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PI3K – From the Bench to the Clinic and Back

Bart Vanhaesebroeck[a], **Peter K. Vogt**[b], and **Christian Rommel**[c]

[a]Centre for Cell Signalling, Institute of Cancer, Queen Mary, University of London, Charterhouse Square, London EC1M 6BQ, UK

[b]The Scripps Research Institute, Department of Molecular and Experimental Medicine, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

[c]Intellikine Inc., 10931 N. Torrey Pines Road, La Jolla, CA 92037, USA

Abstract

From humble beginnings over 25 years ago as a lipid kinase activity associated with certain oncoproteins, PI3K (phosphoinositide 3-kinase) has been catapulted to the forefront of drug development in cancer, immunity and thrombosis, with the first clinical trials of PI3K pathway inhibitors now in progress. Here we give a brief overview of some key discoveries in the PI3K area and their impact, and include thoughts on the current state of the field, and where it could go from here.

PI3K has become a very intense area of research, with over 2000 publications on PI3K in PubMed for 2009 alone. The expectations for a therapeutic impact of intervention with PI3K activity are high, and progress in the clinical arena is being monitored by many. However, targeted therapies almost invariably encounter roadblocks, often exposing unresolved questions in the basic understanding of the target. PI3K will most likely be no exception. Below, we describe some of these early 'surprises' and how these inform and shape basic science investigations.

The discovery of the PI3K signalling pathway and its potential as a therapeutic target

Early work showed that a phosphatidylinositol kinase activity co-purified with various viral oncoproteins expressed in mammalian cells (Macara et al. 1984; Sugimoto et al. 1984) and that cellular transformation mediated by such oncoproteins was to some extent dependent on the association with this lipid kinase activity (Whitman et al. 1985). This oncoprotein-associated lipid kinase could phosphorylate phosphatidylinositol on the 3-OH position of the inositol ring, hereby generating PI3P, a novel type of phosphoinositide (Whitman et al. 1988). This finding was followed by the discovery of PI(3,4,5)P_3 (phosphatidylinositol(3,4,5) trisphosphate; PIP_3) in GPCR-stimulated neutrophils (Traynor-Kaplan et al. 1988; Traynor-Kaplan et al. 1989) and upon acute stimulation with tyrosine kinase agonists (Auger et al. 1989; Hawkins et al. 1992; Jackson et al. 1992). It was not known at the time that agonist-stimulated PI3K is a heterodimer made up of a p110 catalytic subunit and a regulatory subunit, namely p85 in the case of class IA PI3Ks and p101 in the case of the class IB p110γ. Early studies very much focused on a tyrosine-phosphorylated 85 kD protein found in PDGF-stimulated or polyoma middle T-transformed cells which associated with PI3K activity (Courtneidge and Heber 1987; Kaplan et al. 1987). This protein turned out to be the p85 regulatory subunit of PI3K, and its cDNA was cloned by several groups (Escobedo et al. 1991; Otsu et al. 1991; Skolnik et al. 1991). Several teams also purified the PI3K enzyme activity biochemically from various tissues (Carpenter et al. 1990; Fry et al. 1992; Morgan et al. 1990; Shibasaki et al. 1991; Stephens et al. 1994). Protein microsequencing allowed the

design of oligonucleotide probes to isolate the first cDNA of a PI3K catalytic subunit, namely p110α (Hiles et al. 1992). This work revealed that the sequence of p110 was closely homologous to that of the product of vps34, a *S. cerevisiae* gene involved in endosomal sorting of proteins towards the vacuole, the yeast equivalent of the mammalian lysosome (Herman and Emr 1990). Follow-up work revealed that vps34 indeed had PI3K activity, but with a substrate specificity that was different from p110α, in that it can only phosphorylate PI (phosphatidylinositol) but not PI(4,5)P_2 (phosphatidylinositol(4,5)bisphosphate) (Schu et al. 1993).

A concerted effort of many laboratories, using various techniques, including biochemical purification and degenerate PCR approaches, revealed the existence of multiple PI3K isoforms in mammals (Arcaro et al. 1998; Brown et al. 1997; Chantry et al. 1997; Domin et al. 1997; Hu et al. 1993; Misawa et al. 1998; Ono et al. 1998; Stephens et al. 1997; Stoyanov et al. 1995; Vanhaesebroeck et al. 1997b; Virbasius et al. 1996), but also in *D. melanogaster* (MacDougall et al. 1995), *C. elegans* (Morris et al. 1996), *Dictyostelium* (Zhou et al. 1995) and other species, even in plants. These findings led to the realisation that PI3Ks are an evolutionarily conserved family of enzymes which on the basis of structural and biochemical characteristics was divided into 3 classes (Vanhaesebroeck et al. 1997a; Zvelebil et al. 1996). Mammals have eight isoforms of PI3K (class IA: p110α, p110β, p110δ; class IB: p110γ; class II: PI3K-C2α, PI3K-C2β, PI3K-Cγ, and class III: vps34p). A single representative of each of the three PI3K classes is present in *C. elegans* and *D. melanogaster*. In yeast, only a class III PI3K is found (reviewed in Ref. Vanhaesebroeck et al. 2001).

The analysis of PI3K functions in the cell was greatly aided by two small molecule inhibitors, wortmannin and LY294002. Wortmannin was identified as a PI3K inhibitor in 1993 (Arcaro and Wymann 1993; Okada et al. 1994; Powis et al. 1994; Yano et al. 1993), and in 1994, Lilly laboratories published the LY294002 inhibitor (Vlahos et al. 1994). Interestingly, all these papers almost exclusively focused on probing the immunological aspects of PI3K function using these compounds. LY294002 and wortmannin have undoubtedly been instrumental in providing first insights into the cell biology of PI3Ks but may also have generated some false expectations due to lack of specificity (see below).

Concurrent with the isolation of the genes for the different PI3Ks was the realisation that the 3-phosphoinositides could selectively bind to defined target modules in proteins, thereby altering the localisation of such proteins and their conformation and activity. Among numerous protein domains that were defined during this time was the PH (pleckstrin homology) domain, a module that occurs in many proteins (Haslam et al. 1993; Mayer et al. 1993). A major discovery was that some PH domains could bind phosphoinositides (Harlan et al. 1994). The characterisation of other 3-phosphoinositide binding domains soon followed, including the FYVE (Fab 1, YOTB, Vac 1, EEA1) domain (Gaullier et al. 1998; Mu et al. 1995; Stenmark et al. 1996) and PX (Phox) domain (Cheever et al. 2001; Ellson et al. 2001; Kanai et al. 2001; Song et al. 2001; Xu et al. 2001) which both bind PI3P (phosphatidylinositol 3-phosphate).

One of the proteins that was reported (Haslam et al. 1993; Mayer et al. 1993) to have a PH domain was the Ser/Thr kinase Akt, which is the mammalian cellular homologue of the retroviral transforming gene v-Akt (Bellacosa et al. 1991). Akt was also independently cloned as a protein kinase related to PKA and PKC, hence its alternative names PKB (Coffer and Woodgett 1991) and Rac (related to A and C kinases) (Jones et al. 1991). Akt was subsequently confirmed as a PI3K target in cells stimulated with tyrosine kinase agonists, including PDGF and insulin (Burgering and Coffer 1995; Franke et al. 1995), and through its PH domain shown to bind PIP_3 and PI(3,4)P_2 with high specificity and affinity (Andjelkovic et al. 1997; Frech

et al. 1997; Stokoe et al. 1997). An intact PH domain in Akt is crucial for its function (Stocker et al. 2002).

The regulation of Akt itself turned out to be rather complex. The PH domain recruits Akt to PIP_3 and $PI(3,4)P_2$ and the plasma membrane, where it becomes a substrate for the membrane-bound PDK1 kinase, which phosphorylates Akt on Thr308 (Alessi et al. 1997a; Alessi et al. 1997b; Stephens et al. 1998; Stokoe et al. 1997). Very early on, it was documented that Akt is also phosphorylated on Ser473 (Alessi et al. 1996), but it took more than a decade to identify the kinase that performs this phosphorylation. It turned out to be mTOR complexed with the Rictor protein, also referred to as mTORC2 (Sarbassov et al. 2005) (as opposed to mTORC1, the 'classical' mTOR in complex with Raptor).

A next step was to identify downstream substrates of Akt protein kinase activity. Akt was found to control other protein kinases either directly, such as GSKβ (Cross et al. 1995) or indirectly, such as p70 S6 kinase (Burgering and Coffer 1995). One of the Akt substrates turned out to be the pro-apoptotic protein BAD, which is inhibited in its apoptotic function upon phosphorylation by Akt (Datta et al. 1997; del Peso et al. 1997). Given that wortmannin and LY294002 had previously been shown to be able to induce cell death (Yao and Cooper 1995), these observations suggested the existence of a PI3K-Akt cell survival pathway.

It is often overlooked that studies in *D. melanogaster* and especially in *C. elegans* have been instrumental in delineating the generic layout of the PI3K pathway and key aspects of its biology. For example, studies in *C. elegans* uncovered the link between the insulin-receptor, PI3K and the FOXO transcription factors (Ogg et al. 1997) and between Akt and FOXO (Paradis and Ruvkun 1998). FOXO transcription factors were later shown to be a target for direct phosphorylation by Akt in mammalian cells (Brunet et al. 1999; Kops et al. 1999). Further seminal work in model organisms included the identification of AGE-1 as the *C. elegans* p110 paralog with a key function in the control of lifespan (Morris et al. 1996) and the identification of PI3K *in Drosophila* as an important determinant in the regulation of cell growth and size (Leevers et al. 1996).

Work from many groups further uncovered new elements of PI3K signaling, revealing the involvement of other PH domain-containing proteins, including regulators of small GTPases (GEFs and GAPs) (Klarlund et al. 1997; Krugmann et al. 2002; Welch et al. 2002) and various scaffolding and adaptor proteins (such as Gab1, Bam32, DAPP1) (Isakoff et al. 1998). These pathways have received much less attention over the years than Akt, and this may have had the effect of underestimating the importance of Akt-independent biology in PI3K action.

PI3K and human disease

Although the link between oncoproteins, growth factors and PI3K signaling, including the identification of PI3K as a Ras effector (Rodriguez-Viciana et al. 1994; Sjolander et al. 1991) and the demonstration that PI3K could act as a retroviral oncogene (Chang et al. 1997), provided some circumstantial evidence for a role of PI3K in cancer, genetic evidence from human cancer emerged only relatively late. An important breakthrough was the identification of the PTEN tumour suppressor as a PIP_3-phosphatase (Maehama and Dixon 1998). The frequently occurring inactivation of PTEN in cancer leads to constitutive activation of the PI3K pathway. It was not until 2004, however, that cancer-specific activating mutations were reported in *PIK3CA*, which encodes the p110α isoform of PI3K (Campbell et al. 2004; Samuels et al. 2004). Surprisingly, no mutations in non-p110α isoforms have been detected thus far (Parsons et al. 2008; Samuels et al. 2004; TGCA 2008; Thomas et al. 2007; Wood et al. 2007). Mutations in the regulatory subunit, p85α, encoded by *PIK3R1*, have been also discovered, although they occur at low frequency (Jaiswal et al. 2009; Philp et al. 2001; TGCA 2008). Interestingly, these mutations can also activate p110β and p110δ, possibly providing a

broader activation of the class IA PI3K pathway than *PIK3CA* mutations (Jaiswal et al. 2009). The sheer number of mutations directed to PI3K signaling in *PTEN*, *PIK3CA*, *PIK3R1* and several upstream receptor tyrosine kinases makes this pathway one of the most deregulated and druggable biochemical activities in human cancer.

Since the mid nineties, evidence for non-redundant functions of the class IAPI3K isoforms began to emerge (Hill et al. 2000; Roche et al. 1998; Roche et al. 1994; Vanhaesebroeck et al. 1999). Isoform-specific functions were exemplified by mice with inactivated p110γ (Hirsch et al. 2000; Li et al. 2000; Sasaki et al. 2000) or p110δ (Clayton et al. 2002; Jou et al. 2002; Okkenhaug et al. 2002), PI3K isoforms that are preferentially expressed in leukocytes. These mice are viable and fertile but show largely non-overlapping immune phenotypes. The phenotypes of these genetically modified mice identified p110γ and p110δ as targets in immunity and inflammation (Rommel et al. 2007; Ruckle et al. 2006; Soond et al.).

Another area of isoform-specific function and possible therapeutic intervention is represented by the role of p110β in platelet biology and thrombosis (Jackson et al. 2005). The p110β isoform plays a key role in regulating the formation and stability of integrin/adhesion bonds, necessary for shear activation of platelets (Jackson et al. 2005). An isoform-selective p110β inhibitor eliminates occlusive thrombus formation but does not prolong bleeding time *in vivo* (Jackson et al. 2005). These studies defined p110β as a new target for antithrombotic therapy.

The development of PI3K inhibitors for human disease starts to inform basic science

In 2003, the first isoform-selective inhibitor, IC87114, which has high selectivity for p110δ, was published (Sadhu et al. 2003). Over the last decade, ever increasing efforts were made to create both isoform-selective and pan-PI3K inhibitors for therapeutic use, efforts aided by the first crystal structure of a PI3K, that of p110γ (Walker et al. 1999).

Isoform-selective inhibitors for p110δ (CAL101/hematologic malignancies) and p110β (AZD6482/thrombosis) have recently entered early clinical evaluation. Compounds that are effective against all class I PI3K isoforms, including sometimes mTOR, are currently being advanced into cancer patients with solid tumors. PI3K inhibitors have not yet been tested in allergy, inflammation and autoimmunity.

Several PI3K drug candidates have started to raise questions that impact on basic research, especially in the regulation of cell survival by PI3K. Indeed, inhibition of class I PI3K activity with pan-class I PI3K inhibitor compounds does not efficiently induce apoptosis, but rather lead to a G0/G1 cell cycle arrest (Dan et al. 2009; Fan et al. 2007; Guillard et al. 2009; Raynaud et al. 2007). In other words, inhibition of class I PI3K activity appears to be better at slowing down cell proliferation than at killing cells. This observation is reminiscent of what has been found in flies and worms, where inactivation of class I PI3K activity inhibits cell growth but does not induce cell death (Leevers et al. 1996; Morris et al. 1996). Mammalian cells have recently been shown to be able to survive and proliferate normally with extremely reduced levels of class I PI3K activity (Foukas et al. 2010).

Looking back, it is clear that the effect on cell survival has been most prominently associated with PI3K action. It is becoming increasingly clear that, while PI3K and Akt are effective modulators of anti-apoptotic signalling, in many systems, they are neither necessary nor sufficient to protect against cell death (reviewed in Ref. Vanhaesebroeck et al. 2001), These data suggest that the role of PI3K, and especially of Akt, in the control of cell survival and apoptosis may have been overestimated.

It is possible that the apoptosis-inducing activity of the pan-PI3K inhibitor LY294002, seen in some but not all cells, may be due to off-target effects. It is even more likely that cellular stress may have played a role in the outcome of some of the early studies on LY294002, for example when tested on explanted cells such as neurons which are undergoing tissue culture stress (Yao and Cooper 1995). An option for increasing therapeutic effectiveness of PI3K inhibitors in cancer could be to broaden the PI3K target spectrum to include class II and class III PI3Ks whose potential role in cancer is largely unexplored. It might also be of interest to target PI3K-C2α. Indeed, in a recent study, RNAi targeted to this isoform of PI3K led to cell death in half of the panel of cancer cells tested (Elis et al. 2008). PI3K-C2α is relatively resistant to LY294002 (Domin et al. 1997; Virbasius et al. 1996) and might not have been inhibited by the doses of LY294002 that allowed cells to survive in the presence of this compound. The class III PI3K, vps34, may also be an important cancer target, given that it has been implicated in autophagy, a response to which cells under stress can resort to overcome adverse conditions.

Evidence is slowly emerging that, in large panels of cancer cell lines tested *in vitro*, there is a lack of correlation between sensitivity to class I PI3K inhibitors and the mutational status of *PIK3CA* or *PTEN* (Edgar et al.). These data argue against the existence of 'oncogene-addiction' to the PI3K pathway in cultured cancer cells. One possible explanation for this finding could be that a gain-of-function in the PI3K pathway is important in cancer initiation, but that the cancer cells are no longer critically dependent on PI3K once the cancer is established. These observations suggest that patient selection on the basis of mutational status may not be as straightforward as originally hoped for. More work clearly needs to be done to define the molecular parameters that predict sensitivity of cancer cells to PI3K inhibition.

New evidence also shows that in cancer cell lines, there is no good correlation between the presence of *PIK3CA* mutations and the steady state or growth factor-stimulated activity of PI3K and Akt (Morrow et al. 2005; Stemke-Hale et al. 2008; Vasudevan et al. 2009). This is in contrast to engineered cell model systems where gain-of-function mutations in *PIK3CA* are linked to increased PI3K signalling. It is likely that in cancer cells, other signalling networks come into play and that regulatory feedback loops affect the status of the PI3K activities. Interestingly, some cells with mutant *PIK3CA* show a dependency on the PDK1 and SGK3 protein kinases (Vasudevan et al. 2009), and it will be important to determine the genes and signaling pathways that might modulate the sensitivity of PI3K mutant cells to PI3K inhibitors.

If (class I) PI3K inhibition alone does not induce cancer cell death, the question arises what are the cancer-cell intrinsic effects of such inhibition that could be exploited for therapy. A cancer-specific role of PI3K signalling in intracellular nutrient sensing and control of metabolic pathways needs to be considered (Coloff and Rathmell 2006; Foukas et al. 2006; Jones and Thompson 2009; Plas and Thompson 2005). Such a role is also supported by the phenotypes of PI3K inactivation in flies and worms (Leevers et al. 1996; Morris et al. 1996). Inhibition of PI3K *in vivo* has been documented to have a major impact on glucose uptake in tumor cells, as measured by ^{18}fluoro deoxyglucose PET scans (Engelman et al. 2008). Other areas of cell-intrinsic impact of PI3K inhibition such as cell migration, invasion and metastasis also need to be examined.

It is most likely that class I PI3K inhibitors will be clinically effective only in combination with other interventions, such as targeted therapies against the EGF-R or MAPK pathways (Engelman et al. 2008; Faber et al. 2009; Sos et al. 2009), or more generic approaches such as chemo- and radiotherapy. One of the challenges for the future will be to delineate cancer types that might benefit from such combined therapies. An early example of such effective combination strategies is emerging in breast cancer where PI3K inhibitors can overcome resistance to EGF-R-directed therapy (Sergina et al. 2007).

It is important to keep in mind that most of the data on the impact of PI3K inhibition in cancer come from studies with cultured cell lines and xenografts. These conditions may affect the requirement for PI3K which may then differ significantly from the roles of PI3K in an autochthonous tumor growing *in vivo*. Indeed, the impact of PI3K inhibition on the stroma, including immune cells, fibroblasts, and endothelial cells, could be substantial but remains largely unexplored. A role of PI3K in developmental angiogenesis has recently been established (Graupera et al. 2008), but the functions of PI3K in tumor angiogenesis are not defined. An indirect role of PI3K blockade may also underpin the promising results of the phase I trials with the p110δ inhibitor CAL-101, which induced disease stabilisation in a substantial number of patients with B-cell lymphoma (Flinn et al. 2009). The direct impact of p110δ-centered inhibitors on the proliferation and survival of haematological cancer cells is modest, and it is possible that indirect actions of PI3K inhibitors come to play in this clinical setting.

Some outstanding questions in PI3K biology and signalling

While Akt has been the most studied target of PI3K, many questions on its regulation and function remain unanswered. Indeed, we still do not have a full understanding of its activation by PDK1 and mTORC2, of its inactivation and of the many feedback loops that control this kinase. We are largely ignorant of the mechanisms by which Akt regulates its cellular location and affects its many targets, notably those in the nucleus. We also have little definitive understanding of the specific, non-redundant functions of the three Akt isoforms. As aptly captured by Brian Hemmings when reviewing the field ten years after the molecular cloning of Akt, this is still ‘a hard Akt to follow’ (Brazil and Hemmings 2001). It will also be important to re-evaluate the pro-survival and growth-promoting role of Akt and to define the signalling context that would make it a potentially exploitable therapeutic target.

PI3K effectors other than Akt also deserve more attention and scrutiny. Indeed, other than Akt, PI3K regulates other tyrosine kinases (such as Btk) and affects adaptor proteins (such as Gab2) and a plethora of GEFs and GAPs for monomeric GTPases of the Rac, Ras and Arf families (Vanhaesebroeck et al. 2001). The regulation of these GEFs and GAPs is complex and difficult to track experimentally, but some of these proteins could play important roles in PI3K signalling pathways. This is illustrated by P-REX2a, which activates the small GTPase Rac and is regulated by both PIP_3 and the $G_{\beta\gamma}$ subunits of heterotrimeric G proteins, and which has recently been shown to interact with PTEN, inhibiting PTEN function (Fine et al. 2009).

The roles of the PI3K isoforms in human disease need to be further delineated. In a non-cancer context, class I PI3K isoforms have highly non-redundant functions, but it is not clear at this point how such specificity is achieved, as all PI3K isoforms activate Akt indiscriminately. It is possible that PI3K isoforms produce PIP_3 in different cellular compartments, and they could also differentially regulate small GTPases such as RhoA (Papakonstanti et al. 2007; Papakonstanti et al. 2008). In cancer, some of this non-redundancy is lost, possibly because the pathways upstream of the PI3K isoforms have been deregulated (Vanhaesebroeck et al. 2010).

Powerful tools to address some of these questions now available. These include isoform-specific inhibitors for p110β, p110γ and p110δ as well as an array of mutant and transgenic mice. The differential roles of p110 isoforms in cancer remain an important topic. It is not clear why the gene encoding p110α is so selectively mutated in cancer. These mutations increase the activity of p110α by enhanced association with the plasma membrane (Gymnopoulos et al. 2007; Mandelker et al. 2009), or by release from a p85-mediated inhibition (Miled et al. 2007), but the detailed molecular mechanisms of increased downstream signalling remain to be determined. There is suggestive evidence that different mutations can have a differential

biological output such as in breast cancer cells, where the E545K mutation of *PIK3CA* appears to be associated with an enhanced metastatic phenotype compared to the H1047R mutation (Pang et al. 2009).

Thus far, the focus of the field has been on class I PI3Ks and their action through the PH-domain-mediated binding of key effectors to PIP_3 and $PI(3,4)P_2$. Relatively little attention has been paid to class II and III PI3Ks, their physiological roles and possible involvement in disease. These PI3Ks operate through PI3P and its effector proteins which bind this lipid with their PX or FYVE domains. While PH domains are more abundant than PX and FYVE domains, only a very small subset of PH domains binds PIP_3 or $PI(3,4)P_2$ (Lemmon 2008). In contrast, all PX and FYVE domains bind to PI3P. Therefore PI3P has many more effectors than PIP_3 and $PI(3,4)P_2$. These effectors are very diverse and include p40 and p47 subunits of NADPH oxidase and proteins with sorting and scaffolding functions in membrane transport such as early endosome antigen-1 (EEA1), Hrs/vps27, ESCRT components, Alfy, kinesins and sorting nexin family members. PI3P-binding proteins also include the lipid kinase Fab1/PIKfyve (which converts PI3P to $PI(3,5)P_2$), the protein kinase SGK3 and additional GAPs (such as RGS-PX1) (reviewed in Refs. Birkeland and Stenmark 2004; Di Paolo and De Camilli 2006; Hurley 2006; Lemmon 2008; Vanhaesebroeck et al. 2010).

A key question is whether PI3P is involved in acute signalling and to what extent it influences signalling by extracellular agonists. Class II PI3K isoforms have been reported to generate PI3P in an agonist-dependent manner (reviewed in Refs. Falasca and Maffucci 2009; Vanhaesebroeck et al. 2010) and vps34 has been shown to control amino acid-dependent activation of S6 kinase-1 through unknown intermediates (Byfield et al. 2005; Nobukuni et al. 2005). At present there are no small molecule inhibitors of class II and III PI3Ks in the public domain (Shuttleworth et al. 2009). The importance of PI3P in disease is underscored by the observation that germline inactivation of PI3P-phosphatases of the myotubularin family in humans can lead to neuropathies and myopathy (Nicot and Laporte 2008).

Last but not least, we know very little about the production of the PI3K lipids themselves, their levels in disease, their subcellular localisation and their dynamic interconversion to other phosphoinositides. The frequent loss of the tumor suppressor PTEN in cancer demonstrates the importance of 3-phosphoinositide turnover. More recent observations assign important roles to 5-phosphatases of PIP_3, including IPP5E, whose inactivation is involved in ciliopathies (Bielas et al. 2009; Jacoby et al. 2009), and SHIP2, which has been implicated in insulin signalling and glucose homeostasis (Ooms et al. 2009). INPP4 is a 4-phosphatase of $PI(3,4)P_2$; its INPP4B isoform is a tumor suppressor that inhibits PI3K signalling (Gewinner et al. 2009). PI3P turnover is regulated by myotubularin phosphatases, some of which have been implicated in myopathies and neuropathies (Nicot and Laporte 2008). These data show that it will be essential to monitor the levels and species of phosphoinositides in disease, in combination with proteomic and lipidomic profiling. Although it is now possible to monitor the subcellular distribution of 3-phosphoinositides with labelled lipid-binding domains, no progress has been made in the quantification of 3-phosphoinositides. Indeed, over the last decade, the entire field has almost exclusively relied on proxy readouts such as the phosphorylation of Akt. The disconnects between PI3K pathway activation and Akt phosphorylation that starts to surface (Vasudevan et al. 2009) make it imperative to develop new methods for monitoring 3-phosphoinositides in cells.

Concluding remarks

Remarkable progress has been made over the last two decades in our knowledge of PI3K biology and signalling. PI3Ks have been identified as powerful signaling enzymes that respond to diverse upstream inputs and feed into complex downstream networks. Class I PI3Ks generate

the tightly regulated second messenger PIP_3 signaling platform. At the level of cellular signalling, the four PI3K isoforms of class I, despite their identical lipid kinase activities, carry out largely non-redundant tasks, and recent evidence suggests that different isoforms can cooperate in achieving specific effects. The molecular basis for these distinctions and complementations is not understood. The extent to which different isoforms can substitute for each other is also not known.

High points in PI3K studies include genetically engineered mice, high resolution crystal structures, biochemical and cellular high throughput assays, cell-based and *in vivo* imaging assays, human genetics and isoform-selective inhibitors. There is an active debate in the field about selectively targeting single isoforms of PI3K *versus* a broader, pan-PI3K directed approach. First generation drugs against class I PI3K isoforms have entered clinical testing. Several other drugs targeting alternative components of the PI3K signaling network are at a similar stage of development. Despite many open questions, there is hope that an understanding of the genetic signatures that mark a role for PI3K in disease will translate into therapeutic benefits. First generation drugs are often “learning tools” that will be outperformed by better drugs and knowledge. Clinical experience, basic science and drug development are poised to interdigitate and to complement each other as the PI3K field evolves from a cellular signaling specialty to an area of broad medical significance and impact.

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