The Role of Phosphoinositide 3-Kinase Pathway Inhibitors in the Treatment of Lung Cancer

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Abstract

The phosphoinositide 3-kinase signaling network is widely implicated in the pathogenesis of human cancer. This pathway is commandeered by cancer cells to promote unrestrained cellular growth and survival. In this brief review, we speculate about the uses of inhibitors of phosphoinositide 3-kinase signaling as treatments for human cancers, with an emphasis on non–small cell lung cancer.

Therapies that disrupt specific oncogenic processes are being developed for cancer treatments, and the research community has already identified several candidate targets for these interventions. Successful examples of such therapies include imatinib for chronic myelogenous leukemia, gefitinib and erlotinib for epidermal growth factor receptor (EGFR)–mutant non–small cell lung cancer (NSCLC), and trastuzumab or lapatinib for HER2–amplified breast cancer. There are several shared feature among all these examples, First, that the drug target has been activated by a genetic event. Additionally, the cancer is “addicted” to the drug target so that effective target inhibition leads to cellular death. Furthermore, the drug target is simultaneously controlling multiple growth and survival pathways, and its inhibition leads to their down-regulation. One of the critical signaling pathways that is down-regulated upon successful treatment with a targeted therapy is the phosphoinositide 3-kinase (PI3K) signaling pathway. In some epithelial cancers, the PI3K signaling pathway is activated by direct genetic mutation. Additionally, the PI3K signaling pathway seems to be a crucial survival and growth signal in a broad spectrum of cancers, raising speculation that therapies directed against the PI3K signaling pathway may have a wider role in cancers beyond those in which PI3K is directly activated by genetic mutation.

PI3K Signaling in Lung Cancer

The PI3Ks are a family of enzymes that phosphorylate the 3′-OH group on phosphatidylinositol-4,5-bisphosphate (PI4,5-P2). This enzyme is a heterodimer consisting of a p85 regulatory subunit and active p110 catalytic subunit. There are several isoforms of PI3K: PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ. PI3Ks are activated by the binding of PI4,5-P2 to the pleckstrin homology domain–containing protein p85α, which has an SH2 domain that binds to tyrosine-phosphorylated receptors, or on adaptor molecules such as ERBB3 or GAB1 (1). This binding results in its full activation. PI3K, in turn, phosphorylates several cellular proteins, including GSK3α, GSK3β, FOXO transcription factors, MDM2, and BAD, to facilitate cell survival and cell cycle entry (2).

Most widely implicated in cancer, and this review will be limited to signaling cascades initiated by this class of enzymes. Class Iα PI3Ks primarily phosphorylate phosphatidylinositol-4,5-bisphosphate on the plasma membrane to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate in vivo. This enzyme is a heterodimer consisting of a p85 regulatory and a p110 catalytic subunit. There are several isoforms of each subunit (p110α, p110β, p110δ, p50γ, p55γ, p85α, p85β, and p55γ) leading to several potential subunit compositions for the holoenzymes. Class Iα PI3K is most often activated by receptor tyrosine kinase (RTK) signaling, although the p110β–containing holoenzymes have been shown to be activated by G protein–coupled receptors as well (3, 4). The p85 regulatory subunit is critical in mediating class Iα PI3K activation by RTKs. The SH2 domains of p85 bind to phosphotyrosine residues in the sequence context pYxxM on activated RTKs as in the case of the platelet-derived growth factor receptors, or on adaptor molecules such as ERBB3 or GAB1 as in the cases of HER2 and EGFR (5). This binding serves both to recruit the p85/p110 heterodimer to the plasma membrane where its substrate phosphatidylinositol-4,5-bisphosphate resides and to relieve the basal inhibition of p110α by p85. The 3′-phosphatase PTEN dephosphorylates phosphatidylinositol-3,4,5-trisphosphate and thus terminates PI3K signaling.

The production of phosphatidylinositol-3,4,5-trisphosphate initiates potent growth and survival signals. A subset of pleckstrin homology domain–containing proteins, including the protein kinases Akt and PDK1, directly bind to phosphatidylinositol-3,4,5-trisphosphate and are thereby recruited to the plasma membrane. The phosphorylation of Akt at Thr308 by PDK1 and at Ser473 by a mTOR-Rictor dependent process results in its full activation. Akt, in turn, phosphorylates several cellular proteins, including GSK3α, GSK3β, FOXO transcription factors, MDM2, and BAD, to facilitate cell survival and cell cycle entry (for reviews see refs. 1, 6–9). In addition, Akt phosphorylates and inactivates tuberin, a GTPase-activating protein for the Ras homologue Rheb (10). Inactivation of tuberin allows Rheb to accumulate in the GTP-bound state and thereby activate the mTOR/Raptor complex. The mTOR-Raptor complex ultimately regulates protein synthesis and cell growth. Of particular importance, several other pathways converge on mTOR regulation including LKB1/AMPK, MAPK/ERK, and class III PI3K (7, 11, 12). In lung adenocarcinomas, preliminary data suggest that LKB1 mutations are observed in ~30% of cases.
One might predict that LKB1 loss would lead to increased mTOR activity. However, it remains to be determined how class Iα PI3K signaling affects mTOR activity in this subset of lung cancers. One recent report suggests that mTOR activity remains under the control of growth factors even in the LKB1-deficient adenocarcinomas (14). The pharmaceutical and biotechnology sectors are currently developing inhibitors for several of the components in the PI3K signaling pathway, including PI3K, Akt and mTOR, as potential therapies for cancer.

The integral role of this PI3K signaling pathway in cancers has become evident over the past 20 years. In fact, several studies have now shown that genetic mutations that directly activate the PI3K signaling pathway are common in human cancers. The two most common are somatic activating mutations of p110α (PIK3CA) and loss of the tumor suppressor PTEN. Additionally, amplification of PIK3CA and Akt are occasionally observed in epithelial cancers (1). Analogous to the previously cited examples of successful targeted therapies, PI3K pathway inhibitors are particularly attractive for those cancers with mutations that directly activate PI3K signaling. However, in NSCLC, mutations in PIK3CA and PTEN are uncommon (15–17), although there are reports demonstrating evidence for loss of PTEN protein expression (perhaps via PTEN promoter methylation; refs. 18, 19) and PIK3CA amplification (20, 21). Nevertheless, it seems that PI3K/Akt signaling is often activated in lung cancer via mechanisms other than direct genetic modification.

There have been a number of studies suggesting that the PI3K/Akt signaling pathway is central to NSCLC growth and survival. Immunohistochemical analyses of NSCLC specimens suggest that activated Akt correlates with more aggressive disease and shortened survival (22). In NSCLC cell lines, inhibitors of PI3K (e.g., LY294002) dramatically increase sensitivity to apoptosis-promoting chemotherapeutics (23). We have also found that commercially available inhibitors of PI3K completely abrogate the ability of most NSCLC cell lines to form colonies on soft agar and potently inhibit the growth of these cell lines on Petri dishes (data not shown). However, the commonly used PI3K inhibitors, wortmannin and LY294002, inhibit class I, class II (to a lesser extent), and class III PI3Ks as well as other PIK homologues (such as mTOR; ref. 24). Such lack of isoform specificity precludes their utility in determining the effects of inhibiting class Iα PI3K signaling in lung cancers. The development of isoform-specific small molecule inhibitors, the utilization of RNA interference against specific isoforms, and the generation of knockout mice for the various PI3K isoforms will clarify the distinct roles of class Iα PI3K in lung cancer.

**PI3K Pathway Inhibitors for the Treatment of NSCLC**

There are currently no available data from clinical trials assessing the efficacy of PI3K pathway inhibitors for the treatment of NSCLC. However, data from preclinical and clinical models of RTK-addicted cancers reveal that when a cancer is susceptible to a specific targeted therapy, the PI3K signaling pathway is invariably under strict regulation by the target. For example, breast cancers that are sensitive to trastuzumab use HER2 to regulate PI3K signaling. Similarly, lung cancers that are sensitive to EGFR inhibitors have PI3K under the regulation of EGFR. The same holds true for cancers that are sensitive to Met inhibitors (25). In other words, for a cancer to be susceptible to a particular targeted therapy, the target is the primary regulator of critical growth and survival signals, especially the PI3K signaling pathway. In fact, it is difficult to find examples of cancers sensitive to RTK inhibitors in which the therapies do not lead to inhibition of PI3K signaling. Additionally, multiple studies have reported that down-regulation of PI3K is necessary for targeted therapies to be effective (26–28). In contrast, these same RTK inhibitors usually have no effect on PI3K signaling in cancer cells that are resistant to those therapies. Thus, one may speculate that inhibition of PI3K signaling may be an effective way to attack cancers.

So, will PI3K pathway inhibitors be effective treatments for lung cancer? Extrapolating from previous successful experiences regarding targeted therapies, one might hypothesize that those cancers with PTEN loss and/or mutational activation of p110 would be the ones most likely to respond to single-agent therapy. However, even for these cancers, it remains possible that turning off just the PI3K signaling pathway will not be sufficient to cause significant apoptosis. Additionally, as mentioned above, these two genetic events do not seem to be common in NSCLC.

Unlike *EGFR*-mutant lung cancers, most cancers will not likely have one protein that singly controls several of the critical downstream cell survival pathways, and thus, these cancers may not show dramatic responses to a single targeted therapy (Fig. 1). In fact, each of these pathways may be regulated by distinct mechanisms. In such cases, PI3K inhibitors may be effective only when used in combination with inhibitors of other pathways (e.g., the Raf/MAPK signaling pathway). The effects of blocking multiple pathways in combination may begin to approximate the effect of treating *EGFR*-mutant lung cancers with EGFR inhibitors. Thus, caution is warranted when interpreting clinical trials that evaluate the efficacy of single-agent PI3K inhibitors in clinical trials. Should they fail to induce significant responses in lung cancer patients, it remains quite possible that they may achieve a prominent therapeutic role when combined with other pathway inhibitors.

Which therapies should be combined with PI3K inhibitors? Ultimately, empirical preclinical data may drive the development of combinations to be evaluated in clinical trials. A promising approach may be to combine PI3K inhibitors with other intracellular signaling pathway inhibitors such as those that interfere with the MAPK signaling pathway, as has been suggested by preclinical data (27). This combination may be particularly attractive in *K-ras*-mutant lung cancers. Additionally, combining PI3K inhibitors with RTK inhibitors may be effective in those cancers in which the RTK regulates many intracellular signaling pathways except for PI3K, as may be the case in PTEN-deficient or p110α-mutant cancers. Furthermore, activation of PI3K may be a mechanism for acquired resistance to EGFR inhibitors and HER2 inhibitors, and hence, the addition of PI3K inhibitors to these compounds may be an effective means to treat patients that develop resistance to RTK inhibitors. Another potentially effective combination may be to add PI3K inhibitors to mTOR inhibitors. Recent experiments have shown that mTOR-Raptor inhibitors lead to increased PI3K signaling by blocking a negative feedback on PI3K activation (29). Since this augmentation of...
PI3K signaling may limit the effectiveness of mTOR inhibitors the addition of a PI3K inhibitor may be beneficial. Additionally, this combination may prove effective because, as mentioned above, PI3K does not regulate mTOR activity in many cancers, and thus, the combination would block both critical pathways in the cancer.

In summary, the PI3K signaling pathway is clearly an integral pathway in tumorigenesis and maintenance. However, it remains to be determined if inhibitors of this pathway will be effective as single-agent therapies, especially in those cancers that do not have activating p110α mutations or loss of PTEN. However, there is reason to believe that inhibitors of the PI3K signaling pathway will have a role in the treatment of a large subset of cancers when used in combination with therapies that disrupt other signaling pathways.

Open Discussion

Dr. Roman Perez-Soler: Are we sure that the PTEN protein you detect retains its phosphatase activity? Has anybody been able to measure that, because you don’t need to lose the protein to lose the function. Maybe the PTEN protein measured is nonfunctional and that may explain the resistance.

Dr. Engelman: In the examples I showed, PTEN is working, because if we block activation of class I, PI3K, we completely block Akt. If PTEN were absent, you wouldn’t expect to be able to do that so easily. Whether we know if PTEN is working in the cancers is obviously a good question. There are some data about posttranslational modifications, localization, and serine phosphorylation of PTEN, but I have not seen any situation where a PTEN molecule has less than 20% activity.

Dr. Thomas Lynch: There are number of compounds in development against insulin growth factor (IGF) receptor. What do you think of that as a target in lung cancer?

Dr. Engelman: IGF receptor is a valid target. Inhibiting it will effectively block PI3K activation in a large subset of epithelial cancers, but that will probably not be enough to kill the cells. The IGF receptor inhibitors may prove their value in combination with RAD001. If you block IGF receptor, you block the ability of RAD001 to increase Akt activation. We don’t want RAD001 to increase Akt activity and this would be a way of abrogating that.

Dr. John Heymach: Then you are saying you don’t think there are other addictions out there, because if there are other addictions, those are going to be the big responses.

Dr. Engelman: There is not going to be single addictions for most cancers. For GIST there is. For EGF-mutant lung cancers there is, but not for the other 90% of lung cancers.

Dr. Heymach: The idea behind the immunoprecipitation is a very appealing one, but if you pull down IGF receptor, IGF receptor blockade alone is not causing the apoptosis. IGF receptor presumably is not driving the MEK pathway because if it was, then blocking just the IGF receptor alone would have killed the cells. You are selecting for maybe one of the pathways of addiction, but the tumor isn’t addicted to a single molecule here. What do you think is driving MEK, and is there a way you can actually come up with single molecule agents to get responses like imatinib in chronic myelogenous leukemia?

Dr. Engelman: The single molecule targeted therapy approach works where there is one oncogenic process driving all of these pathways. In the vast majority of lung cancers, it is not going to be that way, particularly in KRAS-mutated lung cancers, because I think you have one way of activating PI3K, and KRAS is activating ERK. You are not going to find one molecule that blocks both pathways.

Dr. Heymach: Could you tell us a little about the research on Akt inhibitors, which are about at the same level of preclinical development, I believe, as the compounds targeting the PI3K pathway.

Dr. Engelman: There are three isoforms of Akt; Akt1 tends to be the one that is pro-growth, whereas Akt2 tends to be the one that controls metabolism. When we get these new compounds, a question will be how specifically they target the isoform.

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Dr. Heymach: The appeal of the addiction hypothesis is that there is one initiating event typically that is driving the cell and it remains dependent on a single event. Do you think there are no other addictions beyond EGFR? Is the modified hypothesis, then, that something driving those two pathways is enough to prove the cancer is addicted?
Acknowledgments

I am grateful to Lewis Cantley and Pasi Janne for reading this manuscript and for countless discussions and insights regarding PI3K signaling and lung cancer. I apologize to the many authors whose work we could not cite directly because of space limitations.

References

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