

## THE PHOSPHATIDYLINOSITOL 3-KINASE–AKT PATHWAY IN HUMAN CANCER

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One signal that is overactivated in a wide range of tumour types is the production of a phospholipid, phosphatidylinositol (3,4,5) trisphosphate, by phosphatidylinositol 3-kinase (PI3K). This lipid and the protein kinase that is activated by it — AKT — trigger a cascade of responses, from cell growth and proliferation to survival and motility, that drive tumour progression. Small-molecule therapeutics that block PI3K signalling might deal a severe blow to cancer cells by blocking many aspects of the tumour-cell phenotype.

### POLYOMAVIRUS MIDDLE T ANTIGEN

A membrane-bound peptide that is produced during the lytic phase of polyomavirus infections. It helps to drive oncogenic signalling by recruiting a multimolecular signalling complex to the plasma membrane.

### SH2 DOMAIN

(SRC homology 2 domain). A protein motif that recognizes and binds tyrosine-phosphorylated sequences, and thereby has a key role in relaying cascades of signal transduction.

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For the past decade, much of the cancer-research community has focused on the central importance of **RAS** — the first-identified oncogene — in neoplastic transformation. Extensive biochemical and genetic studies of the signalling components upstream and downstream of this small GTPase in model organisms led to the model of mitogenic signalling by receptor tyrosine kinases (RTKs) through RAS and mitogen-activated protein kinases (**MAPKs**). Conserved through evolution from flies to mammals, the central importance of this pathway in neoplastic cell proliferation in humans has been confirmed by the clinical success of therapeutics that target tyrosine kinases, such as trastuzumab (**Herceptin**) and imatinib (**Gleevec**). In recent years, a second pathway downstream of RTKs (sometimes via RAS) that involves phosphatidylinositol 3-kinase (**PI3K**) and **AKT** has come onto the scene and is reaching similar status as an important regulator of mammalian cell proliferation and survival. Several components of the PI3K–AKT pathway are dysregulated in a wide spectrum of human cancers (TABLE 1); gain- or loss-of-function mutants of several components of the pathway lead to neoplastic transformation in model systems, and therapeutic strategies that target the PI3K pathway are now in development. But how was the central importance of this pathway in human cancer established?

### Activation and regulation of PI3K

PI3K first became a focus in the cancer-research field in the mid-1980s, when it became apparent that PI3K activity was physically and functionally associated with the transforming activity of viral oncogenes, such as the **SRC** tyrosine kinase and POLYOMAVIRUS MIDDLE T ANTIGEN<sup>1</sup>. As the molecular details of the story began to unfold, it became clear that PI3Ks were heterodimers with separate regulatory and catalytic subunits, and that the **p85** regulatory subunit of PI3K was a phosphoprotein substrate of many cytoplasmic and receptor tyrosine kinases. p85 is directly associated with many active tyrosine kinases through the physical interaction of its SH2 DOMAIN with phosphotyrosine residues — in the context of a YXXM consensus sequence — on the kinase. In some cases, the p85–RTK interaction is indirect and occurs through intermediate phosphoproteins, such as the INSULIN RECEPTOR SUBSTRATES **IRS1** and **IRS2** (reviewed in REF. 2). With the molecular cloning of the PI3Ks, it has become clear that this is a large and complex family that contains three classes with multiple subunits and isoforms. Class I PI3Ks catalyse the phosphorylation of inositol-containing lipids, known as phosphatidylinositols (PtdIns), at their 3-position (FIG. 1). The primary *in vivo* substrate is PtdIns(4,5)P<sub>2</sub> (hereafter called PIP<sub>2</sub>), which is converted to PtdIns(3,4,5)P<sub>3</sub> (called PIP<sub>3</sub>). The class I PI3Ks consist of two subgroups, IA and IB, which

**INSULIN RECEPTOR SUBSTRATES**  
Adaptor proteins that bind the activated insulin receptor and recruit downstream signalling molecules.

**SH3 DOMAIN**  
(SRC homology 3 domain). A protein sequence of ~50 amino acids that recognizes and binds sequences that are rich in proline.

**BCR-HOMOLOGY DOMAIN**  
(Breakpoint cluster region homology domain). A protein–protein interaction motif that is homologous to a region of the *BCR* gene, which is the fusion partner for the ABL tyrosine kinase in chronic myeloid leukaemia cells.

Summary

- **The phosphatidylinositol 3-kinase (PI3K) pathway regulates various cellular processes, such as proliferation, growth, apoptosis and cytoskeletal rearrangement.**
- **PI3Ks are heterodimeric lipid kinases that are composed of a regulatory and catalytic subunit that are encoded by different genes. The genes that encode the regulatory domains are also subject to differential splicing.**
- **Class IA PI3Ks are activated by receptor tyrosine kinases, and deregulation of their function has been implicated in several human cancers.**
- **One of the main functions of PI3K is to synthesize the second messenger PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>) from PtdIns(4,5)P<sub>2</sub> (PIP<sub>2</sub>).**
- **AKT — a serine/threonine kinase that has a wide range of substrates — is activated by recruitment to the plasma membrane through direct contact of its pleckstrin-homology (PH) domain with PIP<sub>3</sub>, and phosphorylation at Thr308 and Ser473. Thr308 is phosphorylated by the 3-phosphoinositide-dependent protein kinase PDK1, whereas Ser473 is phosphorylated by a molecularly unidentified kinase, often termed PDK2.**
- **AKT acts downstream of PI3K to regulate many biological processes, such as proliferation, apoptosis and growth, but other signalling pathways are also known to be regulated by PI3K activity and might be involved in PI3K-mediated tumorigenesis.**
- **The available clinical evidence of PI3K-pathway deregulation in various cancers and the identification of downstream kinases that are involved in mediating the effects of PI3K (AKT, mTOR, PDK1 and ILK) provide potential targets for the development of small-molecule therapies.**
- **The importance of lipid–protein interaction domains (such as the PH domains of AKT and PDK1) for the activation of PI3K targets provides another potential strategy for developing targeted therapies.**

transmit signals from tyrosine kinases and G-protein-coupled receptors, respectively. Only class IA PI3Ks will be discussed here, because this group is clearly involved in oncogenesis.

The regulatory subunits of class IA PI3Ks are encoded by one of three genes ( $\alpha$ ,  $\beta$  and  $\gamma$ ), which are also subject to alternative splicing. The best-studied example, **p85 $\alpha$** , encodes an adaptor-like protein that has

two **SH2 domains** and an inter-SH2 domain that binds constitutively to the **p110 catalytic subunit**. Two splice variants (p55 $\alpha$  and p50 $\alpha$ ) retain these regions but lack an amino-terminal **SH3 DOMAIN** and a **BCR (BREAKPOINT CLUSTER REGION)-HOMOLOGY DOMAIN**. The **SH3** and **BCR** domains are postulated to have a negative regulatory role towards the catalytic activity of the p110 subunit, which is consistent with the observation that the p55 $\alpha$  and p50 $\alpha$  subunits are more efficient activators of p110 than is p85 $\alpha$ <sup>3–5</sup>. The p110 catalytic subunit is also encoded by three genes ( $\alpha$ ,  $\beta$  and  $\delta$ ), all of which have the same basic structure. This includes distinct domains that are responsible for interaction with p85 and RAS, a **C2 domain** that might be important for membrane anchoring, and a kinase domain.

PI3K catalytic activity is tightly regulated in normal cells by various mechanisms. The current view is that a pre-formed, inactive p85–p110 complex is present in the cytoplasm of resting cells, poised for activation in response to appropriate cues. For RTKs, this cue comes from ligand-mediated activation of kinase activity and transphosphorylation of the RTK cytoplasmic tail, followed by recruitment of the p85–p110 complex to the receptor by interaction of the SH2 domain of p85 with consensus phosphotyrosine residues on the RTK (or with the IRS1/IRS2 signalling intermediate, in some cases). PI3K becomes active for two reasons. First, the p110 catalytic subunit is now in close proximity to its lipid substrates in the cell membrane. Second, the RTK–p85 interaction might relieve an inhibitory effect of p85 on p110 kinase activity<sup>6</sup>, presumably owing to conformational changes in the p85–p110 complex that might involve the SH3 and BCR domains that are mentioned above (FIG. 2). RTKs can also activate PI3K indirectly through RAS, which can bind and activate the p110 subunit<sup>7,8</sup>. This model of PI3K activation can

Table 1 | Evidence of PI3K-signalling deregulation in human malignancies

Cancer type	Type of alteration	References
Glioblastoma	<i>PTEN</i> mutation	133
Ovarian	Allelic imbalance and mutations of <i>PTEN</i> gene	134
	Elevated AKT1 kinase activity	135
	<i>AKT2</i> amplification and overexpression	71
	PI3K <i>p110<math>\alpha</math></i> amplification	70
	PI3K <i>p85<math>\alpha</math></i> mutation	74
Breast	Elevated AKT1 kinase activity	135
	<i>AKT2</i> amplification and overexpression	71
	<i>RSK</i> amplification and overexpression	78,79
	Loss of heterozygosity at <i>PTEN</i> locus	136
	PI3K and <i>AKT2</i> overactivation	137
Endometrial	<i>PTEN</i> mutation	138
	<i>PTEN</i> silencing	139
Hepatocellular carcinoma	<i>PTEN</i> mutation	140
Melanoma	<i>PTEN</i> mutation	141
	<i>PTEN</i> silencing	142
Digestive tract	Aberrant <i>PTEN</i> transcripts	143
	PI3K <i>p85<math>\alpha</math></i> mutation	74
Lung	<i>PTEN</i> inactivation	144
Renal-cell carcinoma	<i>PTEN</i> mutations	145
Thyroid	<i>PTEN</i> mutations	146–148
	AKT overexpression and overactivation	149
Lymphoid	<i>PTEN</i> mutations	150,151
	p85–EPH fusion (only one case reported)	75

EPH, ephrin; PI3K, phosphatidylinositol 3-kinase.

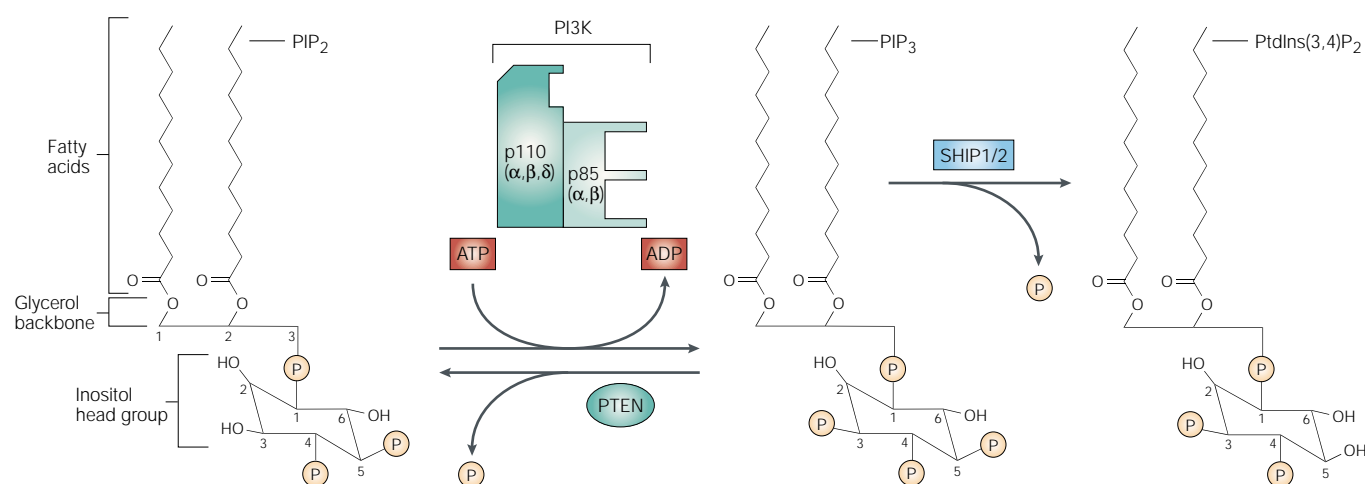


Figure 1 | **Minding your Ps: the PtdIns(4,5)P<sub>2</sub>-PtdIns(3,4,5)P<sub>3</sub> cycle.** Phosphatidylinositol phosphates are composed of a membrane-associated phosphatidic acid group and a glycerol moiety that is linked to a cytosolic phosphorylated inositol head group. Phosphatidylinositol 3-kinase (PI3K) can phosphorylate PtdIns(4,5)P<sub>2</sub> (PIP<sub>2</sub>) at the D3 position to form the second messenger PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>). Phosphorylation at the D3 position is necessary for binding to the pleckstrin-homology domain of AKT (not shown). Dephosphorylation of PIP<sub>3</sub> to regenerate PIP<sub>2</sub> is accomplished by the 3-phosphatase PTEN. Additionally, PIP<sub>3</sub> can be dephosphorylated at the D5 position by SHIP1 or SHIP2 to generate PtdIns(3,4)P<sub>2</sub>, another potential second messenger.

explain much of the current experimental data, but there are many other potential modes of regulation. Precisely how the various isoforms and splice variants of p85 and p110 affect PI3K activity remains to be determined. In addition, we have a limited understanding of how the activated PI3K complex is downregulated. One hypothesis is that tyrosine phosphorylation of p85, which occurs after the p85-p110 complex has been recruited to the active RTK, serves as a negative regulatory signal that leads to a reduction in p110 catalytic activity<sup>9</sup>. A better understanding of these details will undoubtedly provide new insights and opportunities for pharmacological intervention in PI3K-pathway-driven cancers.

#### PIP<sub>3</sub> phosphatases

The primary consequence of PI3K activation is the generation of PIP<sub>3</sub> in the membrane, which functions as a second messenger to activate downstream pathways that involve AKT and other proteins, as described below. PIP<sub>3</sub> levels are barely detectable in mammalian cells under unstimulated growth conditions and are tightly controlled, owing to the combined effects of stringent PI3K regulation and the action of several PIP<sub>3</sub> phosphatases (**PTEN**, **SHIP1** and **SHIP2**) (FIG. 1). The PIP<sub>3</sub> phosphatase that is most clearly involved in oncogenesis is PTEN (also called MMAC1), a 3-position lipid phosphatase that converts PIP<sub>3</sub> back to PIP<sub>2</sub>. This control mechanism is analogous to the regulation of GDP- versus GTP-bound RAS through the opposing effects of guanine nucleotide exchange factors (GEFs; the activators) and GTPase-activating proteins (GAPs; the repressors). **PTEN** was isolated originally as a tumour-suppressor gene in **breast cancer** and glioblastomas using traditional positional-cloning strategies<sup>10,11</sup>, and has subsequently been implicated more broadly in various

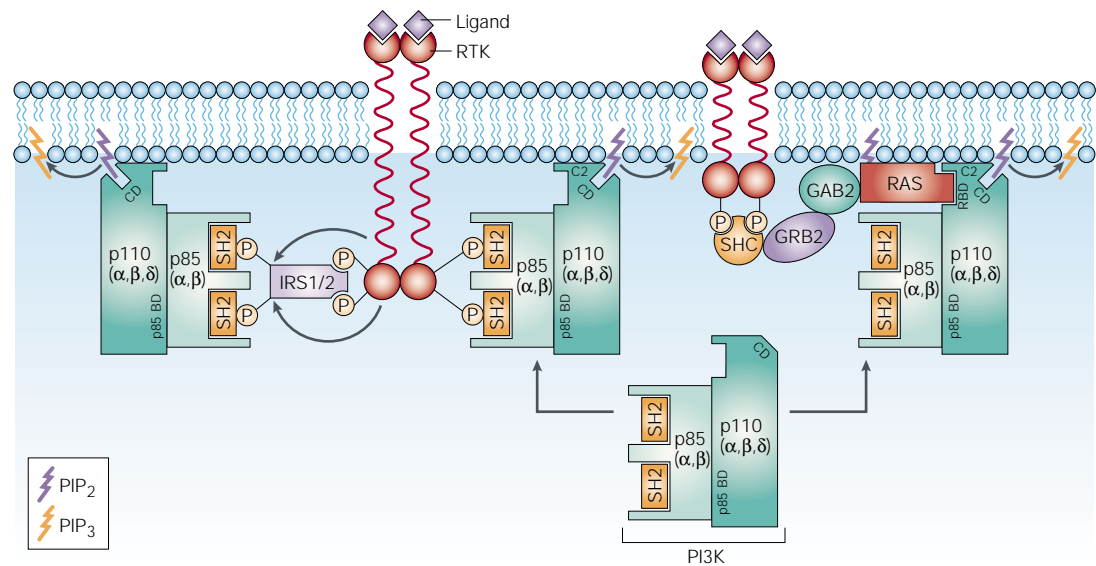
human cancers (see below). Once it became clear that PTEN functions primarily as a PIP<sub>3</sub> lipid phosphatase<sup>12-16</sup>, the central importance of PIP<sub>3</sub> regulation in cancer became indisputable. Although PTEN might also have activity against protein substrates<sup>17,18</sup>, the evidence from mutational studies and from analysis of the hereditary cancer syndrome **COWDEN'S DISEASE** indicates that the PIP<sub>3</sub> phosphatase activity is responsible for the tumour-suppressor function of PTEN (BOX 1).

The SHIP phosphatases also act on PIP<sub>3</sub>, but remove phosphate from the 5-position rather than the 3-position, creating PtdIns(3,4)P<sub>2</sub> (note that PtdIns(3,4)P<sub>2</sub> can also be generated by class II PI3Ks). PtdIns(3,4)P<sub>2</sub> can function as a second messenger (like PIP<sub>3</sub>) to recruit **pleckstrin-homology (PH)-domain**-containing proteins, such as AKT (see below). So, although both PTEN and SHIP reduce the level of PIP<sub>3</sub> in cells, PTEN seems to have primary responsibility for controlling the mitogenic effects of phosphoinositides because it reduces the levels of all those phosphorylated at the D3 position. As expected, knockout mutations in *Pten*, but not *Ship1*, give a strong cancer phenotype in mice. Although useful, this model is likely to be an oversimplification. PIP<sub>2</sub> — the product of the PTEN reaction — might be a second messenger in its own right, as well as being a substrate for several other phosphoinositides that have signalling functions. In addition, *Ship1*-knockout mice can develop myeloproliferative syndromes, indicating that PtdIns(3,4)P<sub>2</sub> can activate certain mitogenic pathways<sup>19-21</sup>.

#### Downstream of PIP<sub>3</sub>: the AKT pathway

Now that the central role of PIP<sub>3</sub> in cancer seems clear, there is renewed emphasis on defining precisely how PIP<sub>3</sub> functions as a second messenger. Much of the recent progress is based on the concept that PIP<sub>3</sub>

**COWDEN'S DISEASE**  
A hereditary predisposition to tumours — especially hamartomas of the skin, mucous membranes, breast and thyroid — that is caused by **PTEN** mutations.



**Figure 2 | Model of PI3K activation.** Autophosphorylation of ligand-activated receptor tyrosine kinases (RTKs) causes recruitment of inactive heterodimeric class IA phosphatidylinositol 3-kinases (PI3Ks) through the interaction of phosphotyrosine residues on the receptor and SRC-homology 2 (SH2) domains on the PI3K p85 regulatory subunit, or the adaptor proteins IRS1 and IRS2. IRS1 and IRS2 are phosphorylated by the activated receptor, generating docking sites for the SH2 domains of p85 and inducing proper assembly of the signalling complex. These SH2–phosphotyrosine interactions bring PI3K in close proximity to its substrate at the plasma membrane and relieve the inhibitory action of p85 on the p110 catalytic subunit, which is then free to convert PtdIns(4,5)P<sub>2</sub> (PIP<sub>2</sub>) into PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>). Alternatively, binding of PI3K to activated RAS can also stabilize its membrane localization and activate the catalytic domain. This occurs by recruitment of the adaptor proteins SHC, GRB2 and GAB2 to activated RTKs. C2, C2 domain; CD, catalytic domain; p85 BD, p85-binding domain; RBD, RAS-binding domain.

functions as a ligand to recruit PH-domain-containing proteins to the inner surface of the cell membrane. PH domains — originally defined on the basis of their presence in the cytoskeletal protein pleckstrin — function as lipid-binding modules in a range of proteins, including several cytoplasmic kinases. Foremost among these in the PIP<sub>3</sub> field is AKT, the cellular homologue of the retroviral oncogene v-Akt, which is also known as protein kinase B (PKB; reviewed in REFS 22,23). Our focus here is to summarize new insights into AKT function, with an emphasis on its links to human cancer. AKT encodes a serine/threonine kinase that has an amino-terminal PH domain, a central catalytic domain and a short carboxy-terminal regulatory domain. There are three members of the AKT family (AKT1, AKT2 and AKT3), which, in general, are broadly expressed, although there are some isoform-specific features. AKT is activated by a dual regulatory mechanism that requires both translocation to the plasma membrane and phosphorylation at Thr308 and Ser473 (REFS 24,25; FIG. 3). The generation of PIP<sub>3</sub> on the inner leaflet of the plasma membrane, following PI3K activation, recruits AKT by direct interaction with its PH domain. At the membrane, another PH-domain-containing serine/threonine kinase named 3-phosphoinositide-dependent protein kinase-1 (PDK1) phosphorylates AKT on Thr308 (REF. 26). Thr308 phosphorylation is necessary and sufficient for AKT activation<sup>27</sup>; however, maximal activation requires additional phosphorylation at Ser473 by PDK2 (REF. 28), a kinase that has been characterized

biochemically but the molecular identity of which remains undetermined. Sequence scanning of the human genome reveals that there are no PDK1 homologues, indicating that PDK2 probably belongs to a different class of kinases. Additional models of AKT activation include autophosphorylation at the PDK2 site and oligomerization that is aided by T-cell leukaemia 1 (TCL1) — the product of an oncogene that is overexpressed in T-cell leukaemias with 14q32-1 translocations<sup>29,30</sup>.

Although the details of AKT activation are fairly clear, there is very little insight into how AKT is down-regulated after activation. So far, no specific AKT phosphatases have been identified; however, treatment of cells with phosphatase inhibitors results in an increase in AKT phosphorylation and activity<sup>31</sup>. AKT can also be inactivated by the recently identified carboxy-terminal modulator protein (CTMP)<sup>32</sup>. CTMP binds AKT, prevents its phosphorylation and blocks downstream signalling. Moreover, overexpression of CTMP can reverse the phenotype of v-Akt-transformed cells. An additional level of AKT regulation is provided by its association with the chaperone protein heat-shock protein 90 (HSP90), which protects AKT from dephosphorylation by the general phosphatase PP2A<sup>33</sup>, thereby preventing its inactivation.

#### Biological effects of AKT activation

The main biological consequences of AKT activation that are relevant to cancer-cell growth can be catalogued loosely into three categories — survival, proliferation (increased cell number) and growth (increased cell size).



## Box 1 | PTEN: a lipid and protein phosphatase

**PTEN (phosphatase and tensin homologue)** is a dual-specificity phosphatase that has activity against lipid and protein substrates. Although PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>) is thought to be the main physiological target of PTEN, inositol 1,3,4,5,6-pentakisphosphate 3-phosphate (IP<sub>5</sub>) has also been reported to be a substrate. PTEN has been shown to dephosphorylate peptide substrates as well, both *in vitro* and *in vivo*. The identification of *PTEN* mutants that have defects in either lipid phosphatase activity, or both lipid and protein phosphatase activities, has made it possible to define the importance of each on different aspects of tumour growth. For example, the C124S mutation inactivates both lipid and protein phosphatase functions. Expression of this mutant in *PTEN*-null cancer cells does not cause growth arrest, indicating that catalytic activity is required for this effect<sup>110</sup>. The G129E mutation in the catalytic domain, which disrupts the lipid-phosphatase activity but does not affect PTEN's ability to dephosphorylate protein targets, is found in patients with Cowden's syndrome, indicating that loss of lipid-phosphatase activity is sufficient to cause the clinical cancer phenotype<sup>12</sup>. The G129E mutant is also defective in causing G1 arrest, indicating that the protein-phosphatase activity is not sufficient to inhibit cell-cycle progression. Additional evidence links the protein-phosphatase activity of PTEN to cell migration. The G129E *PTEN* mutant is sufficient to inhibit cell spreading<sup>18</sup>. PTEN can also reduce phosphorylation of focal adhesion kinase (FAK), which is involved in integrin-induced migration; however, the significance of FAK dephosphorylation by PTEN in tumour development remains controversial.

AKT has additional effects on tumour-induced angiogenesis that is mediated, in part, through hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and vascular endothelial growth factor (VEGF), which will not be covered in detail here owing to space limitations.

**Survival.** Apoptosis, or programmed cell death, is a normal cellular function that controls excessive proliferation by eliminating 'unnecessary' cells. Cancer cells have devised several mechanisms to inhibit apoptosis and prolong their survival. AKT functions in an anti-apoptotic pathway, because DOMINANT-NEGATIVE alleles of AKT block survival that is mediated by insulin-like growth factor 1 (IGF1)<sup>34</sup>, and constitutively active AKT rescues PTEN-mediated apoptosis<sup>35</sup>. The mechanism by which AKT protects cells from death is likely to be multifactorial, because AKT directly phosphorylates several components of the cell-death machinery. For example, BAD is a pro-apoptotic member of the BCL2 FAMILY of proteins that promotes cell death by forming a non-functional heterodimer with the survival factor BCL-X<sub>L</sub>. Phosphorylation of BAD by AKT prevents this interaction<sup>36</sup>, restoring BCL-X<sub>L</sub>'s anti-apoptotic function. Similarly, AKT inhibits the catalytic activity of a pro-death protease, caspase-9, through phosphorylation<sup>37</sup>. Finally, phosphorylation of FKHR — a member of the Forkhead family of transcription factors — by AKT prevents its nuclear translocation and activation of FKHR<sup>38</sup> gene targets, which include several pro-apoptotic proteins such as BIM and FAS ligand.

AKT can also influence cell survival by means of indirect effects on two central regulators of cell death — nuclear factor of  $\kappa$ B (NF- $\kappa$ B)<sup>39,40</sup> and p53 (REFS 41,42). The NF- $\kappa$ B transcription-factor complex promotes survival in response to several apoptotic stimuli. AKT can exert a positive effect on NF- $\kappa$ B function by phosphorylation and activation of I $\kappa$ B kinase (IKK), a kinase that induces degradation of the NF- $\kappa$ B inhibitor, I $\kappa$ B<sup>39</sup>. Degradation

of I $\kappa$ B releases NF- $\kappa$ B from the cytoplasm, allowing nuclear translocation and activation of target genes. AKT can also influence the activity of the pro-apoptotic tumour suppressor p53, through phosphorylation of the p53-binding protein MDM2. MDM2 is a negative regulator of p53 function that targets p53 for degradation by the proteasome through its E3 UBIQUITIN LIGASE activity. This process is regulated, in part, by a negative-feedback loop that controls the level of MDM2 protein, because MDM2 is a transcriptional target gene of p53. Two recent studies provide a new mode of MDM2 regulation through phosphorylation by AKT. Phosphorylated MDM2 translocates more efficiently to the nucleus, where it can bind p53, resulting in enhanced p53 degradation<sup>41,42</sup>. Further interaction between the PI3K pathway and p53 is indicated by the finding that p53 can positively regulate the *PTEN* promoter<sup>43</sup>. Additional genetic studies are required to define the full significance of these various pathways in the AKT survival phenotype.

**Proliferation.** Because most studies of AKT have focused on its role in cell survival, it is often depicted in signalling diagrams as a survival kinase, working in parallel with the well-characterized RAS–MAPK pathway that drives cell proliferation. This artificial division of labour is, however, oversimplified, because AKT can also affect proliferation through signals to the cell-cycle machinery. The cell cycle is regulated by the coordinated action of cyclin–cyclin-dependent kinase (CDK) complexes and CDK inhibitors (CKIs). Cyclin D1 levels, which are important in the G1/S phase transition, are regulated at the transcriptional, post-transcriptional and post-translational level by distinct mechanisms. AKT has an important role in preventing cyclin D1 degradation by regulating the activity of the cyclin D1 kinase glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ). After phosphorylation by GSK3 $\beta$ , cyclin D1 is targeted for degradation by the proteasome. AKT directly phosphorylates GSK3 $\beta$  and blocks its kinase activity, thereby allowing cyclin D1 to accumulate<sup>44</sup>. AKT can also negatively influence the expression of CKIs, such as KIP1 (also known as p27) and WAF1 (also known as CIP1 or p21)<sup>45</sup>. The effects on KIP1 seem to be transcriptional and mediated by FKHR, which represses *CDKN1B* (the gene that encodes KIP1) expression<sup>46,47</sup>. AKT can modulate WAF1 activity by affecting its phosphorylation (presumably through intermediate kinases) and binding to proliferating cell nuclear antigen (PCNA)<sup>48,49</sup>. The functional importance of these biochemical connections between AKT and the cell-cycle machinery are supported by experiments showing that the blockade of PI3K or AKT activity using pharmacological or dominant-negative strategies leads to cell-cycle arrest in certain models<sup>50,51</sup>.

**Cell growth.** In addition to its role in proliferation, there is growing evidence that AKT also affects cell growth. Although these terms might seem synonymous, Schmelzle and Hall have noted that the interchangeable use of growth and proliferation is both confusing and incorrect<sup>52</sup>. Proliferation refers to cell division, which leads to an increase in cell number, whereas growth

## DOMINANT NEGATIVE

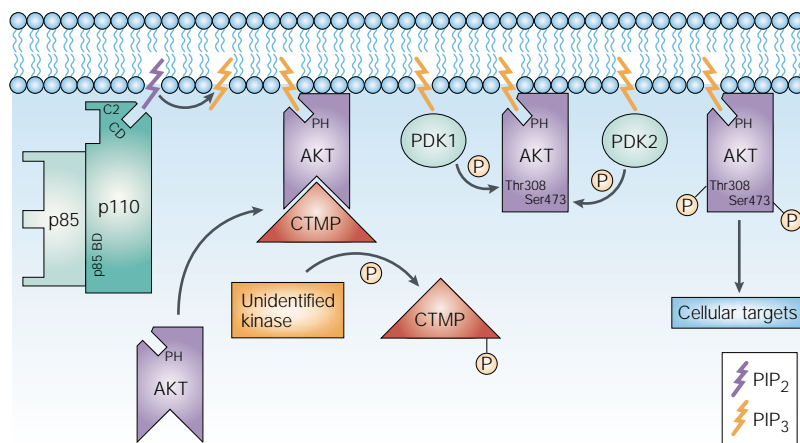
A defective protein that retains interaction capabilities and so distorts or competes with normal proteins.

## BCL2 FAMILY

A family of proteins that determine whether or not a cell commits apoptosis by regulating the exit of cytochrome c from mitochondria. The family comprises both pro-apoptotic and anti-apoptotic members.

## E3 UBIQUITIN LIGASE

The third enzyme in a series — the first two are designated E1 and E2 — that is responsible for ubiquitinating target proteins. E3 enzymes provide platforms for binding E2 enzymes and specific substrates, thereby coordinating ubiquitylation of the selected substrates.



**Figure 3 | Regulation of AKT activity.** Activation of AKT is initiated by membrane translocation, which occurs after cell stimulation and PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>) production. Localization of AKT to the plasma membrane is accomplished by an interaction between its pleckstrin-homology (PH) domain and PIP<sub>3</sub>. At the membrane, association with carboxy-terminal modulator protein (CTMP) prevents AKT from becoming phosphorylated and fully active. Phosphorylation of CTMP by an as yet unidentified kinase releases CTMP from AKT and allows AKT to be phosphorylated by PDK1 and PDK2 at Thr308 and Ser473, respectively. Phosphorylation at these two sites causes full activation of AKT. C2, C2 domain; CD, catalytic domain; p85 BD, p85-binding domain.

refers to the synthesis of macromolecules, which results in increased cell mass or size, a process that is enhanced in cancer cells to meet the biosynthetic requirements that are imposed by the augmented degree of proliferation. One protein that is emerging as a central regulator of cell growth is **mTOR** (the mammalian target of rapamycin, also known as FRAP1), a serine/threonine kinase that serves as a molecular sensor that regulates protein synthesis on the basis of the availability of nutrients. mTOR regulates biogenesis by activating p70 S6 kinase (**RSK**), which enhances the translation of mRNAs that have 5' polypyrimidine tracts, and by inhibiting **4E-BP1** (or PHAS-I) — a translational repressor of mRNAs that bears a 5' CAP structure. mTOR is a direct target of AKT<sup>53</sup>, and its activity can be suppressed by the PI3K inhibitors wortmannin and LY294002 (REF. 54). However, it is still unclear how or whether phosphorylation of mTOR by AKT is a mechanism for activation. Pharmacological studies with the mTOR inhibitor rapamycin indicate that the AKT pathway regulates muscle-cell growth through mTOR. Muscle hypertrophy that is induced by either IGF1 or the expression of a constitutively active form of AKT is reversed by rapamycin treatment<sup>55,56</sup>. However, the PI3K–AKT pathway is unlikely to be the only stimulus that leads to mTOR activation in cancer cells. For example, mTOR can also function as an ATP sensor<sup>57</sup>. In tumours that have increased rates of glycolytic metabolism, mTOR might detect the subsequent rise in ATP level and initiate the signal for increased ribosomal biogenesis that is commonly observed in these cancers. Finally, it is likely that cell growth can also be modulated independently of PI3K and AKT, on the basis of recent biochemical and genetic evidence for a direct link between PDK1 and RSK<sup>58,59</sup>.

**EPISTASIS**

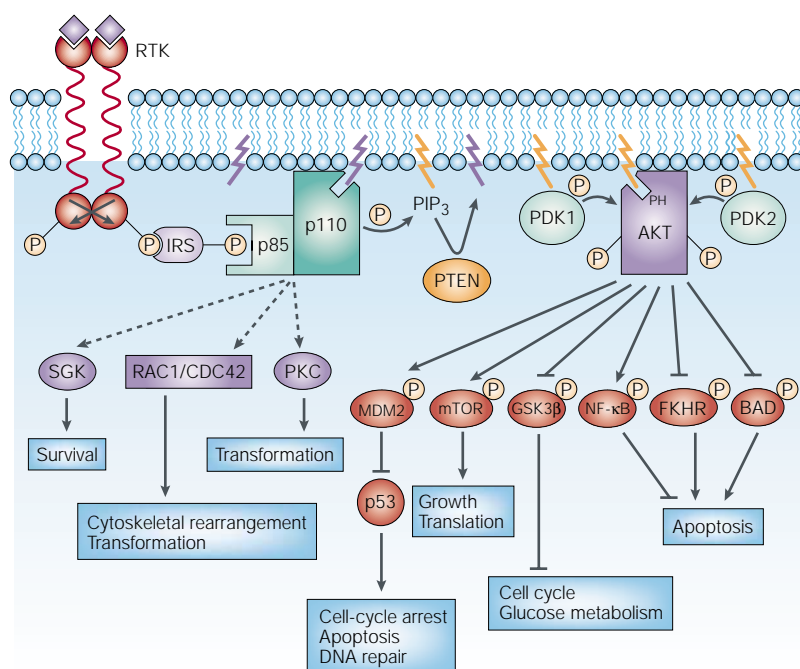
The masking of a phenotype that is caused by a mutation in one gene, by a mutation in another gene. Epistasis analysis can be used to dissect the order in which genes in a genetic pathway act.

**PI3K-dependent, AKT-independent pathways**

As can be surmised from the increasingly popular use of the phrase 'PI3K–AKT pathway' in the scientific literature, there is strong evidence that AKT is a crucial downstream target of PI3K and is likely to be responsible for many of the biological consequences of PI3K activation (FIG. 4). But is AKT the only effector, or do other proteins, in addition to AKT, contribute to the full range of PI3K action? This question is worth consideration for several reasons. First, gain- or loss-of-function mutations in *Pi3k* versus *Akt* give non-overlapping phenotypes in several model systems, including transgenic and knockout mice, indicating that these genes are not purely EPISTATIC (see below). Second, PI3K and PIP<sub>3</sub> can activate a growing list of signalling pathways, many of which have biological properties that are consistent with oncogenesis (FIG. 4). Two of these — activation of the small GTP-binding proteins **CDC42** and **RAC1**, and activation of the serum and glucocorticoid-inducible kinase (**SGK**) — are discussed briefly here.

CDC42 and RAC1 are best known for their role in regulating cytoskeletal movement and cell motility, and can function as oncogenes in fibroblasts when overexpressed. Although they were linked originally to transformation through the RAS pathway, there is growing biochemical and genetic evidence that CDC42 and RAC1 are also regulated by PI3K, independently of AKT<sup>60–62</sup>. For example, cells that have a targeted deletion of *PTEN* show increased CDC42 and RAC1 activity, and this pathway has a functional role in the increased motility that is observed in these cells, perhaps providing a link between *PTEN* loss and tumour invasion<sup>60</sup>. Although the biochemical basis for CDC42/RAC1 activation by PI3K remains to be fully defined, the identification of PIP<sub>3</sub>-sensitive GEFs, such as **VAV1** (REF. 63) and the recently described **PREX1**, provides compelling candidates that might function in this role<sup>64</sup>. PREX1 contains a PH domain and a GEF domain, and is required for reactive oxygen species production (by RAC activation) in neutrophils. Screens that identify novel PIP<sub>3</sub>-binding proteins, such as the recently described phosphoinositide-affinity matrices that identified **ARAP3** (a GAP for the small GTP-binding protein **ARF6**) are likely to shed new light on this area<sup>65</sup>. Although the data from experiments using *Pten*-null cells indicate that CDC42/RAC1 activation is PIP<sub>3</sub>-dependent, there is evidence that association with the p85 regulatory subunit of PI3K is sufficient for CDC42 activation in the absence of catalytic activity<sup>66</sup>.

The SGK family are additional targets of PI3K that have attracted much recent attention because of their high homology to AKT and similar functional effects on survival signalling pathways<sup>67</sup>. The SGKs encode serine/threonine kinases that can be activated by IGF1 (and other stimuli) in a PI3K-dependent manner<sup>68</sup>. However, the mechanism of PI3K-driven SGK activation differs from activation of AKT, because SGKs do not contain a PH domain, which is required for the recruitment of AKT to PIP<sub>3</sub> in the membrane.



**Figure 4 | PI3K signalling: the big picture.** Activation of class IA phosphatidylinositol 3-kinases (PI3Ks) occurs through stimulation of receptor tyrosine kinases (RTKs) and the concomitant assembly of receptor–PI3K complexes. These complexes localize at the membrane where the p110 subunit of PI3K catalyses the conversion of PtdIns(4,5)P<sub>2</sub> (PIP<sub>2</sub>) to PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>). PIP<sub>3</sub> serves as a second messenger that helps to activate AKT. Through phosphorylation, activated AKT mediates the activation and inhibition of several targets, resulting in cellular growth, survival and proliferation through various mechanisms. Additionally, PI3K has been shown to regulate the activity of other cellular targets, such as the serum and glucocorticoid-inducible kinase (SGK), the small GTP-binding proteins RAC1 and CDC42, and protein kinase C (PKC), in an AKT-independent manner through poorly characterized mechanisms. The activity of these targets leads to survival, cytoskeletal rearrangement and transformation. GSK3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; NF- $\kappa$ B, nuclear factor of  $\kappa$ B; PDK1/2, 3-phosphoinositide-dependent protein kinase 1/2.

A full account of the AKT-independent effects of PI3K and their functional significance in cancer will require extensive investigation using genetic models. A recent study in *Drosophila*, by Stocker and colleagues, reports that the phenotype of *Pten* loss in flies (lethality) is rescued by a PH-domain mutant of Akt that lacks the ability to bind PIP<sub>3</sub> (REF 69; see also BOX 2). This result indicates that Akt might be the only important effector of PIP<sub>3</sub>. It remains to be seen if the same is true in mammals.

#### PI3K/AKT deregulation in cancer

As the list of proteins that are involved in the PI3K–AKT pathway grows, the number of these genes that are reported to have structural alterations at the DNA level in human tumours continues to increase (TABLE 1). The gene that encodes the p110 catalytic subunit of PI3K is amplified in some cases of **ovarian cancer**, and amplification of *AKT2* can occur in breast, ovarian and **pancreatic cancers**<sup>70–73</sup>. In both cases, the overexpression of a structurally normal protein is presumed to contribute to transformation, analogous to the amplification of the non-mutant *ERBB2* (also known as *HER2/neu*) RTK in breast cancer. The regulatory p85 subunit of PI3K is also

targeted for mutation in human cancer<sup>74</sup>. A truncated p65 PI3K subunit — isolated originally from a human tumour cell line — causes constitutive activation of PI3K and cell transformation<sup>75</sup>. Some primary human colon and ovarian cancers have mutations in p85 $\alpha$ , which produce deletions in the inter-SH2 region and lead to PI3K activation<sup>74</sup>. These structural alterations presumably release the p85–p110 complex from negative regulation, bypassing the normal role of RTK signalling in PI3K activation. Activating mutations in the RTKs themselves provide additional — although less direct — evidence for the importance of the PI3K–AKT pathway in human cancer. For example, a truncated variant of the epidermal-growth-factor receptor (*EGFR*) that lacks the extracellular domain (*EGFR* viii) potently activates the PI3K–AKT pathway, but not the RAS–MAPK pathway<sup>76</sup>. In addition, downstream effectors of the pathway, such as RSK (S6 kinase), are amplified at the genomic level in breast and ovarian cancer, often in conjunction with the neighbouring gene *ERBB2* (REFS 77–79).

As alluded to earlier, the most compelling evidence for the PI3K–AKT pathway being involved in human cancer comes from studies of the *PTEN* tumour-suppressor gene. Comprehensive surveys of several human cancers for *PTEN* gene deletion or mutations indicate that *PTEN* loss occurs in a wide spectrum of human cancers<sup>80</sup> (TABLE 1). As loss of *PTEN* function can also occur through transcriptional silencing or protein instability<sup>81,82</sup>, it is likely that the frequency of *PTEN* abnormalities in human cancer will rise as additional surveys are conducted using immunohistochemical analysis.

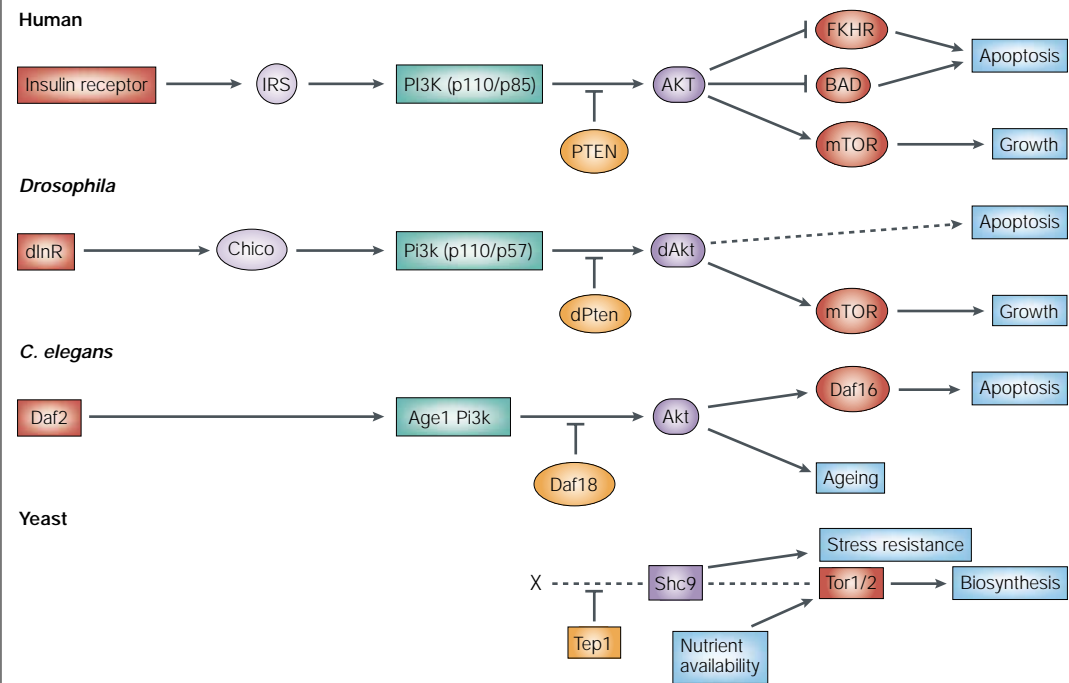
Finally, there might be previously unsuspected connections between known oncogenes and the PI3K–AKT pathway. For example, *TCL1* can bind AKT and promote its constitutive dimerization, phosphorylation and nuclear translocation<sup>30,83</sup>. The functional importance of the AKT pathway in *TCL1* leukaemogenesis remains to be defined, but this observation indicates that the PI3K–AKT pathway might become activated in human tumours by a diverse array of mechanisms. Estimating the true frequency of PI3K–AKT-pathway abnormalities in human cancer will be challenging, particularly in the absence of a full understanding of all the factors that affect pathway activation. Nevertheless, this task is important because the potential application of anticancer drugs that are targeted against the pathway is nearly upon us (see below). One approach might be to measure the activation state of the pathway in large surveys of human tissue using phosphospecific antibodies to key pathway components (such as PIP<sub>3</sub>, AKT, mTOR or RSK), then to determine the molecular basis for that activation later. This approach might also help to develop clinical assays to diagnose PI3K–AKT-pathway activation so that appropriate patients can be selected in clinical trials of drugs that are designed to target this pathway.

#### Gain-of-function mouse models

Finding DNA-based alterations in components of the PI3K–AKT pathway in human tumours provides compelling evidence that this pathway has a causal



Box 2 | Evolutionary conservation of the PI3K–AKT pathway



The importance of using lower organisms for the study of mammalian signalling pathways is beautifully illustrated by the lessons learned from Son of Sevenless (SOS). The discovery of this molecule and its characterization as a mediator of RAS signalling in *Drosophila* led to the characterization of its mammalian counterpart.

**Yeast**

Although class I phosphatidylinositol 3-kinases (PI3Ks) have not been found in yeast, there is some evidence of pathway conservation. Studies in yeast diploid cells that carry a homozygous deletion in the PTEN homologue *Tep1* show enhanced resistance to wortmannin and have a defect in spore-wall formation<sup>111</sup>. Homologues of other members of the PI3K pathway have been described, including two PDK homologues, named *Pkh1* and *Pkh2* (*Pkb*-activating kinase homologues 1 and 2), which are essential for viability and activate human AKT1 *in vitro*<sup>112</sup>, and two *Tor* proteins that regulate biosynthesis<sup>113</sup>. More recently, Fabrizio *et al.* described *Shc9*, an AKT homologue that regulates longevity and stress resistance<sup>114</sup>.

***Caenorhabditis elegans***

In *C. elegans*, the PI3K (or *Age1*) signalling pathway regulates adult longevity and dauer diapause<sup>115</sup> — a larval developmental stage that is induced by unfavourable environmental conditions. The *C. elegans* insulin receptor *Daf2* (REF 116) lies upstream of *Age1* and signals through *Akt1* and *Akt2* in a process that also requires the activity of *Pdk1* (REF 117). The homology of the pathway extends to downstream targets of Akt, such as the forkhead transcription factor *Daf16* (REF 118), and to negative regulators, such as *Daf18*, the *C. elegans* PTEN homologue<sup>119</sup>.

***Drosophila***

*Drosophila* homologues exist for all characterized members of the PI3K pathway, from insulin-like peptides to downstream effectors of Akt<sup>120–123</sup>. Genetic studies have implicated PI3K signalling in cell growth<sup>120,121,123</sup> and proliferation<sup>124,125</sup>. The fly phenotype of loss-of-function mutants in the pathway is a decrease in organ and body size owing to changes in cell size and number. Conversely, mutations in the negative regulator *Pten* cause an increase in cell size and cell proliferation<sup>124</sup>.

role in oncogenesis, but the ultimate proof must come from genetically defined models. Towards this end, several groups have constructed mouse strains that have constitutive activation of the Pi3k–Akt pathway in various tissues, with the expectation that these mice will develop cancer (TABLE 2). The aggregate results are largely in support of this hypothesis, but there are clear differences in the cancer phenotype depending on the genetic manipulation that is used to activate the pathway.

Constitutive activation of Pi3k has been achieved in mice by transgenic expression of an activated form of *p110α* (in the heart) or a truncated allele of *p85α* (in T cells). The *p110α* mice develop large hearts owing to increased cell size (growth) without affecting cell number or apoptosis, whereas the expression of dominant-negative *p110α* gives a small-heart phenotype<sup>84</sup>. Very similar results are observed using constitutively active and dominant-negative alleles of Akt, indicating that Pi3k and Akt are epistatic in



Table 2 | Animal models of the Pi3k–Akt pathway

Approach	Phenotype	References
Cardiac-muscle-specific expression of activated <i>p110</i>	Bigger hearts	84
Cardiac-muscle-specific expression of dominant-negative <i>p110</i>	Smaller hearts	84
Cardiac-muscle-specific expression of constitutively active <i>Akt1</i>	Bigger hearts	85
T-cell-specific expression of activated <i>p85</i> ( <i>p65<sup>PI3k</sup></i> )	Lymphoproliferative disorder with increased memory-T-cell count and reduced apoptosis; lymphomas when crossed with <i>Trp53<sup>-/-</sup></i> mice	86
T-cell-specific expression of <i>gag</i> – <i>Akt</i> fusion (activated Akt)	Increased T-cell survival; increased NF- $\kappa$ B activation	87
$\beta$ -cell-specific expression of myristoylated <i>Akt1</i>	Increased $\beta$ -cell survival, hypertrophy, hyperplasia and hyperinsulinaemia	88,89
Breast-specific expression of myristoylated <i>Akt1</i>	Delay in postpartum mammary-gland involution	90
Targeted deletion of <i>p85<math>\alpha</math></i> regulatory subunit	Insulin hypersensitivity, hypoglycaemia and immunodeficiency	129,152,153
Targeted deletion of <i>p85<math>\alpha</math></i> , <i>p50<math>\alpha</math></i> and <i>p55<math>\alpha</math></i> regulatory subunits	Perinatal lethality	128
Targeted deletion of <i>p110<math>\alpha</math></i> catalytic subunit	Embryonic lethality	101
Targeted deletion of <i>p110<math>\gamma</math></i> catalytic subunit	Impaired T-cell activation and neutrophil migration; colon carcinomas*	126,154–156
Targeted deletion of <i>Akt1</i>	Growth retardation and increased apoptosis	130
Targeted deletion of <i>Akt2</i>	Impaired ability to lower blood glucose	132
Targeted deletion of <i>Pten</i>	Embryonic lethal; heterozygotes develop gonadostromal and germ-line tumours, and cancers of the endometrium, thyroid, prostate, breast, liver and intestine	92–95
Targeted deletion of <i>Ship1</i>	Impaired B-cell development and myeloid hyperplasia	21

\*Only one of the three laboratories who reported this knockout have observed this phenotype. Note that *p110 $\gamma$*  is a class IB PI3K. NF- $\kappa$ B, nuclear factor of  $\kappa$ B; PI3K, phosphatidylinositol 3-kinase.

determining heart size<sup>85</sup>. Mice that express a constitutively active *p85* allele, called *p65<sup>PI3k</sup>*, develop a lymphoproliferative disorder, which progresses to lymphoma when crossed with *Trp53<sup>-/-</sup>* (the gene that encodes p53) mice<sup>86</sup>. The effects of tissue-specific transgenic expression of constitutively active *Akt* alleles have been reported in several different models. T-cell-specific expression gives an increase in T-cell survival, but there is no evidence of malignancy<sup>87</sup>. *Akt* expression in pancreatic islet cells gives a phenotype of hypertrophy, hyperplasia and hyperinsulinaemia, but no islet-cell carcinomas<sup>88,89</sup>. Mice that express *Akt* under the control of the mouse mammary tumour virus long-terminal-repeat promoter (MMTV-LTR) have a delay in postpartum mammary-gland involution, but do not develop mammary tumours. Interestingly, these mice were able to complement a defect in the breast cancer phenotype of MMTV mice that express a mutant allele of SV40 middle T antigen that fails to activate the Pi3k–Akt pathway<sup>90</sup>. One conclusion that is consistent with the data from all these models is that activation of the Pi3k–Akt pathway is insufficient to cause cancer unless combined with an oncogenic lesion in a second pathway. Direct evidence for this hypothesis comes from a study in which retroviral transfer of activated alleles of both *Ras* and *Akt* into glial progenitor cells in the mouse

brain produced glioblastomas, whereas transfer of either gene alone did not<sup>91</sup>.

Constitutive Pi3k–Akt–pathway activation has also been achieved in the mouse by targeted deletion of *Pten*. Homozygous deletion of this gene causes embryonic lethality, indicating a requirement for *Pten* expression during embryonic development. Heterozygous animals are viable, but have a high incidence of T-cell lymphomas, gonadostromal and germ-line tumours, and cancers of the **endometrium, thyroid, prostate, breast, liver** and intestine<sup>92–95</sup>. Analysis of the remaining *Pten* allele in tumours from these mice typically shows loss of function, consistent with the classic KNUDSON'S TWO-HIT MODEL of tumour-suppressor gene function. As expected, these tumours have increased levels of activated Akt. Because of the high frequency and early onset of haematopoietic tumours in these mice, definitive conclusions about the role of PTEN in human tumours (that is, glioblastoma and prostate cancer) must wait until the results from tissue-specific knockouts have been obtained.

When the evidence from mouse models is considered in aggregate, it seems that *Pten* loss produces a more marked cancer phenotype than transgenic expression of either *Pi3k* or *Akt*. Although this observation might provide evidence for Akt-independent signals,

**KNUDSON'S TWO-HIT MODEL**  
In 1971, Alfred Knudson proposed that two successive genetic 'hits', one in each allele of a tumour-suppressor gene, are required to turn a normal cell into a tumour cell, and that one hit was inherited in familial cancers, leading to earlier onset of disease.

## Box 3 | Loss-of-function mouse models

Recent progress in the generation of germ-line knockout alleles of phosphatidylinositol 3-kinase (*Pi3k*) and *Akt* has enhanced our understanding of the role of this pathway in development, and is likely to have implications for the development of drugs that target this pathway. One potential and unexpected conclusion is that the class I PI3Ks might have non-overlapping functions. For example, targeted disruption of *p110 $\alpha$*  results in embryonic lethality, whereas mice deficient for *p110 $\gamma$*  (a class IB PI3K) are viable<sup>101,126,127</sup>. However, the lethal phenotype of the *p110 $\alpha$* -knockout does not rule out non-redundant functions, because *p85* overexpression, which is observed in these mice, could be acting as a dominant-negative for all class Ia PI3Ks. Curiously, one strain of *p110 $\gamma$* -knockout mice developed colon carcinomas, raising the unexpected possibility that this particular *p110* isoform might function as a tumour-suppressor gene<sup>126</sup>. This issue remains controversial, because three other groups have generated *p110 $\gamma$* -knockouts that lack the colon phenotype<sup>127</sup>. Analysis of *p85 $\alpha$* -knockouts indicates another layer of complexity. Complete deletion of *p85 $\alpha$* , and the splice variants *p55 $\alpha$*  and *p50 $\alpha$* , results in perinatal lethality<sup>128</sup>. However, mice that have targeted disruptions that selectively inactivate *p85 $\alpha$* , but not *p55 $\alpha$*  and *p50 $\alpha$* , are viable. These mice have a defect in B-cell development that results in an immunodeficiency syndrome that resembles the knockout phenotype of Bruton's tyrosine kinase<sup>129</sup>. These mice also develop hypoglycaemia and have elevated PIP<sub>3</sub> levels in certain tissues, presumably owing to unrestrained *p55 $\alpha$*  and *p50 $\alpha$*  activity. Although the simplest explanation for this insulin hypersensitivity phenotype is excess PI3K activity in muscle tissue (due to unregulated *p55 $\alpha$* /*p50 $\alpha$*  expression), recent analysis of the perinatal phenotype of the complete *p85 $\alpha$* /*p55 $\alpha$* /*p50 $\alpha$* -knockout indicates that the situation might be more complex. Analysis of *Akt* knockouts indicates a similar degree of complexity, with non-overlapping functions of *Akt1* and *Akt2*. *Akt1*-deficient mice show growth retardation and increased apoptosis<sup>130,131</sup>, whereas *Akt2* knockouts develop hyperglycaemia owing to insulin insensitivity<sup>132</sup>. The *Akt2* phenotype is opposite to that observed in the selective *p85 $\alpha$* -knockout and is consistent with the notion that alterations in PIP<sub>3</sub>-*Akt2* signalling can alter glucose metabolism. These observations might have important implications for the development of PI3k-Akt-pathway inhibitors.

this interpretation must be considered cautiously because many of these studies were conducted in different genetic backgrounds. Definitive conclusions await direct comparisons in isogenic mouse strains. Loss-of-function mouse models in the PI3k-Akt pathway, generated through targeted disruption of the genes that encode different *Pi3k* or *Akt* isoforms (BOX 3), also provide important insights into the role of this pathway in cancer and might be of relevance to the development of targeted therapeutics.

## Translational possibilities

The basic players in the PI3K-AKT pathway have now been defined, and the importance of the pathway in various human cancers is firmly established. These facts should put the issue of developing targeted drugs for the treatment of cancers that have PI3K-AKT pathway dysregulation at the forefront of the translational cancer-research field. Based on the successes that have been seen with small-molecule kinase inhibitors against *BCR-ABL* in **chronic myelogenous leukaemia**, *c-KIT* in **gastrointestinal stromal tumours** and EGFR in lung cancer<sup>96-99</sup>, one obvious approach is to develop kinase inhibitors for PI3K and AKT. Early-generation compounds, such as wortmannin and LY294002, which inhibit the catalytic activity of the *p110* subunit of PI3K, have been widely used *in vitro* for many years, but have not been developed for clinical application.

The reasons for this are not clear, but might reflect unfavourable PHARMACOKINETIC properties in the case of wortmannin, which has a short half-life. Although drug-delivery issues could presumably be solved by intensive efforts in medicinal chemistry, one of the main unanswered questions is whether PI3K inhibition can be achieved with an acceptable therapeutic index. LY294002 administration in mouse tumour models has been shown to confer antitumour activity and to enhance the efficacy of the chemotherapeutic agent **paclitaxel** with relatively modest side effects<sup>100</sup>, but more extensive preclinical or toxicological studies have not been reported. One concern is that first-generation PI3K inhibitors are likely to have broad inhibitory activity against all the *p110* isoforms, as well as more distant PI3K-like kinases, such as *ATM* and *ATR*. A logical strategy to reduce toxicity would be to identify isoform-specific *p110* inhibitors. However, even if such compounds can be isolated, it might turn out that selective inhibition of PI3K is not feasible in the clinic, owing to a narrow (or non-existent) therapeutic index. In support of this view, selective knockout of *p110 $\alpha$*  in mice is lethal during embryogenesis<sup>101</sup> (BOX 3), but it is impossible to predict the toxicity profile of a targeted drug solely on the basis of the knockout phenotype of that target in mice. The recently approved kinase inhibitor Gleevec, which inhibits *ABL*, *c-KIT* and the **platelet-derived growth-factor receptor**, serves as a compelling example. The mouse knockouts of all three of these kinases have severe phenotypes that were not recapitulated in clinical trials of Gleevec<sup>102-104</sup>. Until selective inhibitors of PI3K and/or AKT undergo extensive toxicological evaluation, the question of whether inhibiting the PI3K-AKT pathway can be accomplished safely in humans remains open.

Recent work using rapamycin — an inhibitor of the TOR kinase, which functions downstream of PI3K — offers hope that the PI3K-AKT pathway can be targeted safely, albeit by indirect means. Rapamycin is approved for clinical use as an immunosuppressive agent on the basis of its ability to inhibit T-cell activation and prevent allograft rejection in organ-transplant recipients. Both rapamycin and an ester conjugate, CCI-779, have been shown to have selective antitumour activity against cancers with mutations in *PTEN* and/or upregulation of the PI3K-AKT pathway<sup>105,106</sup>. This activity was observed across a broad panel of human tumour lines with *PTEN* mutations, including glioblastoma, prostate and breast cancer, as well as cells and mice with targeted disruption of *PTEN*. Rapamycin might also have anti-angiogenic activity, by inhibiting endothelial-cell proliferation that is induced by VEGF<sup>107</sup>. Interestingly, VEGF-mediated endothelial-cell proliferation also seems to be AKT dependent<sup>108</sup>.

Working on the assumption that selective targeting of the PI3K-AKT pathway can be achieved with an acceptable therapeutic index, several drug-discovery programmes are actively searching for small-molecule inhibitors of several additional kinases in the pathway. In addition to PI3K and AKT, these include

## PHARMACOKINETICS

The study of the time course of a drug and its metabolites in the body after administration by any route.

PDK1 and integrin-linked kinase (ILK), which might be equally as important in maintaining pathway activation. Although much of the drug-development community is focused on finding ATP-binding-site inhibitors that target the kinases in the pathway, it is important to consider alternative modes for pathway interruption. It might be possible to find small molecules that block the interaction of PIP<sub>3</sub> with the PH domains of effector proteins, such as AKT, thereby preventing downstream propagation of the signal. Furthermore, targeting of AKT could, in theory,

restore p53 function, thereby sensitizing cells to DNA-damaging chemotherapeutics. This effect has already been shown *in vitro*<sup>109</sup>. Another strategy might be to tilt the balance of pathway activation using drugs that prevent the pro-AKT effects of positive regulators such as TCL1, or enhance the anti-AKT effects of negative regulators, such as CTMP. The successes of Gleevec and Herceptin have ushered in a new era of cancer therapeutics. We should soon be in a position to evaluate the clinical success of drugs that are targeted against the PI3K-AKT pathway.

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**Cancer.gov:** [http://www.cancer.gov/cancer\\_information/breast\\_cancer](http://www.cancer.gov/cancer_information/breast_cancer) | chronic myelogenous leukaemia | colon cancer | endometrial cancer | gastrointestinal stromal tumours | liver cancer | ovarian cancer | pancreatic cancer | prostate cancer | thyroid cancer

**LocusLink:** <http://www.ncbi.nlm.nih.gov/LocusLink/> 4E-BP1 | ABL | AKT | AKT1 | AKT2 | AKT3 | ARAP3 | ARF6 | ATM | ATR | BAD | BCL-X<sub>L</sub> | BCR | BIM | caspase-9 | CDC42 | CTMP | cyclin D1 | EGFR | ERBB2 | FAK | FAS ligand | FKHR | GSK3 $\beta$  | HIF-1 $\alpha$  | IGF1 | I $\kappa$ B | IKK | ILK | IRS1 | IRS2 | KIP1 | c-KIT | MAPKs | MDM2 | mTOR | NF- $\kappa$ B | p110 catalytic subunit | p53 | p65 | p85 | p85 $\alpha$  | PCNA | PDK1 | PDK2 | PI3K | platelet-derived growth-factor receptor | PREX1 | PTEN | RAC1 | RAS | RSK | SGK | SHIP1 | SHIP2 | SRC | TCL1 | VAV1 | VEGF | WAF1

**InterPro:** <http://www.ebi.ac.uk/interpro/> C2 domain | pleckstrin-homology domain | SH2 domain | SH3 domain

**Medscape DrugInfo:** <http://promini.medscape.com/drugdb/search.asp> Gleevec | Herceptin | paclitaxel

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