Molecules in medicine mini-review: isoforms of PI3K in biology and disease

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Abstract The PI3K lipid kinases are involved in signal transduction and intracellular vesicular traffic, endowing these enzymes with multiple cellular functions and important roles in normal physiology and disease. In this mini-review, we aim to distil from the vast PI3K literature the key relevant concepts for successful targeting of this pathway in disease. Of the eight isoforms of PI3K, the class I PI3Ks have been implicated in the aetiology and maintenance of various diseases, most prominently cancer, overgrowth syndromes, thrombosis, inflammation and autoimmunity, with emerging potential roles in metabolic, cardiovascular and other disorders. The development of class I PI3K inhibitors, mainly for use in cancer and inflammatory disorders, is a very active area of drug development. In 2014, an inhibitor of the p110δ isoform of PI3K was approved for the treatment of some human B-cell malignancies. The key therapeutic indications of targeting each class I PI3K isoform are summarized and discussed.

Keywords PI3K • Signalling • Vesicular Traffic • Cancer • Overgrowth Syndrome • Immunity • Inflammation • Auto-immunity • Diabetes • Obesity • Metabolic syndrome • Pharmacological inhibitor

The PI3K family

The common and defining feature of PI3Ks is their ability to phosphorylate the 3-hydroxyl group on phosphoinositides that reside on cellular membranes. All eight mammalian PI3Ks **(Figure 1)** share a conserved catalytic domain but differ in their regulation and preferred lipid substrate. PI3K activity is opposed by lipid phosphatases, such as PTEN, INPPs and SHIPs (class I PI3Ks) and myotubularins (class II and III PI3Ks). Loss-of-function of these phosphatases is of key importance in some diseases, such as cancer (the PTEN and INPPs are tumour suppressors) and certain myopathies (myotubularins).

Class I PI3Ks are heterodimers formed of a regulatory and a catalytic (p110) subunit, further referred to as PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ. They are further subdivided into class IA (PI3Kα, PI3Kβ, PI3Kδ) or IB (PI3Kγ) depending on the type of regulatory subunit present in the complex (p85 or p84/p101 for class IA and IB, respectively). All class I PI3Ks couple to cell surface receptors at the plasma membrane where, upon activation, they phosphorylate phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) into the short-lived second messenger $PI(3,4,5)P_3$ (PIP_3). Despite this apparent simplicity, the upstream activation of these isoforms is complex, and includes engagement with phosphotyrosines in receptors and adaptor proteins via the SH2 domains of the p85 regulatory subunit (PI3Kα, PI3Kβ, PI3Kδ), $G_{\beta\gamma}$ subunits released by activated G protein-coupled receptors (GPCRs) (PI3Kβ, PI3Kγ) and small GTPases such as Ras (PI3Kα, PI3Kγ), Rac and cdc42 (PI3Kβ). Class I PI3K activity is transmitted into the cell through the localised accumulation of PIP_3, which induces the recruitment of pleckstrin homology (PH) domain-containing effector proteins to the plasma membrane where they are activated. These effector proteins include protein kinases, adaptor proteins and regulators of small GTPases. The serine/threonine kinase Akt/PKB, a key class I PI3K

effector, is an important signalling node that regulates cell survival, cell cycle, glucose metabolism, protein synthesis and migration.

Class II PI3Ks (PI3K-C2α, PI3K-C2β and PI3K-C2γ) and the class III PI3K (vps34) mainly associate with intracellular membranes where they produce the lipids PI3P and most likely also $PI(3,4)P_2$ **(Figure 1)**. Their regulation is less well understood than that of class I PI3Ks, but they have been implicated in the regulation of vesicular traffic, such as in endocytosis and autophagy. The role of the class II/III PI3Ks in signal transduction by extracellular ligands is not clear, but their activities might directly or indirectly modulate such signalling.

PI3K isoforms in disease

In addition to their catalytic activity, several isoforms of PI3K and their adaptors have scaffolding functions[1,2]. In this review, we only describe the kinase-dependent functions of PI3Ks, given that these, in principle, could be regulated by small molecule pharmacological inhibitors.

At present, only the class I PI3K isoforms have been firmly implicated in disease (**Table I**). This is not the case for class II/III PI3Ks, and these isoforms will therefore not be discussed in detail here. Interestingly, however, organismal inactivation of the kinase activity of PI3K-C2β in mice has recently been reported to result in enhanced insulin sensitivity and glucose tolerance, without detectable side effects[3], indicating that this particular PI3K isoform might be a potential drug target for metabolic disorders. The class III PI3K, vps34, has been implicated in the regulation of autophagy, a process that has been associated with a spectrum of diseases ranging from cancer to neurodegeneration. This has spurred the development of vps34 inhibitors (reviewed in Ref.[4]), but the potential of vps34 inhibition in a disease context still remains to be established.

Below, we describe key features and functions of each class I PI3K isoform.

PI3Kα

p110α, the catalytic subunit of PI3Kα**,** is ubiquitously expressed. *PIK3CA*, the gene encoding p110α, is frequently amplified or somatically mutated in solid tumours/carcinoma but very rarely in soft tissue cancers/sarcoma or haematological malignancies. 'Oncogenic' *PIK3CA* mutations are found across the *PIK3CA* gene but mainly occur in hot-spots and lead to increased lipid binding and/or basal activity of p110α through a multitude of mechanisms that mimic and enhance the dynamic allosteric/intramolecular events that normally activate wild-type PI3Kα (Ref.[5]).

PIK3CA and PI3K pathway components have recently been found to be somatically mutated in a spectrum of congenital or early childhood onset human overgrowth disorders[6-10]. PI3K pathway mutations in these disorders are almost exclusively found in tissue of mesodermal origin, with overgrowth frequently observed in adipose, muscle and skeletal tissue. Interestingly, despite the *PIK3CA* mutations associated with overgrowth disorders being similar to those observed in cancer, individuals with this condition do not appear to be predisposed to cancer. This suggests that the context in which the *PIK3CA* mutations occur determines its role in disease.

The prevalence of *PIK3CA* mutation and amplification in cancer suggest an important role for p110α in cancer development. Two recent reports[11,12] have revealed that expression of mutant *Pik3ca* induces multipotency in breast cancer progenitor cells, possibly contributing to intratumoural heterogeneity. At present, it is not entirely clear how critical *PIK3CA* alterations are in *established* cancer. Indeed, whereas the presence of *PIK3CA* mutation/amplification in cancer cell

lines has some predictive value in determining sensitivity to PI3K inhibitors, this correlation is not absolute and other genetic parameters also control this response[13]. This is in fact not surprising given that cancer cells have multiple ways of activating the PI3K pathway, other than through mutational activation of p110α, including through mutation of the p85 regulatory subunits, upstream activators such as tyrosine kinases and Ras, and downstream effectors such as Akt, or by loss of the PTEN tumour suppressor. Indeed, cancer cells almost invariably "hijack" the PI3K pathway, with mutation of *PIK3CA* being only one of several ways of achieving this. An overview of the genomic determinants of PI3K pathway inhibitor response in cancer is given in Ref.[14].

Under normal physiological conditions, PI3Kα is a key effector molecule in insulin/IGF-1 signalling (Refs.[15,16]). Indeed, heterozygous genetic inactivation of p110α leads to insulin resistance and glucose intolerance in young mice[15]. Interestingly, upon ageing, the heterozygous genetic inactivation of p110α leads to better overall glucose homeostasis compared to that in control aged mice[17]. Similarly, sustained low-level pharmacological inactivation of p110α (together with p110δ) reduces obesity and ameliorates metabolic syndrome in obese mice and monkeys[18], uncovering pharmacological inhibition of PI3K/p110α as a potential anti-obesity intervention.

PI3Kβ

p110β, the catalytic subunit of PI3Kβ, also has a broad tissue distribution but is absent or expressed at low levels in some cell types, such as B- and T-lymphocytes (Ref.[19] and Klaus Okkenhaug, personal communication).

p110β was the target of one of the first developed isoform-selective PI3K inhibitors[20], namely for antithrombotic therapy. p110β plays a specific role downstream of collagen and integrin receptors in platelet aggregation and p110β inhibitors interfere with thrombosis without inducing bleeding[20-22]; reviewed in Ref.[23]).

Recently, mutations similar to those in *PIK3CA* have also been found in *PIK3CB* (the gene for p110β) in cancer, albeit at a much lower frequency[24,25]. It is important to keep in mind that non-mutated p110β could be activated by mutations in p85 (Ref.[26]). PI3Kβ has been reported to be the main mediator of enhanced PI3K activity induced upon the inactivation and loss of PTEN in cancer (reviewed in Ref.[14]), but this appears to be dependent on the genetic context, as PI3Kα (Ref.[27,28]) and PI3Kδ (Ref.[29]) are also capable of contributing to biology induced by PTEN loss.

There is increasing evidence for a role of PI3Kβ in prostate cancer, in which PTEN inactivation is a common event. PI3Kβ has been shown to positively regulate androgen receptor transactivation in prostate cancer cell lines[30] as well as in Sertoli cells in the regulation of mouse fertility[31]. In line with the contribution of PI3Kβ and PI3Kδ (Ref.[29]) to enhanced PI3K activity upon PTEN loss, a PI3Kβ/δ inhibitor was found to be very effective in a preclinical study of prostate cancer, particularly in combination with hormonal therapy[32]. Another study[33] showed that the combined inhibition of PI3Kα/β and androgen receptor is effective at inhibiting *PTEN* mutant prostate cancer cells.

p110β is highly expressed in myeloid cells in which it has been shown to regulate Fcγ receptor-driven responses, in concert with p110δ under certain conditions (Refs.[34,35]). This could be exploited in the context of inflammatory disorders resulting from the deposition of immune complexes, which, when not cleared effectively, lead to tissue damage and non-resolving inflammation. This therapeutic potential for p110β inhibition is illustrated by the observation that mice lacking PI3Kβ activity are protected in an experimental model of autoimmune skin blistering disease[34].

PI3Kδ

p110δ, like p110γ, is highly expressed in leukocytes[36,37] but is also present at intermediate levels in other tissues, such as neurons[38] and some transformed epithelial cells[39,40]. Its predominant expression in the haematopoietic compartment correlates with a variety of immune functions, mainly in the adaptive immune system, with important roles in B- and T-cells[41], but also in mast cells[42] and myeloid cells (such as neutrophils and macrophages)[43,34].

Recently, germline mutations in *PIK3CD* have been identified in a rare disease called APDS (Activated p110delta syndrome)[44] or PASLI (p110δ-activating mutation causing senescent T-cells, lymphadenopathy and immunodeficiency)[45] (reviewed in Ref.[46]). This disease is an autosomally-dominant primary immune deficiency, which often (but not always) predisposes to respiratory infections and airway damage, and can lead to early death from infection-related causes and possibly lymphoma. The *PIK3CD* mutations in APDS are similar to the hot-spot mutations found in *PIK3CA* in that they activate the p110δ kinase. These patients could therefore benefit from the use of p110δ-selective inhibitors.

Apart from a very low frequency mutation found in diffuse large B-cell lymphoma[47], the *PIK3CD* gene is mostly non-mutated in cancer. Like wild-type p110α and p110β, p110δ may become activated by mutations in its associated p85 regulatory subunit[26]. Whereas the expression level of p110δ in leukocytes does not appear to significantly increase upon transformation, some solid tumour cell lines express high levels of the p110δ protein, where it might contribute to migration and epithelial polarity[48,39,40,49,50].

Given the high expression levels of p110δ in leukocytes, p110δ inhibitors were developed to treat blood cancers in the hope that they would induce an anti-proliferative/cytotoxic effect across all haematological malignancies. However, the cytotoxic/cytostatic effects of p110δ inhibitors in transformed leukocytes turned out to be modest, at least *in vitro.* Despite this, p110δ inhibitors have shown impressive clinical impact in some human B-cell malignancies, such as CLL, and a PI3Kδ inhibitor (Idelalisib/Zydelig) from Gilead is the first approved (2014) PI3K inhibitor[51]. The mechanism of action of PI3Kδ inhibition in B-cell malignancies is based on interference with signalling by stimuli from the B-cell antigen receptor, co-stimulatory receptors, adhesion receptors and chemokines, on which some B-cell malignancies depend (reviewed in Ref.[52]). There is no evidence for a direct cytotoxic effect of PI3Kδ inhibition on leukaemic cells in patients.

An interesting recent finding is that inhibition of PI3Kδ can stimulate immune responses against solid tumours, thereby broadening the utility of PI3Kδ inhibitors in cancer treatment beyond haematological malignancies[53], a concept that will be tested in human clinical trials in the near future (NCT02540928 and NCT02468557 on ClinicalTrials.gov). Mechanistically, inhibition of PI3Kδ in cancer preferentially reduces the immune-suppressive function of regulatory T-cells, allowing an anti-tumour cytotoxic T-cell response to develop[53]. PI3Kδ inhibition also dampens myeloid-derived suppressor cells in cancer[53]. As a potential therapeutic approach, it would be interesting to combine PI3Kδ inhibitors with surgery (which allows the immune system to deal with micrometastases once the primary tumour is resected, as was demonstrated in Ref.[53]), irradiation (which can generate neo-antigens) or other immuno-modulatory agents, such as immune checkpoint blockers or tumour vaccines.

PI3Kγ

As for p110δ, expression of this PI3K isoform is enriched in leukocytes, in which it mainly regulates the innate immune system. The literature on p110γ is complex, as it describes both scaffold-

dependent and –independent roles of this PI3K. Below, we summarize what is known about the kinase-dependent roles of p110γ.

There are multiple immune-related disease indications for p110γ (**Table I**; reviewed in Refs.[54,55]) but it has turned out to be challenging to make truly isoform-selective inhibitors for this PI3K. This means that the early promise of p110γ as a drug target in disease[56] has not (yet) been fulfilled.

Other than in leukocytes, p110γ is also expressed at low levels (compared to leukocytes) in cardiomyocytes, smooth muscle cells and endothelial cells. Interplay between all these p110γ-expressing cells has been implicated in cardiovascular biology. Indeed, interference with p110γ activity could be beneficial in cardiovascular disease under certain conditions, for example by alleviating myocardial inflammation and preventing maladaptive matrix remodelling (for a review, see Ref.[57]).

Whereas a cancer-cell-intrinsic role for p110γ has not been clearly established, a role for this PI3K isoform in cancer-associated inflammation is emerging[58]. Indeed, p110γ regulates the recruitment and activation of myeloid cells by diverse tumour-derived stimuli, allowing these cells to invade into the tumour and stimulate tumour angiogenesis, growth and progression[58].

Interference with PI3K activity in disease: isoform-selective or multi-PI3K-targeted inhibitors?

PI3Ks play important roles in normal physiology and therefore identifying a suitable therapeutic window of PI3K inhibition at the organismal level is an important issue. This is disease-dependent, with different toxicity profiles accepted for example in cancer *versus* in more chronic conditions, such as in inflammatory, auto-immune and metabolic disorders.

Pharma has been very effective in developing a range of inhibitors that target single or multiple PI3K isoforms. Given the importance of PI3K in cell biology, a consideration has been the toxicity profile of pan-class I PI3K isoform inhibitors. These have turned out to be reasonably well tolerated in a cancer setting, although the level of on-target inhibition that was achieved in these trials has not always been fully established. Toxicity of isoform-selective PI3K inhibitors is generally expected to be less wide-ranging than that of pan-PI3K inhibitors, allowing tolerance of higher drug doses, resulting in more complete PI3K target inhibition. However, as illustrated by the colitis induced by long-term PI3Kδ inhibition in CLL patients[59], drug tolerability can be an issue upon interference with a single PI3K isoform, as a result of mechanism-based, on-target side effects. Potential ways to overcome this is through topical application of a PI3K inhibitor, as exemplified by the ongoing development of an inhaled PI3Kδ inhibitor by GSK as an anti-inflammatory agent for the treatment of inflammatory airway diseases[60,61], avoiding systemic exposure to the drug.

PI3K isoforms can also cooperate, especially under physiological conditions where cells are exposed to multiple stimuli, simultaneously signalling through different types of receptors. For example, some immune functions of p110δ are executed in concert with other class I PI3K isoforms, such as with p110γ in the respiratory burst of human neutrophils[43] or with p110β in FcRγ receptor signalling[34,35]. This interplay between class I PI3K isoforms in inflammation and immunity is complex (reviewed in Refs.[62,54,63,64]) and therapeutically explored by the ongoing development of dual PI3K inhibitors, such as dual PI3Kβ/δ or dual PI3Kγ/δ (IPI-145; Duvelesib)[65] inhibitors. A risk

of this strategy is the induction of overwhelming immune suppression, especially in cancer trial settings in which patients may have previously undergone immune-compromising therapies.

PI3K isoforms can also compensate for each other, as best documented in cancer. A key way in which this phenomenon arises is through feedback mechanisms: these regulatory loops exist to counterbalance the PI3K pathway that is inhibited by an isoform-selective PI3K inhibitor, and lead to the activation of the remaining, non-inhibited PI3K isoform(s). Recently published examples include the 'rebound' of PI3Kα activity upon blockade of PI3Kβ (Refs.[48,33]). These data suggest that the use of pan-class I PI3K inhibitors may be needed to counterbalance class I PI3K-dependent compensatory mechanisms developed by cancer cells. Another key consideration for using PI3K inhibitors for cancer treatment is the finding that cells can survive and proliferate with very low levels of class I PI3K activity[66]. Moreover, because PI3Kα is critical for insulin signalling, a serious problem upon administration of PI3K inhibitors is the compensatory, increased systemic insulin production, which can stimulate cancer cell proliferation. Of course, upon sufficient inhibition of PI3K activity in the cancer cells, their responsiveness to insulin-stimulated PI3K would be expected to be blocked, but insulin could still activate other signalling pathways in the cancer cells, such as MAPK.

Conclusion

Despite extensive PI3K drug development efforts, only one PI3K inhibitor (Idelasib/Zydelig against PI3Kδ, for use in some B-cell malignancies) has been approved for human therapy to date[51], but this is expected to change in the near future, especially as part of combination therapies in cancer. Indeed, some promising combination treatments with PI3K inhibitors have recently been reported, such as with inhibitors of PARP (reviewed in Ref.[67]) or CDK4/6 (Ref.[68]) in breast cancer. Another exciting example is the observation that PI3Kα inhibitors can enhance the responsiveness of oestrogen receptor-positive breast cancer cells to hormone therapy[69]. The exploitation of the roles that PI3Ks play in the immune system is also likely to be a further fertile area of drug development, not only in chronic inflammation as initially intended (mainly PI3Kγ and PI3Kδ), but also in cancer, such as in immunotherapy (PI3Kδ) or modulation of cancer-induced inflammation (PI3Kγ). The use of PI3K inhibitors to interfere with angiogenesis[70] in cancer, for example by modulating the tumour microvasculature to increase chemotherapy delivery[71], remains to be more extensively explored.

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Figure Legend: Shown are the different classes and isoforms of PI3K, their generic roles in cell biology and potential disease implications.

Table I - Therapeutic indications for class I PI3K isoforms: Key indications for interference with selected PI3K isoforms in disease. This is an oversimplification, given that PI3K isoforms could be targeted in combination, as described in more detail in the main text.

PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ
• Solid tumours • Overgrowth syndromes • Obesity, metabolic syndrome	• Solid tumours (PTEN null?) • Thrombosis • Inflammation: antigen/antibody immune complex-driven immune dysfunction	• B-cell malignancies • Solid tumours (through immune stimulation) • Autoimmunity/inflammatory disorders (rheumatoid arthritis, allergy, airway inflammation, …) • Activated p110delta Syndrome	• Inflammatory disorders (rheumatoid arthritis, allergy, airway inflammation, obesity-related inflammation, atherosclerosis, …) • Cardiovascular disease • Cancer-associated inflammation

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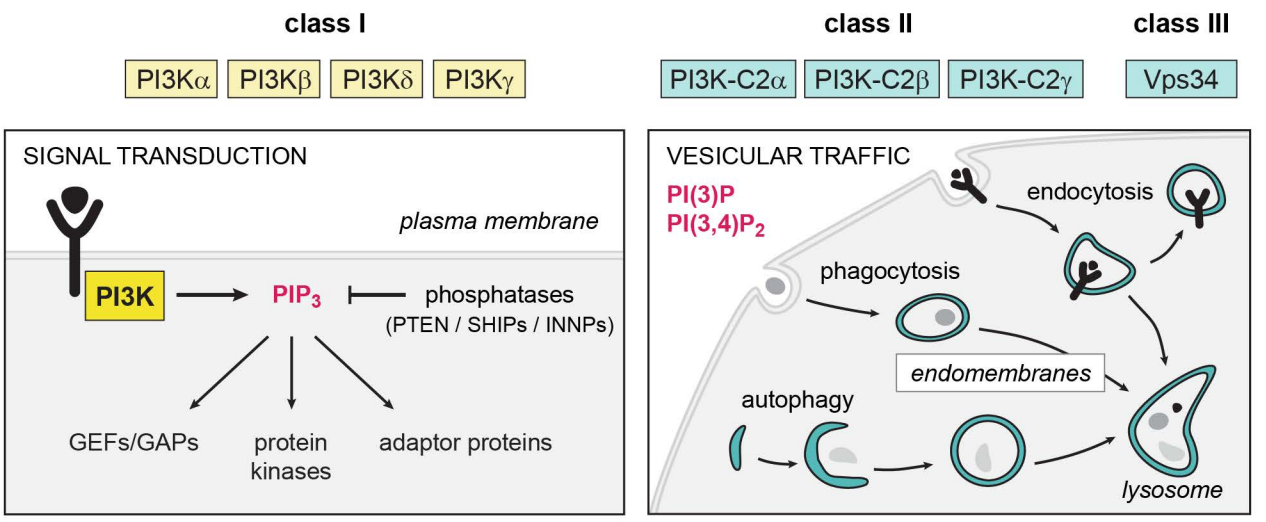

cancer, overgrowth, metabolic syndromes, thrombosis, autoimmunity & inflammation

disease indications: ?