PI3 Kinases in Cancer: From Oncogene Artifact to Leading Cancer Target
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Sci. STKE **2006** (365), pe52. [DOI: 10.1126/stke.3652006pe52]

PI3 Kinases in Cancer: From Oncogene Artifact to Leading Cancer Target

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(Published 12 December 2006)

Introduction

One of the foremost achievements of signaling research has been the elucidation of the signaling pathways originating from receptor tyrosine kinases (RTKs) that shed light on how the constitutive activation of these pathways drives tumor formation. In response to growth factor stimulation, the tyrosine kinase activity of RTKs is activated through ligand-induced dimerization. Subsequent receptor autophosphorylation creates a series of phosphotyrosine (p-tyr)-containing motifs on the receptor. Downstream effector molecules are then recruited to these p-tyr motifs on the activated RTKs through interactions of various p-tyr binding domains found on the effectors themselves or on intermediate adapter proteins, which serve to bridge the activated receptors and effectors by physically interacting with each. Perhaps the most famous effector is the small guanosine triphosphatase, Ras, which is mutationally activated in a large number of human cancers. Another such effector, termed phosphatidylinositide 3-kinase (PI3K), will be the focus of this Perspective (see Fig. 1 for a schematic of PI3K roles in signaling). Recent findings have suggested that, like Ras, PI3K plays a particularly important role in human cancers.

A Brief History of the Discovery of PI3Ks

PI3Ks are a family of lipid kinases that are distinguished from one another by their substrates, expression patterns, and regulation. What we now call class 1A PI3Ks were originally described as a phosphatidylinositol kinase (PIK) activity in partially purified preparations of the oncoprotein $pp60^{v\text{-}src}$ (*1*). A similar activity was found in immunoprecipitates of another oncoprotein, polyoma virus middle T antigen (MTag), which was known to function by binding and activating $pp60^{c\text{-}src}$ (*2*, *3*). In both cases, the copurifying PIK activity was only a small fraction of the total PIK activity in the cell and was considered by many to be an isolation artifact. Notably, however, the PIK activity was found associated with all transformation-competent alleles of MTag but not with a number of nontransforming alleles (*3*). In time, it was realized that the small fraction of oncoprotein-bound PIK activity, designated type I PIK, possessed unique biochemical properties that distinguished it from the bulk of the PIKs in the cell (*4*). The presence of type I PIK activity in immunoprecipitates of MTag was tightly correlated with the presence of an 85-kD protein (*5*). More importantly, both the type 1 PIK and p85 were physically associated with various activated RTKs, and this association was essential for the biological functions of both RTKs and oncoproteins (*5*–*7*). With the cloning of the gene encoding p85, it became clear that p85 is an SH2 (Src homology 2) domain–containing adapter protein that couples the type I PIK to activated RTKs and to oncoproteins such as MTag through its interactions with specific p-tyr motifs (*8*, *9*).

The next flurry of discoveries identified and characterized the p110 catalytic subunits of the type I PIKs. Classical PIKs were known to phosphorylate phosphatidylinositol (PI) on the 4′ position on the inositol ring to generate PI(4)P (phosphatidylinositol 4-phosphate). However, the observation that type 1 PIK could use PI(4)P as a substrate (*10*) suggested that type 1 PIK was not simply phosphorylating the 4′ position on the inositol ring. Instead, the type 1 enzyme phosphorylated the 3′ position to generate phosphatidylinositol 3,4-bisphosphate $PI(3,4)P_2$, leading to renaming the enzyme PI3K (*10*). Purified PI3K that was associated with RTKs and oncoproteins was found to consist of two subunits: the p85 adapter plus a 110-kD catalytic subunit (*11*). With further purification and molecular cloning of the PI3Ks, it became clear that there was actually a family of PI3Ks (*12*, *13*). The family was divided into three groups: class I PI3Ks, which could use PI, PI(4)P, and $PI(4,5)P_2$ as substrates in vitro; class II PI3Ks, which used PI and PI(4)P; and class III PI3Ks, restricted to PI as a substrate [see reviews (*14*) and (*15*)]. Notably, class 1 enzymes seem to be limited to the use of $PI(4,5)P_2$ as a substrate in vivo. Class 1 was further divided into the three class 1A enzymes, each consisting of the p85 adapter subunit complexed with one of three p110 catalytic subunits (α, β, or δ) and capable of associating with RTKs and oncoproteins, and class 1B, a single species that features a different subunit composition and is largely confined to leukocytes. The discovery in the late 1990s that firmly established the class 1A PI3Ks as oncogenes was the finding that p110α had been captured by a tumorigenic avian retrovirus, rendering it oncogenic (*16*). Subsequently, an artificially activated form of p110α was found to be capable of driving tumor formation when expressed in telomerase-immortalized human epithelial cells (*17*).

The Discovery of the PTEN Tumor Suppressor Ties PI3K to Human Cancer

The discovery that the tumor suppressor PTEN (phosphatase and tensin homolog deleted from chromosome 10) works by antagonizing PI3K provided the first direct link between PI3K activation and human cancer. After the positional cloning of PTEN, it was obvious from its sequence that PTEN was a phosphatase (*18*, *19*). Although PTEN possesses protein tyrosine phosphatase activity (*19*), it subsequently emerged that PTEN was also a lipid phosphatase capable of specifically removing the 3′ phosphate from $PI(3,4,5)P_3$ (*20*). Further analysis of PTEN mutations showed that this activity was essential to its function as a tumor suppressor [reviewed by (*21*)]. Thus, PTEN seems to act as a brake for the class 1 PI3Ks. Not surprisingly, PI3K signaling is highly activated in PTEN-null tumor cell lines and primary tumors. PTEN is inactivated in a large percentage of common hu-

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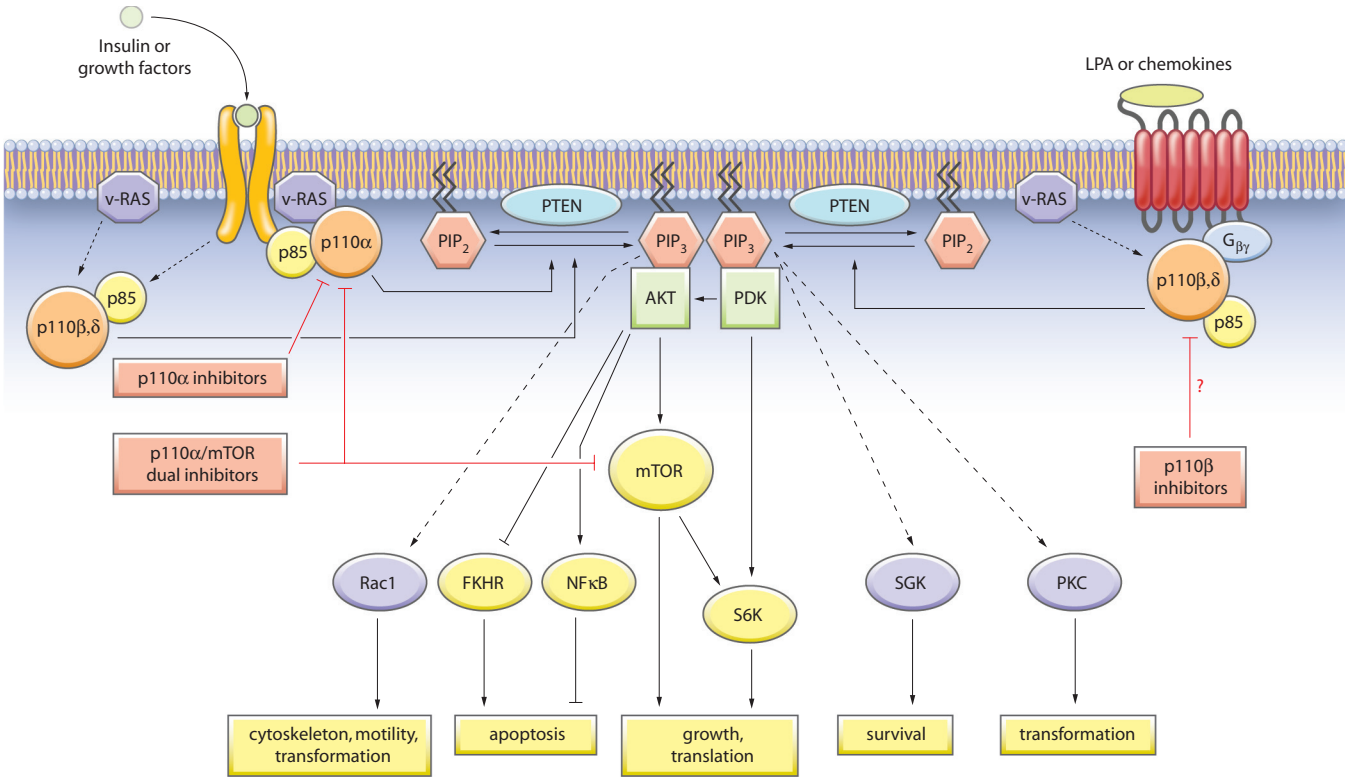

Fig. 1. The roles of PI3K isoforms in signaling and transformation. Where is inhibition wise? Each isoform of class 1A PI3K has distinct roles in receptor signaling. The p110α isoform carries much of the signal from RTKs and certain oncogenes such as Ras. Thus, inhibitors specific for p110α may well have a marked effect on tumors driven by mutant or amplified growth factor receptors but may also have an inhibitory effect on insulin function. A key goal for the future development of PI3K inhibitors is determining which isoform(s) to inhibit in a given tumor. Targeting the PI3K pathway at several places simultaneously may both make it harder for tumors to generate resistance mutants and create a wider therapeutic window by allowing each targeted element of the pathway to retain residual activity.

man cancer types, including tumors of the prostate (30 to 50%) (*22*), brain (at least 30%) (*23*), and breast (more than 20%) (*24*).

Activating Mutations in Human Tumors Make the Case for Class 1A PI3Ks as Cancer Targets

Although the discovery of the lipid phosphatase activity of PTEN heightened interest in PI3K as a potential drug target in cancer, the recognition of the importance of this class of enzymes in cancer was cemented with the discovery that the *PIK3CA* gene, which encodes p110α, was frequently mutated in a number of the most common forms of cancer, including colon, breast, prostate, liver, and brain tumors (*25*). Moreover, studies in several model systems showed that the mutations commonly found in p110α in human tumors are activating (*26*–*29*). Although the exact molecular mechanism(s) by which these mutations activate p110α has not been determined, current data point to a model in which they release p110α from inhibition mediated through its interaction with p85. Notably, mutations have not been found in the genes encoding the other class 1 PI3Ks, even though several groups have demonstrated that these enzymes are capable of acting as oncogenes in model systems (*26*, *27*). This apparent difference in oncogenic potential may reflect the difficulty of activating the other isoforms through single mutational events, as has been demonstrated for PIK3CB (*26*). However, the *PIK3CB* gene, encoding p110β, has been found to be amplified in several tumor types (*30*, *31*).

Loss of Function Studies Reveal Both the Therapeutic Potential and the Potential Hazards of PI3K Inhibitors

As drug companies prepare to attack the PI3K pathway, an examination of what we have learned from genetic mouse models and chemical genetics might facilitate the design of optimal inhibitors for PI3K. Traditional knockout of p110δ or knockin of an inactive form of p110δ yields mice that grow to adulthood but have severely impaired T and B cell function (*32*, *33*). Genetic ablation of either p110α or p110β gives an early embryonic lethal phenotype (*34*, *35*), limiting the information that can be gained about the roles of these isoforms in adults. However, although heterozygous knockout of either p110α or p110β had no effect on insulin signaling, mice with heterozygous loss of both isoforms showed a noticeably impaired insulin response (*36*). Recent studies have shown that heterozygous knockin of a kinase-dead allele of p110α yields small adult mice with markedly impaired insulin signaling (the homozygous knockin is, as expected, early embryonic lethal) (*37*). When p110α is totally ablated in mouse embryo fibroblasts (MEFs) prepared from a p110α conditional knockout, signaling in response to various growth factors, including insulin, IGF-1 (insulin-like growth factor 1), EGF (epidermal growth factor), and PDGF (platelet-derived growth factor), is impaired (*38*). A chemical genetic study using partially specific inhibitors of the various p110 isoforms also suggested that p110α functions in insulin signaling, whereas inhibition of p110β appeared not to affect

insulin signaling but rather to block LPA (lysophosphatidic acid) signaling (*39*). Because most if not all of the first inhibitors in clinical studies will block all p110 isoforms (and perhaps other kinases related to the PI3Ks as well), some target-directed side effects maybe encountered. These might include impaired immune function caused by loss of p110δ function as well as problems in insulin response caused by inhibition of p110α. A more complete understanding of the effects of p110β loss awaits further studies with conditional knockout mice and mice with a knockin of a kinase-dead allele. However, none of the potential side effects of a pan-inhibitor need necessarily abolish its therapeutic usefulness if such an inhibitor can be used for a relatively limited course of treatment (perhaps weeks or months instead of the years of treatment used for some protein kinase inhibitors).

As we look to the future, it may be worthwhile to speculate on the methods that might be used to minimize any potential toxicity associated with attacking the PI3K pathway while maximizing the benefits of pathway ablation. Again, there is some information that may be gleaned from the knockout mice. Making an inhibitor that spares p110δ is one possibility. Such an inhibitor would presumably have no (or fewer) adverse effects on the immune response, even on prolonged dosage. Unfortunately, this plan is not without risk, because at least some tumors express p110δ and may be at least partially dependent upon it. Also of interest are p110α-specific inhibitors, which could be useful against tumors with activating mutations in the *PIK3CA* gene. In addition, experiments with p110α knockout MEFs have revealed that p110α ablation reduces transformation and tumorigenesis induced by mutant RTKs, including activated EGFR alleles from human lung tumors (*38*). This suggests that an inhibitor targeted at p110α might be effective against various tumors. Side effects of inhibitors targeted toward p110α on insulin signaling might be mitigated by treatment with peroxisome proliferator-activated receptor γ (PPARγ) agonists. Once again, we await data from p110β knockout animals to help us assess its importance in cancer.

To further enhance the antineoplastic effects of PI3K inhibitors, it might be useful to target key additional points in the PI3K signaling pathway. This might be achieved by treating with a PI3K inhibitor in combination with a separate compound aimed at a downstream pathway component, such as a rapamycin analog targeting mTOR (mammalian target of rapamycin), or an inhibitor of Akt. Indeed, because mTOR itself is a member of the greater family of PI3K-like kinases, it has been possible to use single compounds that target both p110 α and mTOR to good effect in preclinical models (*40*). Whatever the final composition of inhibitors used to attack the PI3K pathway, we can have high hope that inhibiting PI3K will be as effective in fighting cancer as activating it is in cancer growth.

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Citation: J. J. Zhao, T. M. Roberts, PI3 kinases in cancer: From oncogene artifact to leading cancer target. *Sci. STKE* 2006, pe52 (2006).