Inhibiting the phosphoinositide 3-kinase pathway for cancer treatment

P. Workman

Cancer Research UK Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, U.K.

Abstract

There is extensive evidence from the molecular and genomic analysis of human cancers that the PI 3-kinase (phosphoinositide 3-kinase)–Akt/PKB (protein kinase B) pathway is deregulated in malignant progression. Furthermore, the causal involvement of PI 3-kinase is supported by gene-knockout mouse models. Prototype inhibitors show evidence of anticancer activity in vitro and in vivo animal models. The recent development of isofom-selective inhibitors shows considerable promise for cancer treatment.

The emergence of genome-based cancer therapeutics

Cancer treatment has traditionally been based on cytotoxic drugs that damage DNA directly, block its synthesis or interfere with the mechanics of cell division; for example, by inhibiting microtubule function. The major exceptions are agents that interfere with the biology of oestrogens and androgens in hormone-dependent breast and prostate cancer. These set a precedent for other anti-signalling drugs.

However, the epidemiology, genetics, genomics and molecular pathology of cancer all point to a large number of abnormalities in the structure and expression of cancer-inducing genes as being causally involved in malignant progression [1]. The current dominating strategy in cancer drug discovery seeks to exploit our growing knowledge of the genes and cognate biochemical pathways that drive the disease [2,3]. The expectation is that by targeting the molecular abnormalities that are responsible for various cancers, drugs will be identified that are both more effective and have fewer side effects than those developed in the cytotoxic era.

The success of the new genome-based approach to cancer therapeutics is exemplified by the regulatory approval of pioneering examples of the genre, together with the preclinical and clinical development of a large number of others [4]. The best known drug of this type is imatinib (Gleevec) which inhibits the kinase activity of Bcr-Abl and is approved for the treatment of chronic myeloid leukaemia – a disease driven by this genetically activated oncogenic kinase [5]. Imatinib is also approved for the treatment of GISTs (gastrointestinal stromal tumours) because of its inhibitory activity on the c-Kit kinase that is commonly mutated in this malignancy. Gefitinib (Iressa) inhibits the EGFR (epidermal growth factor receptor) tyrosine kinase and is approved for the treatment of non-small-cell lung cancer [6]. The drug also shows activity in other malignancies such as hormone-resistant prostate cancer, and head and neck cancer. Both of the above agents are ATP-competitive small-molecule tyrosine kinase inhibitors. In addition, the humanized monoclonal antibody trastuzumab (Herceptin) is approved for the treatment of ErbB2/HER2-positive breast cancer [6]. This drug was, in fact, the first drug to gain regulatory approval for use in a genomically defined subset of cancer patients.

Other drugs in development include small-molecule inhibitors of cyclin-dependent kinases, which are frequently deregulated in human cancers [7], and also those that act on MEK1/2 [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase 1/2] and Raf-1 in the Ras → Raf → MEK → ERK1/2 cascade [8,9]. Drugs acting on the Raf family of serine/threonine kinases are of current interest in view of the discovery of activating BRAF mutations in melanoma and other cancers [10]. BRAF mutations were detected by systematic high-throughput sequencing of the genomes of cancer cell lines and tissues [10]. Similarly, large-scale sequencing of tyrosine kinase in colorectal cancers has revealed several genes that are mutated and may represent potential drug targets.

We have therefore entered an era in which drugging the cancer genome, and particularly the cancer kinase, has become an experimental and clinical reality. Having established the precedent that a high degree of sensitivity for one kinase compared with another can be achieved with small-molecule ATP-competitive agents, and that disease modulation can be achieved in animal models and in the clinic, there is enthusiasm to develop other genome-based drugs for cancer treatment.

Two important aspects of the development of kinase inhibitors and other molecular therapeutics for cancer are worthy of being highlighted here. The first concerns the issue of how selectivity for malignant compared with normal cells can be achieved when the molecular targets are commonly expressed and functional in normal cells, albeit at a lower level and in a non-mutated or less activated form. The best...
is overexpressed and activated, e.g. in breast, ovarian and cervical cancers. The downstream kinase Akt/PKB (protein kinase B) is amplified and overexpressed in ovarian and cervical cancers. (i) The P110α catalytic subunit is overexpressed and activated, e.g. in colorectal and pancreatic cancer. (ii) The PI 3-kinase pathway in human cancer is summarized below (for more details see [18,20]). (i) The P110α catalytic subunit is overexpressed and activated, e.g. in breast, ovarian and pancreatic cancer. (iii) Upstream receptor tyrosine kinases that activate PI 3-kinase are commonly mutated, amplified and overexpressed, e.g. the EGFR and ErbB2 in breast, ovarian and lung cancer. (iv) The Ras family members which are involved in PI 3-kinase activation are frequently mutated, e.g. in colorectal and pancreatic cancer. (v) The PTEN (phosphatase and tensin homologue deleted on chromosome 10) gene, which encodes a phosphatase that reverses the PI 3-kinase reaction, is the second most commonly mutated tumour suppressor gene after p53 and is deregulated particularly frequently in glioblastoma, prostate and endometrial cancer, and also in melanoma. (vi) Akt/PKB phosphorylates the cyclin-dependent kinase inhibitor p27Kip1 and blocks entry into the nucleus, and cytoplasmic localization correlates with poor clinical outcome in breast cancer. (vii) Akt/PKB also phosphorylates the oncoprotein Mdm2, promoting nuclear entry and causing degradation of p53.

In addition to the large body of compelling correlative data as summarized above, strong direct proof of the involvement of deregulated PI 3-kinase signalling in malignancy comes from mouse genetic models (for details see [20]). Constitutive activation of PI 3-kinase by transgenic expression of the activated form of p110α causes a large heart phenotype and a dominant-negative p110α results in a small heart. Similar results with Akt constructs indicate that PI 3-kinase p110α and Akt are epistatic in this respect. Specific expression of activated Akt in T-cells increases their survival, and similar expression in the pancreas causes hyperplasia, but in both cases, no malignancy is seen. Mice with a constitutively activated p85 regulatory subunit of PI 3-kinase develop a lymphoproliferative disorder, progressing to malignant lymphoma when crossed with p53-knockout mice. Thus it can be concluded that the PI 3-kinase pathway can contribute to cancer, but is insufficient alone to cause malignant progression. In further support of this, retroviral introduction of Akt and Ras together, but not either gene alone, caused glioblastomas in mice. Although homozygous deletion of Pten in the mouse is embryonic lethal, heterozygous mice are viable and exhibit a high frequency of T-cell lymphomas, gonadostomal tumours, germ-line cancers, and malignancies of the endometrium, thyroid, prostate, breast, liver and intestinal tract. These are associated with loss of the second Pten allele and activation of Akt. Taken together, the genetic and biological data described in this section provide extremely strong validation for the PI 3-kinase pathway as a target for cancer drug development.

**Development of inhibitors of the PI 3-kinase pathway for cancer**

The development of inhibitors of the PI 3-kinase–Akt pathway is eagerly awaited [24]. Rapamycin analogues that inhibit the mTOR (mammalian target of rapamycin) kinase in a non-catalytic fashion are showing activity in animal models and clinical trials [20,25]. Of interest, some degree of selectivity was seen towards PTEN-null compared
With PTEN-negative tumours with rapamycin analogues [25].

To date, inhibitors of the PI 3-kinase family or Akt have not been developed for clinical trials [24]. The first generation of PI 3-kinase inhibitors, in particular the fungal metabolite wortmannin and the flavone-based compound LY294002 have been used widely as pharmacological tools to provide evidence for the involvement of the PI 3-kinase pathway [26,27]. Of note, however, is that these agents are poorly selective, inhibiting not only the lipid PI 3-kinases, but also other members of the PI 3-kinase superfamily and, indeed, other enzymes. For example, kinase profiling has shown that wortmannin potently inhibits myosin light-chain kinase and LY294002 similarly inhibits casein kinase-2 [28]. Furthermore, both agents are known to suffer from problematic pharmaceutical properties, particularly in relation to stability and pharmacokinetics. In addition, although wortmannin is a potent inhibitor, LY294002 is not.

Nevertheless, despite these reservations, wortmannin and LY294002 have been shown not only to inhibit the growth of cancer cells in vitro, but also to exert therapeutic effects in animal tumour models [29,30]. In addition, potentiation of the effects of cytotoxic drugs and ionizing radiation has been seen, although this may occur through inhibition of DNA-PK (DNA-activated protein kinase) and ATM (ataxia telangiectasia mutated), kinases involved in DNA repair that are closely related to the PI 3-kinases [31].

Thus, despite the limitations of wortmannin and LY294002, these compounds have not only proved to be useful as pharmacological reagents, but also they have provided a degree of proof of concept for the anticancer activity of PI 3-kinase inhibitors.

The challenge of isoform-selective PI 3-kinase inhibitors

Given the large numbers of PI 3-kinase family members and their wide range of biological activities, it is clear that isoform-selective inhibitors could potentially have a major impact. Studies with other classes of kinases have clearly shown that selectivity can even be achieved between quite closely related enzymes. Insight into how selectivity might potentially be designed has been provided by the solving of the X-ray co-crystal structures of PI 3-kinase p110γ bound to various inhibitors [32]. The results show that wortmannin, LY294002 and quercetin compete at the ATP-binding site. Wortmannin binds irreversibly and the other two agents bind reversibly without inducing conformational changes, but surprisingly do so in opposite orientations with respect to each other, despite their structural similarity.

Even with the availability of the crystal structure of at least one member of the PI 3-kinase family, together with a detailed knowledge of the general rules of kinase-inhibitor design [33], the discovery of isoform-specific PI 3-kinase inhibitors has remained challenging. However, a number of patents have been published recently that suggest that the ‘Holy Grail’ of PI 3-kinase pharmacology may become attainable (reviewed in [24]). Although further biological data are awaited, promising drug-like compounds that show the potential for isoform-selectivity are now clearly emerging.

In the cancer therapeutic area, the evidence suggests that inhibition of the class IA enzymes p110α and p110β appear to be most promising approaches. The majority of the biological interest in cancer has been focused on the p110α isoform;
however, recent studies using siRNA (small interfering RNA) have shown that knockdown of P110β leads to anticancer effects [34]. However, care must be taken in the interpretation of these experiments since knockdown of one PI 3-kinase species can also have an impact on expression and function of other isoforms.

In a collaboration involving my own laboratory and that of Professor Peter Parker (Cancer Research UK London Research Institute), Professor Mike Waterfield (Ludwig Institute for Cancer Research, University College, London) and the Yamanouchi Pharmaceutical Company, we have identified two series of compounds that inhibit p110α PI 3-kinase with a high degree of selectivity. These inhibitors are the imidazopyridines and the pyridofuropyrimidines (Figure 1; [35,36]). They are now being developed under the auspices of PIramed Ltd.

A series of compounds closely related to LY294002 were disclosed by Thrombogenics to exhibit isoform-selectivity, including quinolones and pyridopyrimidines with 100-fold selectivity for p110α/β versus p110γ (Figure 1; [37]). ICOS Corporation have disclosed quinazolines that show selectivity for p110α versus p110β/γ (Figure 1; [38,39]).

Thus there are now exciting prospects for the development of isoform-selective inhibitors for the treatment of cancer and other diseases. These have potential for maintaining anticancer activity and avoiding possible toxicities associated with certain isoforms.

**Concluding remarks**

PI 3-kinase inhibitors have the potential not only for inhibiting the proliferation of cancer cells and inducing cell cycle arrest, but also for inducing apoptosis and overcoming drug resistance, as well as inhibiting invasion, angiogenesis and metastasis. Thus such drugs would attack several, if not all, of the characteristic ‘hallmark traits’ of the malignant phenotype [40]. The pre-clinical and development of these agents is eagerly awaited.

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**References**


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