308 (Thr309 in AKT2 and Thr305 in AKT3) in the catalytic domain by PDK1, another pleckstrin homology domain containing colocalized kinase²⁴ and at Ser473 in the C-terminal hydrophobic motif (or Ser474 in AKT2 and Ser472 in AKT3) by the mTORC2 complex.²⁵ Activated AKT is released from the plasma mem-

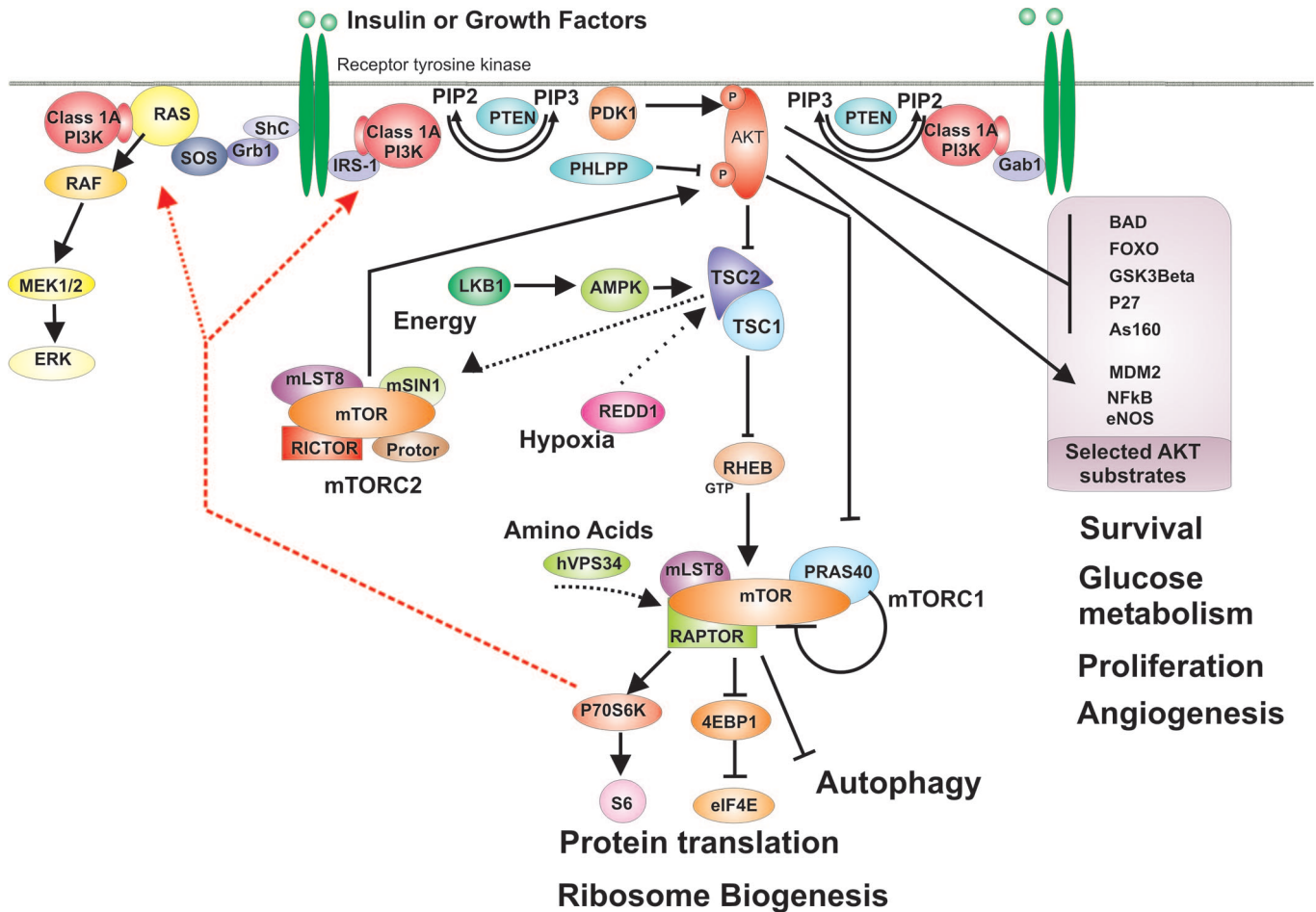


FIGURE 1. Signaling through the class IA phosphatidylinositol-3 kinases/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) axis integrates extracellular signals from insulin and growth factors together with energy status, oxygenation and nutrient availability to modulate processes including cell growth, survival, proliferation, glucose metabolism, and angiogenesis. The topology of this signaling network, genetic alterations of components of the PI3K pathway in non-small cell lung cancer (NSCLC) and presence of feedback loops (indicated by the broken red arrows) are described in the text.

brane and phosphorylates multiple nuclear and cytoplasmic targets (over 100 putative AKT substrates have been reported [www.phosphosite.org]) resulting in pleiotropic effects on cellular homeostasis (Figure 1 and reviewed by Manning and Cantley²⁶). The phosphatase PHLPP inactivates AKT by dephosphorylating AKT at Ser473.²⁷

The effects of AKT on cell growth and metabolism are mediated largely by activation of the mTORC1 complex which acts as a master regulator of protein synthesis in response to multiple inputs.^{28,29} mTORC1 contains mTOR complexed with Raptor, PRAS40, and mLST8 and is sensitive to inhibition by rapamycin and its analogues including RAD001 and CCI-779.²⁸ mTOR also exists in a distinct complex termed mTORC2 where it is combined with Rictor, mSIN1, Protor and mLST8.^{28,30} This complex is resistant to rapamycin, lies upstream of AKT²⁵ and has other less well-characterized effects including regulation of actin organization.³¹

Activation of mTORC1 by AKT involves phosphorylation of TSC2 (which exists in a complex with TSC1) leading to accumulation of the GTP bound form of the GTPase RheB

which activates mTORC1. AKT also phosphorylates and inactivates PRAS40, an inhibitory component of the mTORC1 complex.³² mTOR may be modulated by energy status via LKB1/STK11 and AMPK,³³ hypoxia through REDD1 signaling³⁴ and nutrient availability by hVPS34¹⁰ (Figure 1). mTORC1 acts on substrates including p70 S6 kinase and eIF4E-BP1 to influence translation of mRNA encoding key proteins such as cyclin D1, MYC, and HIF-1 α . It also stimulates ribosome biogenesis and inhibits autophagy.^{35,36}

Role of Cross-Talk and Feedback

The class IA PI3K/AKT/mTOR axis is a critical hub for cross talk with co-operating pathways and negative feedback loops within a complex and interconnected signaling environment. It is intimately linked with the RAS/RAF/MAPK pathway at its apex where RAS binds to and directly activates class IA PI3K²¹ and through interactions between many downstream components of each pathway.³⁷ Indeed, the p110 α isoform is critical for RAS driven tumorigenesis and inhibition of both PI3K and MEK seems critical for targeting

of *KRAS* driven lung tumors.^{22,38} There is also significant cross talk with other pathways including the JNK and LKB1/AMPK pathways.^{39,40} Adding further complexity is an extensive and context dependent network of feedback loops (reviewed in Carracedo et al.⁴¹) that act as rheostats to maintain exquisite control of the pathway in normal cells.

Genetic Alterations of the PI3K Pathway in NSCLC

Phosphorylation of AKT is evident in 50 to 70% of NSCLCs indicating that activation of class IA PI3K/AKT signaling is a frequent event in this malignancy.^{42–44} Constitutive PI3K activation may occur as a consequence of genetic changes in upstream signaling components (e.g., *EGFR* mutations or copy number gain and *KRAS* mutations), mutations or amplification of *PIK3CA*, *PTEN* loss or activation of downstream elements of the pathway (summarized in Table 1).

Although activating mutations in *PIK3CA* have been described in many human cancers⁴⁵ and occur at high frequency in breast (27%), colorectal (15%), and endometrial cancer (24%),^{14,15} *PIK3CA* mutations occur in only about 3% of NSCLC.^{14,46–49} However, increased copy number of the *PIK3CA* gene is a more frequent event in NSCLC being observed in 33% (46 of 139) of squamous cell carcinomas⁴⁹ and 5.9% of adenocarcinomas (12 of 195)⁴⁹ with high-level copy number gains (>fivefold) seen exclusively in squamous cell carcinomas in multiple studies.^{48–51}

Inactivating mutations or deletions of *PTEN* are uncommon in NSCLC (<5%).^{52–54} However, reduced or absent *PTEN* protein expression is frequent⁵⁵ and may be explained

by promoter hypermethylation which is reported to occur in 25 to 40% of cases.^{55,56}

Mutations in two downstream components of the class IA PI3K/AKT/mTOR pathway have been described in NSCLC. An uncommon activating mutation in the pleckstrin homology domain of *AKT1* (E17K), initially identified in breast, ovarian, and colorectal cancers,⁵⁷ has been described in about 1% of NSCLC (with reported cases demonstrating squamous histology).^{58–60} More common inactivating mutations in *LKB1/STK11* are seen in approximately 11% of NSCLC. These mutations are more frequent in tumors from Caucasians than Asians, adenocarcinomas and large cell carcinomas than squamous cell carcinomas and are associated with smoking history and with *KRAS* mutations.^{61–66} Loss of *LKB1* has been shown to potently synergize with *KRAS* mutations in lung tumorigenesis.⁶⁷

Challenges in Targeting PI3K Signaling in Lung Cancer

The first generation of PI3K inhibitors, wortmannin and LY2994002, had preclinical antitumor activity, but suffered from poor specificity,^{68,69} poor pharmacological properties and toxicities that precluded their clinical use. Novel PI3K inhibitors have been developed^{70,71} and join mTOR inhibitors in the armamentarium of agents to target the PI3K pathway. Clinical trials with SF-1126 (a prodrug of LY294002); PX-866 (a pegylated wortmannin derivative); XL147, a selective PI3K inhibitor; as well as BEZ235, BGT226, and XL765 (dual inhibitors of PI3K and mTOR) have been initiated (www.clinicaltrials.gov). Of significance, the presence of feedback and crosstalk within the circuitry of class IA PI3K/AKT/mTOR signaling imply that maximal therapeutic effect is likely require pathway inhibition at multiple levels and/or inhibition of multiple pathways simultaneously. For example, inhibition of mTORC1 by rapamycin analogues leads to increased AKT activation demonstrable in some tumors^{72–74} due to a feedback loop where S6K1 phosphorylates and transcriptionally represses IRS-1.⁷⁵ Inhibition of mTORC1 may also lead to activation of the ERK-MAPK pathway via a feedback loop involving S6K-IRS-1-PI3K-RAS.⁴¹ Indeed agents that inhibit both PI3K and mTOR^{76,77} and combinations of PI3K inhibitors with MEK inhibitors^{38,78,79} have proved to be promising therapeutic strategies in preclinical models. Appreciation of the intricacies of this complex and interconnected signaling network is required to develop clinical strategies that effectively target class IA PI3K signaling in NSCLC.

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TABLE 1. Genetic Alterations of the PI3K Pathway in Non-small Cell Lung Cancer

Genetic Change in PI3K Pathway Component	Frequency in NSCLC	Reference
<i>EGFR</i> mutation	26% (in about 10% of Caucasian patients; 20–40% of Asian patients)	80–82
Increased <i>EGFR</i> copy number	30–40%	81, 83
<i>KRAS</i> mutation	18% (20–30% of adenocarcinomas)	80, 82
<i>MET</i> mutation	2%	84, 85
<i>MET</i> amplification	2–7% (nb. higher frequency in patients with acquired resistance to <i>EGFR</i> TKIs)	84, 86–88
<i>EML4-ALK</i> rearrangements	2–8%	89–93
<i>PIK3CA</i> mutation	3% of NSCLC	14, 46–49
<i>PIK3CA</i> amplification	15–20% (7% in adenocarcinomas; 35% in SCC)	48–51
<i>PTEN</i> loss	Promoter hypermethylation in 26–35% of NSCLC	55, 56
<i>AKT1</i> mutation (E17K (c.49G>A))	1% (4/363)	58–60
<i>LKB1</i> mutation	11% of NSCLC	61–66

Genetic changes in components of the PI3K pathway in NSCLC. Figures in bold are from the catalogue of somatic mutations in cancer (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>).

NSCLC, non-small cell lung cancer; PI3K, phosphatidylinositol-3 kinases; TKI, tyrosine kinase inhibitor; *EGFR*, epidermal growth factor receptor.

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