Unlocking the Mystery of Cancer:

Do the Mutant p110α Subunits of PI3-Kinase Hold the Key?

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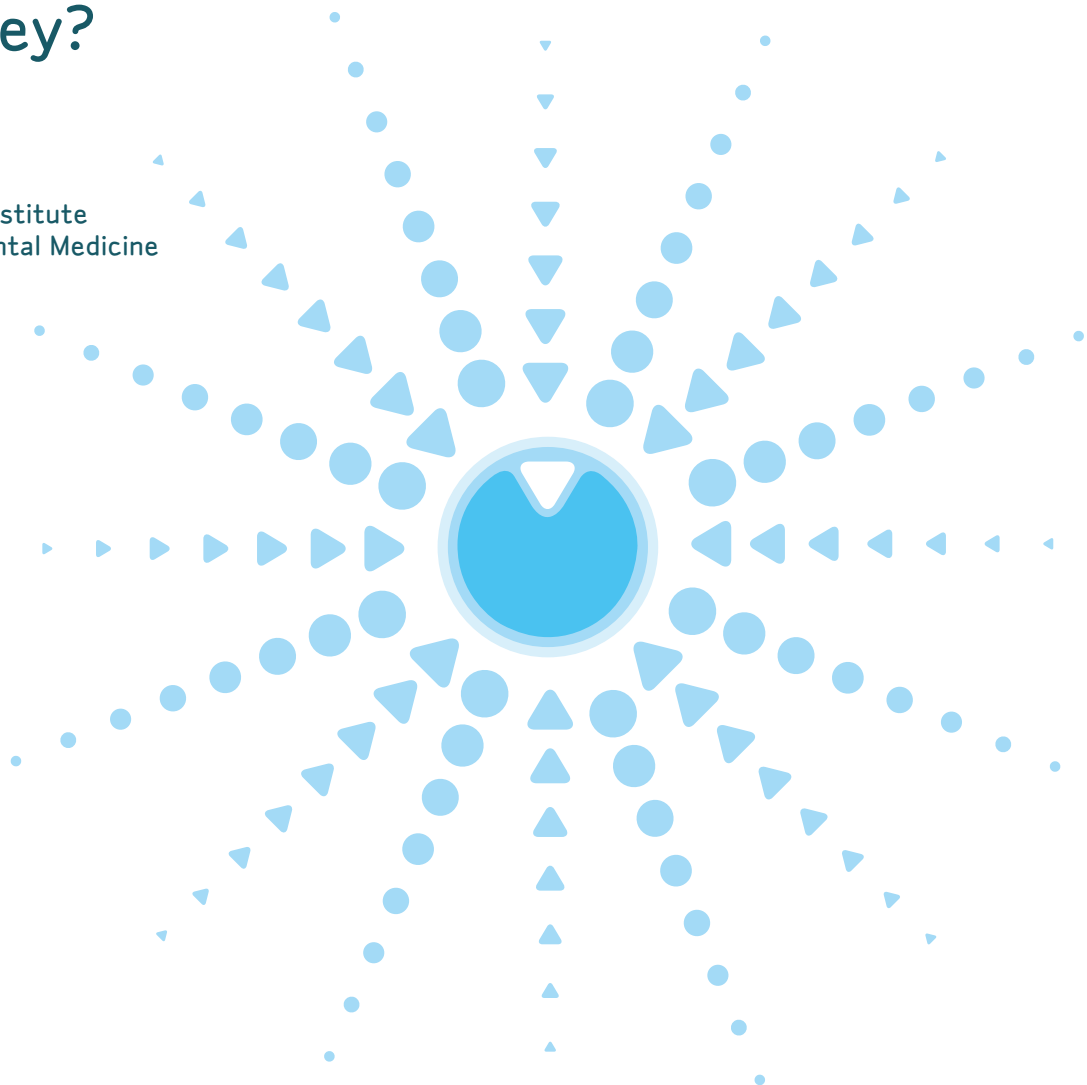


INTRODUCTION

The discovery of cancer-specific mutations in PIK3CA, the gene coding for the catalytic subunit p110α of PI3-kinase, has transformed the field of lipid kinases from an area of basic biochemistry into a focus of intense interest for drug development, yet the new opportunities depend largely on the availability of purified, mutant p110α.

PIP3 is an important second messenger in the cell. Because of its unique link to PIP3, class I PI3Ks occupy a central position in cellular signaling and are among the most intensively studied lipid kinases.

This paper provides an overview of PI3-kinases and what makes them unique, their implications for cancer, their roles in signal transduction and the challenges researchers face today with advancing this research.

WHAT ARE PI3KS?

Phosphatidylinositol 3-kinases (PI3Ks) form a family of enzymes that phosphorylate the inositol ring of phospholipids at the D3 position. These lipid kinases have been grouped into three classes (I - III), distinguished by their structure and function [Figures 1 and 2] [1,2].

They are dimeric enzymes, consisting of a catalytic and a regulatory (adaptor) subunit[3]. An important distinction between classes of PI3K is substrate specificity [Figure 2]:

- Class I accepts three substrates: unphosphorylated phosphoinositides, 4-monophosphate and 4,5-bisphosphate of the inositol ring.
- Class II uses only the non-phosphorylated and monophosphorylated inositol ring.
- Class III is restricted in its activity to the non-phosphorylated substrate[2].

Thus, only class I PI3K generates the 3,4,5-trisphosphate, also referred to as PIP_3. PIP_3 is an important second messenger in the cell, and because of this unique link to PIP_3, class I PI3Ks occupy a central position in cellular signaling and are among the most intensively studied lipid kinases[4, 6, 7].

CATALYTIC SUBUNIT OF CLASS I PI3KS, p110 — IDENTIFICATION OF FIVE STRUCTURE-FUNCTION DOMAINS:

- Amino-terminal adaptor-binding domain that provides the principal interaction surface with the regulatory subunit
- Ras-binding domain mediating interaction between p110 and Ras-GTP and contributing to the stimulation of the PI3K and the Ras-driven signaling pathways
- C2 domain with affinity for lipid membranes
- Helical domain of still undetermined function
- Carboxy-terminal kinase domain [Figure 1][4]

The standard regulatory subunit of class I PI3Ks is p85, a protein that contains several modular protein-protein interaction domains:

- Two Src-homology 2 (SH2) domains
- Src-homology 3 (SH3) domain
- Poly-proline stretches (PP)
- BCR homology domain (BH)
- Inter-SH2 domain [Figure 3][8, 9]

The latter is the primary p110-interacting surface. The regulatory subunit links p110 to upstream signals, interacting with tyrosine receptor kinases and G protein-coupled receptors[2, 4]. A mutant version of the p85 regulatory subunit, p65, has been shown to induce the constitutive activation of PI3K and contribute to cellular transformation[5].

CELLULAR ACTIVITIES CONTROLLED BY CLASS I PI3K

Class I PI3K controls numerous cellular activities that are connected to growth, replication and survival, differentiation, movement and invasiveness, immune signaling and metabolism[2, 6, 7]. Figure 4 shows three important signaling chains that originate in PI3K. All three are mediated by the downstream target Akt, which together with its main activating kinase, PDK1, is recruited to the plasma membrane through its affinity for the product of class I PI3K, PIP_3. Akt serves as a branching point for these signals that are

Figure 1. Three Classes of PI3K

The domain structure of the catalytic subunits is shown. ABD = Adaptor-Binding Domain, RBD = Ras-Binding Domain

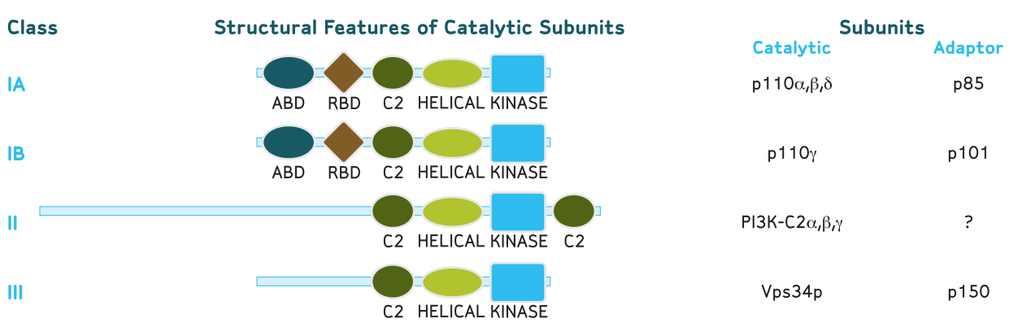


Figure 2. Substrate Specificity of PI3Ks

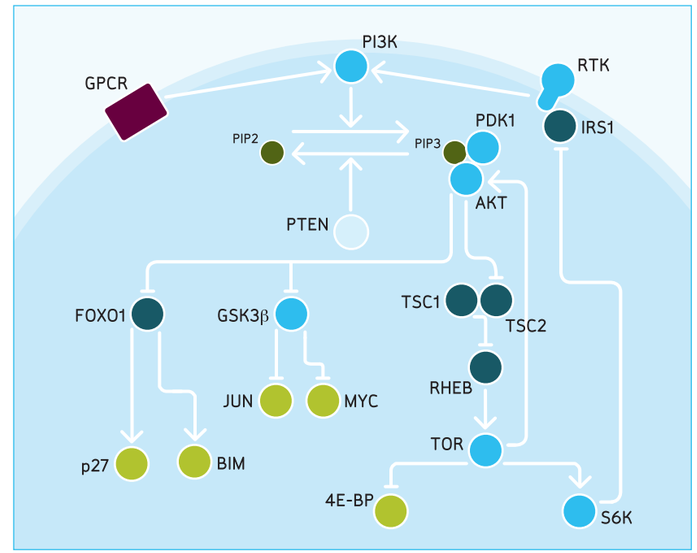
PtdIns = Phosphotidylinositol

Class I	Class II	Class III
(p110 α,β,δ,γ)	(PI3KC2 α,β,γ)	(Vps34p)
	Substrates:	
PtdIns	PtdIns	PtdIns
PtdIns 4-P	PtdIns 4-P	
PtdIns (4,5)–P_2		
	Products:	
PtdIns 3-P (PIP)	PtdIns 3-P (PIP)	PtdIns 3-P (PIP)
PtdIns 3,4-P_2 (PIP_2)	PtdIns 3,4-P_2 (PIP_2)	
PtdIns 3,4,5-P_3 (PIP_3)		

Figure 3. Domain Structure of p85

SH2 = Src-homology 2 Domain
SH3 = Src-homology 3 Domain
PP = Poly-proline Stretches
BCR = BCR Homology Domain
iSH2 = Inter-SH2 Domain

Figure 4. Canonical PI3K Signaling Pathways



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directed toward FOXO1, GSK3β and mTOR[10]. Akt-mediated phosphorylation of FOXO1 leads to the inactivation of this proapoptotic protein and a downregulation of its growth-attenuating targets, p27 and BIM.[11, 12] Akt-dependent phosphorylation also inhibits GSK3β[13] and lifts the GSK3β-mediated inhibition of Jun[14] and of Myc[15]. In a third signaling chain, Akt phosphorylates and thereby inhibits the TSC2 protein downregulating its GTPase-activating functions that negatively regulate the Ras-like protein Rheb. The result is activation of Rheb and of the downstream kinase mTOR[16]. mTOR has multiple functions; among them is the enhancement of protein synthesis.[17] This is accomplished by activating the positive regulator of protein synthesis, p70S6K, and interfering with the negative regulator, 4E-BP[10, 16, 17]. The mTOR-mediated stimulation of protein synthesis preferentially affects the translation of mRNAs that contain complex 5′ untranslated regions. The mRNAs of many growth-promoting proteins belong in this category. The overall effect of these PI3K signals is an upregulation of growth-stimulatory and a reduction of growth-inhibitory cellular functions. Figure 4 presents selected examples of the multiple cellular activities controlled by PI3Ks. There are numerous others, notably metabolic activities that are not considered here.

PI3K SUBUNIT p110 AND ITS LINK TO CANCER

PI3K has been tied to cancer for a long time. The first sign of an involvement of PI3K in cancer was an association of lipid kinase activity with the viral oncoproteins, Src and middle T. In these complexes the PI3K regulatory subunit p85 functions as a connector, binding phosphotyrosine residues on the oncoproteins and recruiting the catalytic PI3K subunit p110 [18, 19]. The oncogenic potential of p110α was then clearly demonstrated by an avian retrovirus, ASV16, that carries a copy of the cellular gene coding for p110α as an oncogene. This viral version of p110α is fused to viral sequences that direct the protein to the cell membrane, making it independent of upstream signaling and hence constitutively active[20]. Membrane-localized p110α is oncogenic.

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Most human cancers show a gain of function in PI3K signaling[10]. The increased activity can result from changes in PI3K or in its downstream target Akt or in the PI3K antagonist PTEN. Akt can be overexpressed, amplified, or its level of activating phosphorylation can be increased. PTEN shows frequent loss of function in cancer, either by mutation or by transcriptional silencing. The regulatory or catalytic subunits of PI3K are often differentially expressed or mutated in cancer[10]. Of particular interest are specific mutations in PIK3CA, the gene coding for the catalytic subunit p110α of PI3K[21]. These mutations occur in many cancers [Table 1] and together with other diverse experimental data they single out the α isoform of class I PI3K as a particularly important contributor to oncogenesis[22-26, 27]. However, the non-α isoforms of class I PI3K may have relevance for cancer as well[28]. Although no cancer-specific mutations have been identified in the non-α isoforms, they show oncogenic potential when overexpressed in avian cells[28], and may also become contributing factors in human cancer by differential expression.

IDENTIFYING "HOT SPOT" MUTATIONS

Some 20 to 30 percent of cancers of the breast, colon, prostate, and endometrium contain a mutated PIK3CA [Table 1][29]. Strikingly, about 80 percent of the mutations occur in one of three "hot spots" in the coding sequence of PIK3CA. Each of the "hot spot" mutations is a single nucleotide substitution leading to a single amino acid substitution[21]. The overwhelming prevalence of these three distinct mutations in cancer strongly suggests that p110α carrying one of these mutations bestows the cell with a strong selective advantage in growth and survival. Indeed, p110α with a "hot spot" mutation shows gain of function in enzymatic activity[30] and signals constitutively through Akt and mTOR. It can also induce oncogenic transformation of cultured cells and tumors in animals[22, 23]. The mutations in PIK3CA have thus all the attributes of "driver" mutations, responsible for neoplastic properties of the cancer cell that harbors them. Mutated p110α has emerged as arguably the most promising cancer target currently available.

5 Salient Features of the "Hot Spot" Mutations in PIK3CA:

- Cancer-specific; not found in normal tissue
- Occurring at high frequencies
- Show a gain of function, which is far easier to correct than loss of function
- They are "driver" mutations
- Mutant protein is a kinase, readily targetable by small molecules

GAINING A DEEPER UNDERSTANDING OF CANCER AND ITS THERAPIES

Mutated p110α offers unique opportunities for gaining a deeper understanding of the cancer process and for developing novel, highly targeted cancer therapies. Two of three "hot spot" mutations, E542K and E545K, map to the helical domain of p110α, and the H1047R mutation is located in the kinase domain. Genetic analyses suggest that the kinase domain mutation induces an oncogenic gain of function by a molecular mechanism different from that of the helical domain mutations[31]. The p110α protein carrying the kinase domain mutation H1047R functions independently of an interaction with Ras but requires binding to the regulatory subunit p85. Conversely, p110α with one of the helical domain mutations, E542K or E545K still depends on Ras binding but no longer needs to interact with p85 [2][31]. In contrast, wild-type p110α is not oncogenic.

Table 1. Incidence of Mutation in PIK3CA in Various Cancers.

(Compiled from the catalogue of somatic mutations in cancer www.sanger.ac.uk/genetics/CGP/cosmic/)

Primary Tissue	Percent Tumors with Mutations in p110α
Endometrium	23
Prostate	29
Breast	27
Colon	23
Urinary tract	17
Liver	12
Ovary	8
Stomach	8
Oesophagus	7
Pancreas	6
Brain	5
Lung	3
Hematopoietic tissue	4

Table 2. Two Different Mechanisms for the Gain of Function in Helical and Kinase Domain Mutations of p110α.

	Requirements for Oncogenicity in Cell Culture	
	Binding to Ras	Binding to p85
p110α E542K or E545K	Yes	No
p110α H1047R	No	Yes

Thus it is already clear that the mutant enzyme behaves very differently from wild-type and that helical domain mutations are distinct from the kinase domain mutation. The substantial differences between mutant and wild-type enzyme augur well for the possibility of obtaining mutant-specific inhibitors of therapeutic quality.

CHALLENGES IN ADVANCING RESEARCH

The new opportunities offered by the cancer-specific mutations in PIK3CA depend largely on the availability of purified, recombinant mutant p110α. This essential resource has not been commercially available until recently, when Millipore first made PI3Ks available to the market. Prior to this, researchers had to set up in-house production, requiring insect cell culture, design of appropriate expression constructs, and co-expression of the regulatory subunit. All of this is achievable only with dedicated and experienced personnel and facilities for cell culture and protein purification at a significant expense. Production of the mutant enzymes may face additional challenges of protein expression levels and protein stability.

Millipore has applied its extensive experience in the production and purification of lipid kinases to meet the need for high-quality PI3Ks, including the prevalent cancer specific mutants of p110α: E542K, E545K, and H1047R.

Mutated p110α offers unique opportunities for gaining a deeper understanding of the cancer process and for developing novel, highly targeted cancer therapies.

ABOUT MILLIPORE

Millipore's high-quality lipid kinases, assays and profiling services are helping today's leading researchers unlock the mystery of cancer.

Millipore's enzyme production experts and assay developers have many years of industry-leading research and validation experience. In fact, Millipore's collection of high-quality human and murine PI3 kinases represents the largest panel commercially available, and includes the most important cancer-specific PI3Kα mutants. All are available for sale to researchers for in-house study, and most are available for outsourced screening through the Millipore **Kinase**Profiler™ service, giving researchers all the tools they need to advance their work.

Homogeneous Time-Resolved Fluorescence (HTRF®) technology, co-developed by Millipore's assay developers, has been accepted as the preferred platform for PI3K studies. Millipore's PI3K HTRF 384 well assay kits, broad panel of validated PI3 kinases and outsourced screening and assay development solutions significantly lower the barriers to successful PI3 kinase drug development by eliminating the need to develop these proteins and assays in-house.

Features of Millipore's PI3 Kinase Products and Services:

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PI3 Kinase (p110α(E542K)/p85α)	Human	14-782
PI3 Kinase (p110α(E545K)/p85α)	Human	14-783
PI3 Kinase (p110α(H1047R)/p85α)	Human	14-792
PI3 Kinase (p110α/p65α) •	Mouse	14-786
PI3 Kinase (p110α/p85α) •	Mouse	14-785
PI3 Kinase (p110α(E542K)/p85α) •	Mouse	14-791
PI3 Kinase (p110α(E545K)/p85α) •	Mouse	14-781
PI3 Kinase (p110α(H1047R)/p85α) •	Mouse	14-787
PI3 Kinase (p110β/p85α) •	Human	14-603
PI3 Kinase (p110β/p85α) •	Mouse	14-794
PI3 Kinase (p110β/p85β) •	Mouse	14-788
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PI3 Kinase HTRF® Assay (5 x 384 assay points)	33-017
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mTOR (1362-end), active	14-770
Anti-PI3 Kinase, p85	06-195
Anti-PI3 Kinase, p85, N-SH3, clone AB6	05-212
Anti-PI3 Kinase, p110α	07-658
Anti-PI3 Kinase, p110β	06-568
Anti-PI3 Kinase, p110δ, clone AW103	05-703

About the Author

Peter K. Vogt has worked on the replicative strategies and oncogenic functions of tumor-inducing retroviruses. In the course of these studies, he discovered viral oncogenes and their cellular counterparts. His work now concentrates on dominant oncogenes in human cancer, notably on phosphatidylinositol 3-kinase, one of the most promising therapeutic targets currently available.

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