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[1]. 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_2 (hereafter called PIP_2), which is converted to PtdIns(3,4,5)P_3 (called PIP_3). 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)P3 (PIP_3) from PtdIns(4,5)P_2 (PIP_2).
- 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_3, 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 (α, β and γ), which are also subject to alternative splicing. The best-studied example, p85α, 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 (p55α and p50α) 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 p55α and p50α subunits are more efficient activators of p110 than is p85α[3–5]. The p110 catalytic subunit is also encoded by three genes (α, β and δ), 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[6], 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[7,8]. This model of PI3K activation can

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

Cancer type	Type of alteration	References
Glioblastoma	*PTEN* mutation	133
Ovarian	Allelic imbalance and mutations of *PTEN* gene	134
	Elevated AKT1 kinase activity	135
	AKT2 amplification and overexpression	71
	PI3K *p110α* amplification	70
	PI3K *p85α* mutation	74
Breast	Elevated AKT1 kinase activity	135
	AKT2 amplification and overexpression	71
	RSK amplification and overexpression	78,79
	Loss of heterozygosity at *PTEN* locus	136
	PI3K and AKT2 overactivation	137
Endometrial	*PTEN* mutation	138
	PTEN silencing	139
Hepatocellular carcinoma	*PTEN* mutation	140
Melanoma	*PTEN* mutation	141
	PTEN silencing	142
Digestive tract	Aberrant *PTEN* transcripts	143
	PI3K *p85α* mutation	74
Lung	PTEN inactivation	144
Renal-cell carcinoma	*PTEN* mutations	145
Thyroid	*PTEN* mutations	146–148
	AKT overexpression and overactivation	149
Lymphoid	*PTEN* mutations	150,151
	p85–EPH fusion (only one case reported)	75

EPH, ephrin; PI3K, phosphatidylinositol 3-kinase.

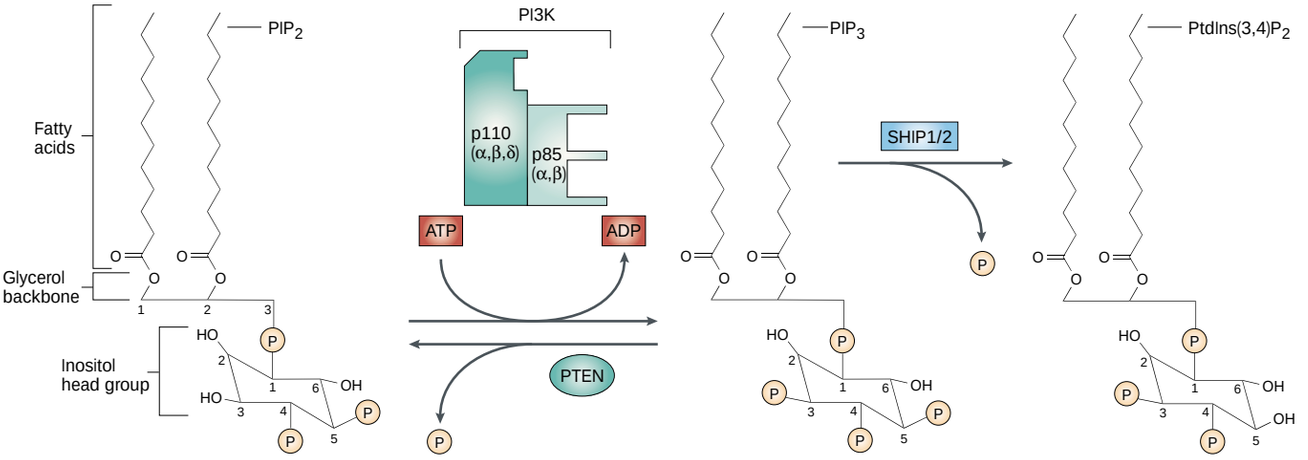


Figure 1 | **Minding your Ps: the PtdIns(4,5)P_2–PtdIns(3,4,5)P_3 cycle.** Phosphatidylinositol phosphates are composed of a membrane-associated phosphatidic acid group and a glycerol moiety that is linked to a cytosolic phosphorylated inositol head group. Phosphatidylinositol 3-kinase (PI3K) can phosphorylate PtdIns(4,5)P_2 (PIP_2) at the D3 position to form the second messenger PtdIns(3,4,5)P_3 (PIP_3). Phosphorylation at the D3 position is necessary for binding to the pleckstrin-homology domain of AKT (not shown). Dephosphorylation of PIP_3 to regenerate PIP_2 is accomplished by the 3-phosphatase PTEN. Additionally, PIP_3 can be dephosphorylated at the D5 position by SHIP1 or SHIP2 to generate PtdIns(3,4)P_2, 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[9]. A better understanding of these details will undoubtedly provide new insights and opportunities for pharmacological intervention in PI3K-pathway-driven cancers.

PIP_3 phosphatases

The primary consequence of PI3K activation is the generation of PIP_3 in the membrane, which functions as a second messenger to activate downstream pathways that involve AKT and other proteins, as described below. PIP_3 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_3 phosphatases (PTEN, SHIP1 and SHIP2) (FIG. 1). The PIP_3 phosphatase that is most clearly involved in oncogenesis is PTEN (also called MMAC1), a 3-position lipid phosphatase that converts PIP_3 back to PIP_2. 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[10,11], and has subsequently been implicated more broadly in various human cancers (see below). Once it became clear that PTEN functions primarily as a PIP_3 lipid phosphatase[12–16], the central importance of PIP_3 regulation in cancer became indisputable. Although PTEN might also have activity against protein substrates[17,18], the evidence from mutational studies and from analysis of the hereditary cancer syndrome COWDEN'S DISEASE indicates that the PIP_3 phosphatase activity is responsible for the tumour-suppressor function of PTEN (BOX 1).

The SHIP phosphatases also act on PIP_3, but remove phosphate from the 5-position rather than the 3-position, creating PtdIns(3,4)P_2 (note that PtdIns(3,4)P_2 can also be generated by class II PI3Ks). PtdIns(3,4)P_2 can function as a second messenger (like PIP_3) to recruit pleckstrin-homology (PH)-domain-containing proteins, such as AKT (see below). So, although both PTEN and SHIP reduce the level of PIP_3 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_2 — the product of the PTEN reaction — might be a second messenger in its own right, as well as being a substrate for several other phosphinositides that have signalling functions. In addition, *Ship1*-knockout mice can develop myeloproliferative syndromes, indicating that PtdIns(3,4)P_2 can activate certain mitogenic pathways[19–21].

Downstream of PIP_3: the AKT pathway

Now that the central role of PIP_3 in cancer seems clear, there is renewed emphasis on defining precisely how PIP_3 functions as a second messenger. Much of the recent progress is based on the concept that PIP_3

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

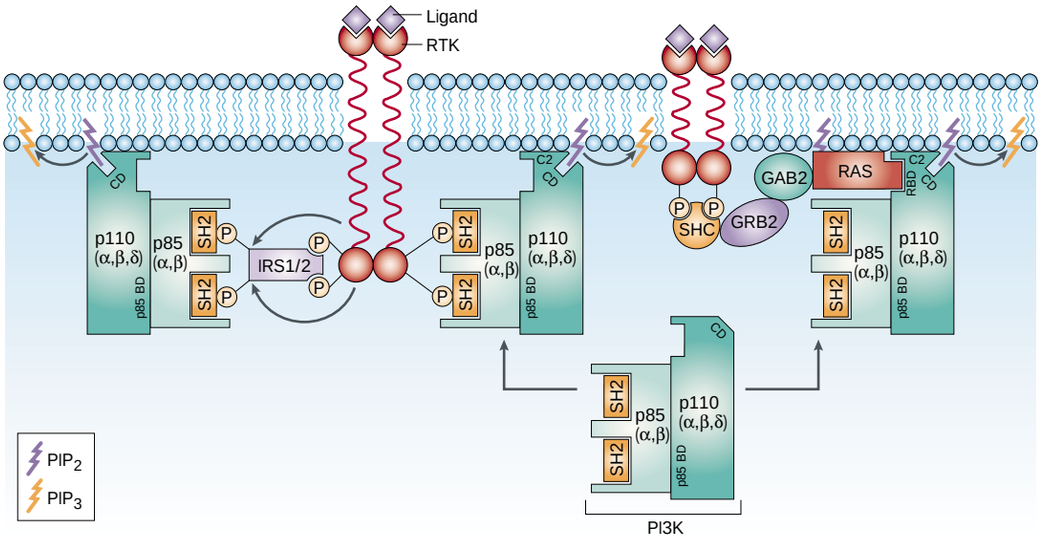


Figure 2 | **Model of PI3K activation.** Autophosphorylation of ligand-activated receptor tyrosine kinases (RTKs) causes recruitment of inactive heterodimeric class IA phosphatidylinositol 3-kinases (PI3Ks) through the interaction of phosphotyrosine residues on the receptor and SRC-homology 2 (SH2) domains 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_2 (PIP_2) into PtdIns(3,4,5)P_3 (PIP_3). 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_3 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_3 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[27]; 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[29,30].

Although the details of AKT activation are fairly clear, there is very little insight into how AKT is downregulated 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[31]. AKT can also be inactivated by the recently identified carboxy-terminal modulator protein (CTMP)[32]. 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[33], 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_3 (PIP_3) is thought to be the main physiological target of PTEN, inositol 1,3,4,5,6-pentakisphosphate 3-phosphate (IP_5) 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[110]. 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[12]. 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[18]. 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-1α (HIF-1α) 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)[34], and constitutively active AKT rescues PTEN-mediated apoptosis[35]. 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_L. Phosphorylation of BAD by AKT prevents this interaction[36], restoring BCL-X_L's anti-apoptotic function. Similarly, AKT inhibits the catalytic activity of a pro-death protease, caspase-9, through phosphorylation[37]. Finally, phosphorylation of FKHR — a member of the Forkhead family of transcription factors — by AKT prevents its nuclear translocation and activation of FKHR[38] 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 κB (NF-κB)[39,40] and p53 (REFS 41,42). The NF-κB transcription-factor complex promotes survival in response to several apoptotic stimuli. AKT can exert a positive effect on NF-κB function by phosphorylation and activation of IκB kinase (IKK), a kinase that induces degradation of the NF-κB inhibitor, IκB[39]. Degradation of IκB releases NF-κB 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[41,42]. Further interaction between the PI3K pathway and p53 is indicated by the finding that p53 can positively regulate the *PTEN* promoter[43]. 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-3β (GSK3β). After phosphorylation by GSK3β, cyclin D1 is targeted for degradation by the proteasome. AKT directly phosphorylates GSK3β and blocks its kinase activity, thereby allowing cyclin D1 to accumulate[44]. AKT can also negatively influence the expression of CKIs, such as KIP1 (also known as p27) and WAF1 (also known as CIP1 or p21)[45]. The effects on KIP1 seem to be transcriptional and mediated by FKHR, which represses *CDKN1B* (the gene that encodes KIP1) expression[46,47]. AKT can modulate WAF1 activity by affecting its phosphorylation (presumably through intermediate kinases) and binding to proliferating cell nuclear antigen (PCNA)[48,49]. 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[50,51].

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[52]. 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 ubiquitylating target proteins. E3 enzymes provide platforms for binding E2 enzymes and specific substrates, thereby coordinating ubiquitylation of the selected substrates.

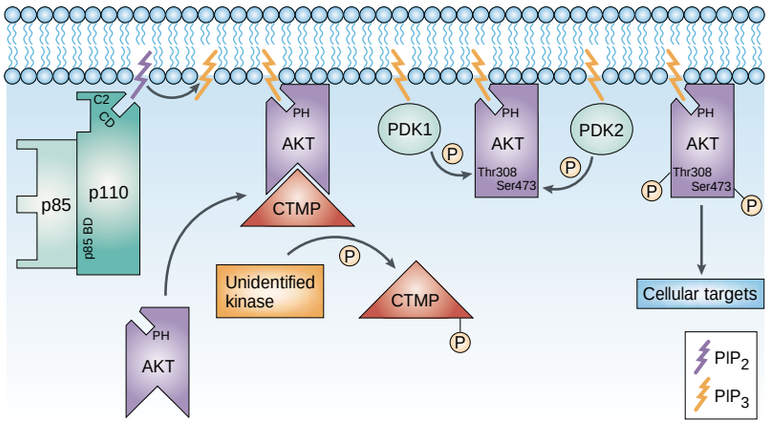


Figure 3 | **Regulation of AKT activity.** Activation of AKT is initiated by membrane translocation, which occurs after cell stimulation and PtdIns(3,4,5)P_3 (PIP_3) production. Localization of AKT to the plasma membrane is accomplished by an interaction between its pleckstrin-homology (PH) domain and PIP_3. 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[53], 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[55,56]. 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[57]. 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[58,59].

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_3 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[60–62]. 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[60]. Although the biochemical basis for CDC42/RAC1 activation by PI3K remains to be fully defined, the identification of PIP_3-sensitive GEFs, such as VAV1 (REF. 63) and the recently described PREX1, provides compelling candidates that might function in this role[64]. 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_3-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[65]. Although the data from experiments using *Pten*-null cells indicate that CDC42/RAC1 activation is PIP_3-dependent, there is evidence that association with the p85 regulatory subunit of PI3K is sufficient for CDC42 activation in the absence of catalytic activity[66].

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[67]. The SGKs encode serine/threonine kinases that can be activated by IGF1 (and other stimuli) in a PI3K-dependent manner[68]. 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_3 in the membrane.

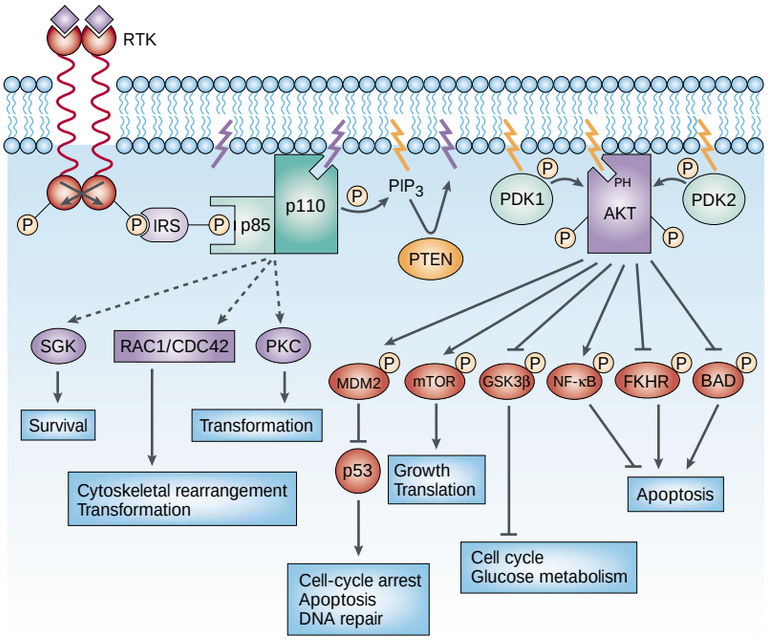


Figure 4 | **PI3K signalling: the big picture.** Activation of class IA phosphatidylinositol 3-kinases (PI3Ks) occurs through stimulation of receptor tyrosine kinases (RTKs) and the concomitant assembly of receptor–PI3K complexes. These complexes localize at the membrane where the p110 subunit of PI3K catalyses the conversion of PtdIns(4,5)P_2 (PIP_2) to PtdIns(3,4,5)P_3 (PIP_3). PIP_3 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. GSK3β, glycogen synthase kinase-3β; NF-κB, nuclear factor of κB; 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_3 (REF. 69; see also BOX 2). This result indicates that Akt might be the only important effector of PIP_3. 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[70–73]. 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[74]. A truncated p65 PI3K subunit — isolated originally from a human tumour cell line — causes constitutive activation of PI3K and cell transformation[75]. Some primary human colon and ovarian cancers have mutations in p85α, which produce deletions in the inter-SH2 region and lead to PI3K activation[74]. 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[76]. 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).

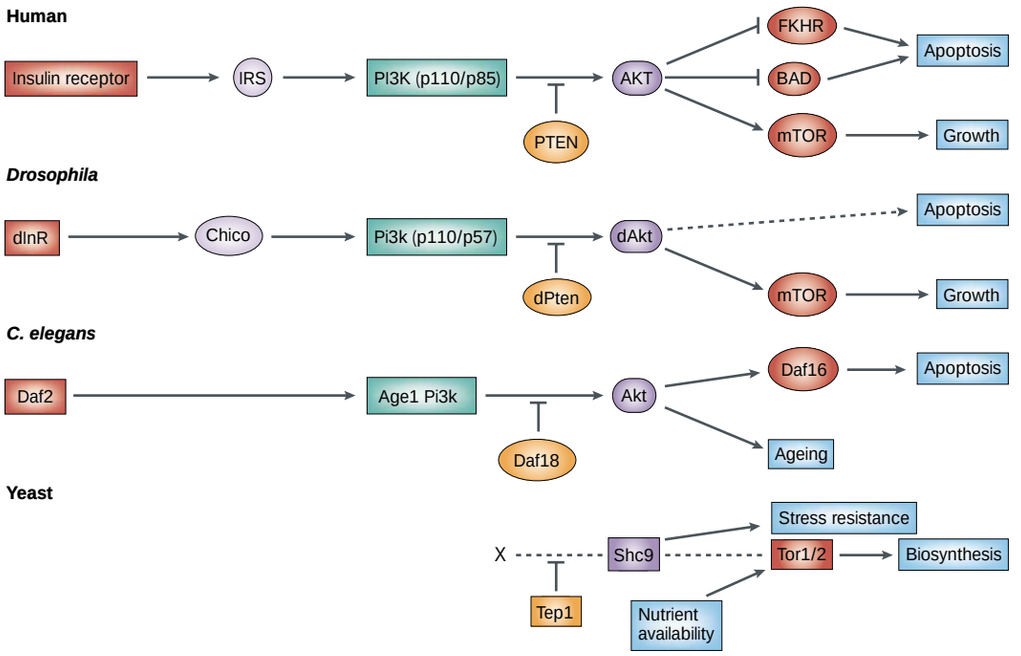
As alluded to earlier, the most compelling evidence for the PI3K–AKT pathway being involved in human cancer comes from studies of the *PTEN* tumour-suppressor gene. Comprehensive surveys of several human cancers for *PTEN* gene deletion or mutations indicate that *PTEN* loss occurs in a wide spectrum of human cancers[80] (TABLE 1). As loss of PTEN function can also occur through transcriptional silencing or protein instability[81,82], 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[30,83]. 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_3, 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[111]. 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*[112], and two Tor proteins that regulate biosynthesis[113]. More recently, Fabrizio *et al.* described Shc9, an AKT homologue that regulates longevity and stress resistance[114].

Caenorhabditis elegans

In *C. elegans*, the PI3K (or Age1) signalling pathway regulates adult longevity and dauer diapause[115] — 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 [119].

Drosophila

Drosophila homologues exist for all characterized members of the PI3K pathway, from insulin-like peptides to downstream effectors of Akt[120–123]. Genetic studies have implicated PI3K signalling in cell growth[120,121,123] and proliferation[124,125]. 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[124].

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[84]. 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 *p110*	Bigger hearts	84
Cardiac-muscle-specific expression of dominant-negative *p110*	Smaller hearts	84
Cardiac-muscle-specific expression of constitutively active *Akt1*	Bigger hearts	85
T-cell-specific expression of activated *p85* (*p65^{Pi3k}*)	Lymphoproliferative disorder with increased memory-T-cell count and reduced apoptosis; lymphomas when crossed with *Trp53$^{-/-}$* mice	86
T-cell-specific expression of *gag–Akt* fusion (activated Akt)	Increased T-cell survival; increased NF-κB activation	87
β-cell-specific expression of myristoylated *Akt1*	Increased β-cell survival, hypertrophy, hyperplasia and hyperinsulinaemia	88,89
Breast-specific expression of myristoylated *Akt1*	Delay in postpartum mammary-gland involution	90
Targeted deletion of *p85α* regulatory subunit	Insulin hypersensitivity, hypoglycaemia and immunodeficiency	129,152,153
Targeted deletion of *p85α*, *p50α* and *p55α* regulatory subunits	Perinatal lethality	128
Targeted deletion of *p110α* catalytic subunit	Embryonic lethality	101
Targeted deletion of *p110γ* catalytic subunit	Impaired T-cell activation and neutrophil migration; colon carcinomas*	126,154–156
Targeted deletion of *Akt1*	Growth retardation and increased apoptosis	130
Targeted deletion of *Akt2*	Impaired ability to lower blood glucose	132
Targeted deletion of *Pten*	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 *Ship1*	Impaired B-cell development and myeloid hyperplasia	21

*Only one of the three laboratories who reported this knockout have observed this phenotype. Note that p110γ is a class IB PI3K. NF-κB, nuclear factor of κB; Pi3k, phosphatidylinositol 3-kinase.

determining heart size[85]. Mice that express a constitutively active *p85* allele, called *p65^{Pi3k}*, develop a lymphoproliferative disorder, which progresses to lymphoma when crossed with *Trp53$^{-/-}$* (the gene that encodes p53) mice[86]. 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[87]. *Akt* expression in pancreatic islet cells gives a phenotype of hypertrophy, hyperplasia and hyperinsulinaemia, but no islet-cell carcinomas[88,89]. 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[90]. 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[91].

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[92–95]. 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 p110α results in embryonic lethality, whereas mice deficient for p110γ (a class IB PI3K) are viable[101,126,127]. However, the lethal phenotype of the *p110α*-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 *p110γ*-knockout mice developed colon carcinomas, raising the unexpected possibility that this particular p110 isoform might function as a tumour-suppressor gene[126]. This issue remains controversial, because three other groups have generated *p110γ*-knockouts that lack the colon phenotype[127]. Analysis of *p85α*-knockouts indicates another layer of complexity. Complete deletion of *p85α*, and the splice variants *p55α* and *p50α*, results in perinatal lethality[128]. However, mice that have targeted disruptions that selectively inactivate p85α, but not p55α and p50α, 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[129]. These mice also develop hypoglycaemia and have elevated PIP_3 levels in certain tissues, presumably owing to unrestrained p55α and p50α activity. Although the simplest explanation for this insulin hypersensitivity phenotype is excess PI3K activity in muscle tissue (due to unregulated p55α/p50α expression), recent analysis of the perinatal phenotype of the complete *p85α/p55α/p50α*-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[130,131], whereas *Akt2* knockouts develop hyperglycaemia owing to insulin insensitivity[132]. The *Akt2* phenotype is opposite to that observed in the selective *p85α*-knockout and is consistent with the notion that alterations in PIP_3–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[96–99], 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[100], 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 *p110α* in mice is lethal during embryogenesis[101] (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[102–104]. 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[105,106]. 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[107]. Interestingly, VEGF-mediated endothelial-cell proliferation also seems to be AKT dependent[108].

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_3 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*[109]. 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.

1. Whitman, M., Kaplan, D. R., Schaffhausen, B., Cantley, L. & Roberts, T. M. Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* **315**, 239–242 (1985).
2. White, M. F. The IRS-signalling system: a network of docking proteins that mediate insulin action. *Mol. Cell. Biochem.* **182**, 3–11 (1998).
3. Inukai, K. *et al.* p85α gene generates three isoforms of regulatory subunit for phosphatidylinositol 3-kinase p50α, p55α, and p85α, with different PI 3-kinase activity elevating responses to insulin. *J. Biol. Chem.* **272**, 7873–7882 (1997).
4. Kaliman, P. *et al.* Insulin-like growth factors require phosphatidylinositol 3-kinase to signal myogenesis: dominant negative p85 expression blocks differentiation of L6E9 muscle cells. *Mol. Endocrinol.* **12**, 66–77 (1998).
5. Ueki, K., Algenstaedt, P., Mauvais-Jarvis, F. & Kahn, C. R. Positive and negative regulation of phosphoinositide 3-kinase-dependent signaling pathways by three different gene products of the p85 α regulatory subunit. *Mol. Cell. Biol.* **20**, 8035–8046 (2000).
6. Yu, J. *et al.* Regulation of the p85/p110 phosphatidylinositol 3′-kinase: stabilization and inhibition of the p110α catalytic subunit by the p85 regulatory subunit. *Mol. Cell. Biol.* **18**, 1379–1387 (1998).
Shows that p85 can both extend the half-life of p110 and inhibit its activity. This inhibitory effect was relieved by the binding of phosphotyrosine peptides to the SH2 domain of p85.
7. Rodriguez-Viciana, P. *et al.* Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* **370**, 527–532 (1994).
Links RAS to the PI3K–AKT pathway.
8. Kodaki, T. *et al.* The activation of phosphatidylinositol 3-kinase by Ras. *Curr. Biol.* **4**, 798–806 (1994).
9. Cuevas, B. D. *et al.* Tyrosine phosphorylation of p85 relieves its inhibitory activity on phosphatidylinositol 3-kinase. *J. Biol. Chem.* **276**, 27455–27461 (2001).
10. Steck, P. A. *et al.* Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nature Genet.* **15**, 356–362 (1997).
11. Li, J. *et al.* PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275**, 1943–1947 (1997).
References 10 and 11 describe the molecular cloning of *PTEN* on the basis of breast cancer and glioma studies, respectively, and reports a high frequency of *PTEN* mutation by various cancer cell lines, xenografts and primary tumours.
12. Myers, M. P. *et al.* The lipid phosphatase activity of PTEN is critical for its tumor supressor function. *Proc. Natl Acad. Sci. USA* **95**, 13513–13518 (1998).
13. Haas-Kogan, D. *et al.* Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor PTEN/MMAC. *Curr. Biol.* **8**, 1195–1198 (1998).
14. Stambolic, V. *et al.* Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* **95**, 29–39 (1998).
Shows that PTEN is a negative regulator of AKT and can reduce intracellular levels of PIP_3 and dephosphorylate PIP_3 *in vitro*.
15. Wu, X., Senechal, K., Neshat, M. S., Whang, Y. E. & Sawyers, C. L. The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. *Proc. Natl Acad. Sci. USA* **95**, 15587–15591 (1998).
16. Maehama, T. & Dixon, J. E. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* **273**, 13375–13378 (1998).
The first report that PTEN has lipid phosphatase actitivty.
17. Gu, J. *et al.* Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. *J. Cell Biol.* **146**, 389–403 (1999).
18. Tamura, M. *et al.* Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science* **280**, 1614–1617 (1998).
19. Scheid, M. P. *et al.* Phosphatidylinositol(3,4,5)P_3 is essential but not sufficient for PKB activation: phosphatidylinositol(3,4)P_2 is required for PKB phosphorylation at Ser473. Studies using cells from *Ship*$^{-/-}$ knockout mice. *J. Biol. Chem.* **7**, 7 (2002).
20. Helgason, C. D. *et al.* A dual role for Src homology 2 domain-containing inositol-5-phosphatase (SHIP) in immunity: aberrant development and enhanced function of B lymphocytes in SHIP$^{-/-}$ mice. *J. Exp. Med.* **191**, 781–794 (2000).
21. Liu, Q. *et al.* SHIP is a negative regulator of growth factor receptor-mediated PKB/AKT activation and myeloid cell survival. *Genes Dev.* **13**, 786–791 (1999).
Shows that Ship-deficient mice have hyperproliferation of myeloid cells, increased survival of neutrophils, and enhanced PIP_3 accumulation and Akt activation upon engagement of certain cytokine receptors.
22. Datta, S. R., Brunet, A. & Greenberg, M. E. Cellular survival: a play in three Akts. *Genes Dev.* **13**, 2905–2927 (1999).
23. Scheid, M. P. & Woodgett, J. R. PKB/AKT: functional insights from genetic models. *Nature Rev. Mol. Cell Biol.* **2**, 760–768 (2001).
24. Andjelkovic, M. *et al.* Role of translocation in the activation and function of protein kinase B. *J. Biol. Chem.* **272**, 31515–31524 (1997).
25. Bellacosa, A. *et al.* Akt activation by growth factors is a multiple-step process: the role of the PH domain. *Oncogene* **17**, 313–325 (1998).
26. Vanhaesebroeck, B. & Alessi, D. R. The PI3K–PDK1 connection: more than just a road to PKB. *Biochem. J.* **346**, 561–576 (2000).
27. Stokoe, D. *et al.* Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. *Science* **277**, 567–570 (1997).
Shows that PIP_3 is necessary for AKT recruitment to the membrane and phosphorylation of AKT on the PDK1 site (Thr308).
28. Alessi, D. R. *et al.* Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase-Bα. *Curr. Biol.* **7**, 261–269 (1997).
29. Toker, A. & Newton, A. C. Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. *J. Biol. Chem.* **275**, 8271–8274 (2000).
30. Laine, J., Kunstle, G., Obata, T., Sha, M. & Noguchi, M. The protooncogene TCL1 is an Akt kinase coactivator. *Mol. Cell* **6**, 395–407 (2000).
31. Andjelkovic, M. *et al.* Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC–PK/PKB) promoted by serum and protein phosphatase inhibitors. *Proc. Natl Acad. Sci. USA* **93**, 5699–5704 (1996).
32. Maira, S. M. *et al.* Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. *Science* **294**, 374–380 (2001).
33. Sato, S., Fujita, N. & Tsuruo, T. Modulation of Akt kinase activity by binding to Hsp90. *Proc. Natl Acad. Sci. USA* **97**, 10832–10837 (2000).
34. Dudek, H. *et al.* Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* **275**, 661–665 (1997).
35. Li, J. *et al.* The PTEN/MMAC1 tumor suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. *Cancer Res.* **58**, 5667–5672 (1998).
36. Datta, S. R. *et al.* Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* **91**, 231–241 (1997).
Shows that growth-factor-induced activation of AKT phosphorylates BAD and inhibits BAD-induced apoptosis in primary neurons.
37. Cardone, M. H. *et al.* Regulation of cell death protease caspase-9 by phosphorylation. *Science* **282**, 1318–1321 (1998).
38. Brunet, A. *et al.* Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96**, 857–868 (1999).
39. Romashkova, J. A. & Makarov, S. S. NF-κB is a target of AKT in anti-apoptotic PDGF signalling. *Nature* **401**, 86–90 (1999).
Shows that PDGF stimulation of fibroblasts causes phosphorylation and activation of IKK by AKT and subsequent activation of NF-κB. This finding links the PI3K–AKT pathway to anti-apoptotic transcription.
40. Kane, L. P., Shapiro, V. S., Stokoe, D. & Weiss, A. Induction of NF-κB by the Akt/PKB kinase. *Curr. Biol.* **9**, 601–604 (1999).
41. Mayo, L. D. & Donner, D. B. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc. Natl Acad. Sci. USA* **98**, 11598–11603 (2001).
Shows that phosphorylation of MDM2 by AKT enhances its nuclear translocation, resulting in destabilization of the p53 protein.
42. Zhou, B. P. *et al.* HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nature Cell Biol.* **3**, 973–982 (2001).
43. Stambolic, V. *et al.* Regulation of *PTEN* transcription by p53. *Mol. Cell* **8**, 317–325 (2001).
44. Diehl, J. A., Cheng, M., Roussel, M. F. & Sherr, C. J. Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev.* **12**, 3499–3511 (1998).
45. Graff, J. R. *et al.* Increased AKT activity contributes to prostate cancer progression by dramatically accelerating prostate tumor growth and diminishing p27Kip1 expression. *J. Biol. Chem.* **275**, 24500–24505 (2000).
46. Dijkers, P. F. *et al.* Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27(KIP1). *Mol. Cell. Biol.* **20**, 9138–9148 (2000).
47. Medema, R. H., Kops, G. J., Bos, J. L. & Burgering, B. M. AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. *Nature* **404**, 782–787 (2000).
48. Lawlor, M. A. & Rotwein, P. Insulin-like growth factor-mediated muscle cell survival: central roles for Akt and cyclin-dependent kinase inhibitor p21. *Mol. Cell. Biol.* **20**, 8983–8995 (2000).
49. Rossig, L. *et al.* Akt-dependent phosphorylation of p21(Cip1) regulates PCNA binding and proliferation of endothelial cells. *Mol. Cell. Biol.* **21**, 5644–5657 (2001).
50. Vemuri, G. S. & Rittenhouse, S. E. Wortmannin inhibits serum-induced activation of phosphoinositide 3-kinase and proliferation of CHRF-288 cells. *Biochem. Biophys. Res. Commun.* **202**, 1619–1623 (1994).
51. Castoria, G. *et al.* PI3-kinase in concert with Src promotes the S-phase entry of oestradiol-stimulated MCF-7 cells. *EMBO J.* **20**, 6050–6059 (2001).
Shows that the induction of PI3K triggers oestrogen-dependent S-phase entry in breast cancer cells.
52. Schmelzle, T. & Hall, M. N. TOR, a central controller of cell growth. *Cell* **103**, 253–262 (2000).

53. Nave, B. T., Ouwens, M., Withers, D. J., Alessi, D. R. & Shepherd, P. R. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem. J.* **344**, 427–431 (1999).
54. Brunn, G. J. *et al.* Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *EMBO J.* **15**, 5256–5267 (1996).
55. Bodine, S. C. *et al.* Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy *in vivo*. *Nature Cell Biol.* **3**, 1014–1019 (2001).
56. Rommel, C. *et al.* Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nature Cell Biol.* **3**, 1009–1013 (2001).
57. Dennis, P. B. *et al.* Mammalian TOR: a homeostatic ATP sensor. *Science* **294**, 1102–1105 (2001).
58. Radimerski, T. *et al.* dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. *Nature Cell Biol.* **4**, 251–255 (2002).
59. Pullen, N. *et al.* Phosphorylation and activation of p70s6k by PDK1. *Science* **279**, 707–710 (1998).
60. Liliental, J. *et al.* Genetic deletion of the *Pten* tumor suppressor gene promotes cell motility by activation of Rac1 and Cdc42 GTPases. *Curr. Biol.* **10**, 401–404 (2000).
61. Jiang, K. *et al.* Pivotal role of phosphoinositide-3 kinase in regulation of cytotoxicity in natural killer cells. *Nature Immunol.* **1**, 419–425 (2000).
62. Welch, H., Eguinoa, A., Stephens, L. R. & Hawkins, P. T. Protein kinase B and Rac are activated in parallel within a phosphatidylinositide 3OH-kinase-controlled signaling pathway. *J. Biol. Chem.* **273**, 11248–11256 (1998).
63. Han, J. *et al.* Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanosine triphosphatases by Vav. *Science* **279**, 558–560 (1998).
Shows that phosphorylation of the RAC-specific guanine nucleotide exchange factor (GEF) VAV1 is enhanced by PIP_3 and inhibited by PIP_2. These findings link a GEF to PI3K-mediated RAC activation.
64. Welch, H. C. *et al.* P-Rex1, a PtdIns(3,4,5)P(3)- and Gβγ-regulated guanine-nucleotide exchange factor for Rac. *Cell* **108**, 809–821 (2002).
65. Krugmann, S. *et al.* Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices. *Mol. Cell* **9**, 95–108 (2002).
66. Jimenez, C. *et al.* Role of the PI3K regulatory subunit in the control of actin organization and cell migration. *J. Cell Biol.* **151**, 249–261 (2000).
67. Brunet, A. *et al.* Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). *Mol. Cell. Biol.* **21**, 952–965 (2001).
68. Park, J. *et al.* Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. *EMBO J.* **18**, 3024–3033 (1999).
69. Stocker, H. *et al.* Living with lethal PIP_3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science* **28**, 28 (2002).
Uses fly genetics to argue that Akt might be the sole effector of PIP_3 action.
70. Shayesteh, L. *et al.* PIK3CA is implicated as an oncogene in ovarian cancer. *Nature Genet.* **21**, 99–102 (1999).
71. Bellacosa, A. *et al.* Molecular alterations of the *AKT2* oncogene in ovarian and breast carcinomas. *Int. J. Cancer* **64**, 280–285 (1995).
72. Cheng, J. Q. *et al.* Amplification of *AKT2* in human pancreatic cells and inhibition of *AKT2* expression and tumorigenicity by antisense RNA. *Proc. Natl Acad. Sci. USA* **93**, 3636–3641 (1996).
73. Ruggeri, B. A., Huang, L., Wood, M., Cheng, J. Q. & Testa, J. R. Amplification and overexpression of the *AKT2* oncogene in a subset of human pancreatic ductal adenocarcinomas. *Mol. Carcinog.* **21**, 81–86 (1998).
74. Philp, A. J. *et al.* The phosphatidylinositol 3′-kinase p85α gene is an oncogene in human ovarian and colon tumors. *Cancer Res.* **61**, 7426–7429 (2001).
75. Jimenez, C. *et al*. Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. *EMBO J.* **17**, 743–753 (1998).
76. Moscatello, D. K., Holgado-Madruga, M., Emlet, D. R., Montgomery, R. B. & Wong, A. J. Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring mutant epidermal growth factor receptor. *J. Biol. Chem.* **273**, 200–206 (1998).
77. Watanabe, T. *et al.* A novel amplification at 17q21-23 in ovarian cancer cell lines detected by comparative genomic hybridization. *Gynecol. Oncol.* **81**, 172–177 (2001).
78. Barlund, M. *et al.* Multiple genes at 17q23 undergo amplification and overexpression in breast cancer. *Cancer Res.* **60**, 5340–5344 (2000).
79. Barlund, M. *et al.* Detecting activation of ribosomal protein S6 kinase by complementary DNA and tissue microarray analysis. *J. Natl Cancer Inst.* **92**, 1252–1259 (2000).
80. Ali, I. U., Schriml, L. M. & Dean, M. Mutational spectra of *PTEN/MMAC1* gene: a tumor suppressor with lipid phosphatase activity. *J. Natl Cancer Inst.* **91**, 1922–1932 (1999).
A thorough review of the frequency of *PTEN* mutation in various cancers.
81. Georgescu, M. M., Kirsch, K. H., Akagi, T., Shishido, T. & Hanafusa, H. The tumor-suppressor activity of PTEN is regulated by its carboxyl-terminal region. *Proc. Natl Acad. Sci. USA* **96**, 10182–10187 (1999).
82. Whang, Y. *et al.* Frequent transcriptional silencing of the tumor suppressor *PTEN/MMAC1* gene in prostate cancer xenografts. *Proc. Natl Acad. Sci. USA* **95**, 5246 (1998).
83. Pekarsky, Y. *et al.* Tcl1 enhances Akt kinase activity and mediates its nuclear translocation. *Proc. Natl Acad. Sci. USA* **97**, 3028–3033 (2000).
84. Shioi, T. *et al.* The conserved phosphoinositide 3-kinase pathway determines heart size in mice. *EMBO J.* **19**, 2537–2548 (2000).
Shows that cardiac-specific expression of constitutively active p110 causes an enlargement of the heart and that dominant-negative p110 causes a reduction in heart size.
85. Shioi, T. *et al.* Akt/protein kinase B promotes organ growth in transgenic mice. *Mol. Cell. Biol.* **22**, 2799–2809 (2002).
86. Borlado, L. R. *et al.* Increased phosphoinositide 3-kinase activity induces a lymphoproliferative disorder and contributes to tumor generation *in vivo*. *FASEB J.* **14**, 895–903 (2000).
87. Jones, R. G. *et al.* Protein kinase B regulates T lymphocyte survival, nuclear factor κB activation, and Bcl-x_L levels *in vivo*. *J. Exp. Med.* **191**, 1721–1734 (2000).
88. Tuttle, R. L. *et al.* Regulation of pancreatic β-cell growth and survival by the serine/threonine protein kinase Akt1/PKBα. *Nature Med.* **7**, 1133–1137 (2001).
89. Bernal-Mizrachi, E., Wen, W., Stahlhut, S., Welling, C. M. & Permutt, M. A. Islet β-cell expression of constitutively active Akt1/PKBα induces striking hypertrophy, hyperplasia, and hyperinsulinemia. *J. Clin. Invest.* **108**, 1631–1638 (2001).
90. Hutchinson, J., Jin, J., Cardiff, R. D., Woodgett, J. R. & Muller, W. J. Activation of Akt (protein kinase B) in mammary epithelium provides a critical cell survival signal required for tumor progression. *Mol. Cell. Biol.* **21**, 2203–2212 (2001).
91. Holland, E. C. *et al.* Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. *Nature Genet.* **25**, 55–57 (2000).
Shows that co-expression of activated Ras and Akt in neural progenitor cells in the mouse brain induces glioblastoma.
92. Stambolic, V. *et al.* High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in *Pten*$^{+/-}$ mice. *Cancer Res.* **60**, 3605–3611 (2000).
93. Suzuki, A. *et al.* High cancer susceptibility and embryonic lethality associated with mutation of the *PTEN* tumor suppressor gene in mice. *Curr. Biol.* **8**, 1169–1178 (1998).
94. Podsypanina, K. *et al.* Mutation of *Pten/Mmac1* in mice causes neoplasia in multiple organ systems. *Proc. Natl Acad. Sci. USA* **96**, 1563–1568 (1999).
95. Di Cristofano, A., Pesce, B., Cordon-Cardo, C. & Pandolfi, P. P. Pten is essential for embryonic development and tumour suppression. *Nature Genet.* **19**, 348–355 (1998).
The first report of a *Pten*-knockout mouse phenotype. Homozygous deletion causes embryonic lethality, whereas heterozygous animals are viable but develop various tumors.
96. Goldman, J. M. & Melo, J. V. Targeting the BCR–ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* **344**, 1084–1086 (2001).
97. Druker, B. J. *et al.* Activity of a specific inhibitor of the BCR–ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N. Engl. J. Med.* **344**, 1038–1042 (2001).
98. van Oosterom, A. T. *et al.* Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* **358**, 1421–1423 (2001).
99. Baselga, J. *et al*. in *2001 AACR-NCI–EORTC International Conference* **128** (American Association for Cancer Research, Fontainebleau Hilton Hotel. Miami Beach, Florida, 2001).
100. Hu, L., Hofmann, J., Lu, Y., Mills, G. B. & Jaffe, R. B. Inhibition of phosphatidylinositol 3′-kinase increases efficacy of paclitaxel in *in vitro* and *in vivo* ovarian cancer models. *Cancer Res.* **62**, 1087–1092 (2002).
Shows that non-toxic doses of LY294002 can increase the efficacy of the chemotherapeutic paclitaxel in an ovarian cancer xenograft model.
101. Bi, L., Okabe, I., Bernard, D. J., Wynshaw-Boris, A. & Nussbaum, R. L. Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110α subunit of phosphoinositide 3-kinase. *J. Biol. Chem.* **274**, 10963–10968 (1999).
Describes the phenotype of the p110α-targeted deletion.
102. Tybulewicz, V. L., Crawford, C. E., Jackson, P. K., Bronson, R. T. & Mulligan, R. C. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-Abl proto-oncogene. *Cell* **65**, 1153–1163 (1991).
103. Reith, A. D. *et al.* W mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-Kit receptor. *Genes Dev.* **4**, 390–400 (1990).
104. Soriano, P. The PDGFα receptor is required for neural crest cell development and for normal patterning of the somites. *Development* **124**, 2691–2700 (1997).
105. Neshat, M. S. *et al.* Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc. Natl Acad. Sci. USA* **98**, 10314–10319 (2001).
Shows that loss of *PTEN* or stable expression of constitutively active *AKT* sensitizes tumours to inhibition of mTOR.
106. Podsypanina, K. *et al.* An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in *Pten*$^{+/-}$ mice. *Proc. Natl Acad. Sci. USA* **98**, 10320–10325 (2001).
Shows that pre-neoplastic uterine lesions fail to develop or regress in *Pten*$^{+/-}$ mice that are treated with an inhibitor of mTor.
107. Guba, M. *et al.* Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nature Med.* **8**, 128–135 (2002).
108. Chan, J. *et al.* Dissection of angiogenic signaling in zebrafish using a chemical genetic approach. *Cancer Cell* **1**, 257–267 (2002).
109. Mayo, L. D., Dixon, J. E., Durden, D. L., Tonks, N. K. & Donner, D. B. PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. *J. Biol. Chem.* **277**, 5484–5489 (2002).
110. Li, D. M. & Sun, H. PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. *Proc. Natl Acad. Sci. USA* **95**, 15406–15411 (1998).
111. Heymont, J. *et al. TEP1*, the yeast homolog of the human tumor suppressor gene *PTEN/MMAC1/TEP1*, is linked to the phosphatidylinositol pathway and plays a role in the developmental process of sporulation. *Proc. Natl Acad. Sci. USA* **97**, 12672–12677 (2000).
112. Casamayor, A., Torrance, P. D., Kobayashi, T., Thorner, J. & Alessi, D. R. Functional counterparts of mammalian protein kinases PDK1 and SGK in budding yeast. *Curr. Biol.* **9**, 186–197 (1999).
113. Cardenas, M. E., Cutler, N. S., Lorenz, M. C., Di Como, C. J. & Heitman, J. The TOR signaling cascade regulates gene expression in response to nutrients. *Genes Dev.* **13**, 3271–3279 (1999).
114. Fabrizio, P., Pozza, F., Pletcher, S. D., Gendron, C. M. & Longo, V. D. Regulation of longevity and stress resistance by Sch9 in yeast. *Science* **292**, 288–290 (2001).
115. Morris, J. Z., Tissenbaum, H. A. & Ruvkun, G. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* **382**, 536–539 (1996).
Shows that mutations in *Age1*, the *C. elegans* Pi3k homologue, causes dauer formation and an extension of lifespan.
116. Kimura, K. D., Tissenbaum, H. A., Liu, Y. & Ruvkun, G. *daf2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**, 942–946 (1997).
117. Paradis, S., Ailion, M., Toker, A., Thomas, J. H. & Ruvkun, G. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. *Genes Dev.* **13**, 1438–1452 (1999).
118. Lin, K., Hsin, H., Libina, N. & Kenyon, C. Regulation of the *Caenorhabditis elegans* longevity protein DAF16 by insulin/IGF1 and germline signaling. *Nature Genet.* **28**, 139–145 (2001).
119. Mihaylova, V. T., Borland, C. Z., Manjarrez, L., Stern, M. J. & Sun, H. The *PTEN* tumor suppressor homolog in *Caenorhabditis elegans* regulates longevity and dauer formation in an insulin receptor-like signaling pathway. *Proc. Natl Acad. Sci. USA* **96**, 7427–7432 (1999).
120. Verdu, J., Buratovich, M. A., Wilder, E. L. & Birnbaum, M. J. Cell-autonomous regulation of cell and organ growth in *Drosophila* by Akt/PKB. *Nature Cell Biol.* **1**, 500–506 (1999).
121. Zhang, H., Stallock, J. P., Ng, J. C., Reinhard, C. & Neufeld, T. P. Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. *Genes Dev.* **14**, 2712–2724 (2000).
122. Montagne, J. *et al. Drosophila* S6 kinase: a regulator of cell size. *Science* **285**, 2126–2129 (1999).
123. Miron, M. *et al.* The translational inhibitor 4E-BP is an effector of PI(3)K/Akt signalling and cell growth in *Drosophila*. *Nature Cell Biol.* **3**, 596–601 (2001).
124. Goberdhan, D. C., Paricio, N., Goodman, E. C., Mlodzik, M. & Wilson, C. *Drosophila* tumor suppressor PTEN controls cell size and number by antagonizing the Chico/PI3-kinase signaling pathway. *Genes Dev.* **13**, 3244–3258 (1999).

Shows that in *Drosophila*, Pten is a negative regulator of Pi3k and mutations in *Pten* result in increased cell size and cell number.
125. Gao, X., Neufeld, T. P. & Pan, D. *Drosophila* PTEN regulates cell growth and proliferation through PI3K-dependent and -independent pathways. *Dev. Biol.* **221**, 404–418 (2000).
126. Sasaki, T. *et al.* Colorectal carcinomas in mice lacking the catalytic subunit of PI(3)Kγ. *Nature* **406**, 897–902 (2000).
127. Barbier, M. *et al.* Tumour biology. Weakening link to colorectal cancer? *Nature* **413**, 796 (2001).
128. Fruman, D. A. *et al.* Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85α. *Nature Genet.* **26**, 379–382 (2000).
Shows that targeted deletion of all isoforms of Pi3k regulatory subunits results in lethality, unlike deletions of individual isoforms.
129. Suzuki, H. *et al.* Xid-like immunodeficiency in mice with disruption of the p85α subunit of phosphoinositide 3-kinase. *Science* **283**, 390–392 (1999).
130. Cho, H., Thorvaldsen, J. L., Chu, Q., Feng, F. & Birnbaum, M. J. Akt1/PKBα is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J. Biol. Chem.* **276**, 38349–38352 (2001).
131. Chen, W. S. *et al.* Growth retardation and increased apoptosis in mice with homozygous disruption of the *Akt1* gene. *Genes Dev.* **15**, 2203–2208 (2001).
Shows that *Akt1*-deficient mice are smaller than wild-type littermates, have a shorter lifespan after exposure to genotoxic stress and show more susceptibility to apoptosis in the testes and thymus.
132. Cho, H. *et al.* Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBβ). *Science* **292**, 1728–1731 (2001).
133. Wang, S. I. *et al.* Somatic mutations of *PTEN* in glioblastoma multiforme. *Cancer Res.* **57**, 4183–4186 (1997).
134. Saito, M. *et al.* Allelic imbalance and mutations of the *PTEN* gene in ovarian cancer. *Int. J. Cancer* **85**, 160–165 (2000).
135. Sun, M. *et al.* AKT1/PKBα kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. *Am. J. Pathol.* **159**, 431–437 (2001).
136. Teng, D. H. *et al. MMAC1*/*PTEN* mutations in primary tumor specimens and tumor cell lines. *Cancer Res.* **57**, 5221–5225 (1997).
137. Sun, M. *et al.* Phosphatidylinositol-3-OH Kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor-α (ERα) via interaction between ERα and PI3K. *Cancer Res.* **61**, 5985–5991 (2001).
138. Yokoyama, Y. *et al.* Expression of *PTEN* and PTEN pseudogene in endometrial carcinoma. *Int. J. Mol. Med.* **6**, 47–50 (2000).
139. Salvesen, H. B. *et al.* PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. *Int. J. Cancer* **91**, 22–26 (2001).
140. Kawamura, N. *et al. PTEN*/*MMAC1* mutations in hepatocellular carcinomas: somatic inactivation of both alleles in tumors. *Jpn. J. Cancer Res.* **90**, 413–418 (1999).
141. Celebi, J. T., Shendrik, I., Silvers, D. N. & Peacocke, M. Identification of *PTEN* mutations in metastatic melanoma specimens. *J. Med. Genet.* **37**, 653–657 (2000).
142. Zhou, X. P. *et al.* Epigenetic *PTEN* silencing in malignant melanomas without *PTEN* mutation. *Am. J. Pathol.* **157**, 1123–1128 (2000).
143. Chang, J. G. *et al.* Mutation analysis of the *PTEN*/*MMAC1* gene in cancers of the digestive tract. *Eur. J. Cancer* **35**, 647–651 (1999).
144. Forgacs, E. *et al.* Mutation analysis of the *PTEN*/*MMAC1* gene in lung cancer. *Oncogene* **17**, 1557–1565 (1998).
145. Alimov, A. *et al.* Somatic mutation and homozygous deletion of *PTEN*/*MMAC1* gene of 10q23 in renal cell carcinoma. *Anticancer Res.* **19**, 3841–3846 (1999).
146. Dahia, P. L. *et al.* Somatic deletions and mutations in the Cowden disease gene, *PTEN*, in sporadic thyroid tumors. *Cancer Res.* **57**, 4710–4713 (1997).
147. Halachmi, N. *et al.* Somatic mutations of the *PTEN* tumor suppressor gene in sporadic follicular thyroid tumors. *Genes Chromosom. Cancer* **23**, 239–243 (1998).
148. Hsieh, M. C. *et al.* Mutation analysis of *PTEN*/*MMAC1* in sporadic thyroid tumors. *Kaohsiung J. Med. Sci.* **16**, 9–12 (2000).
149. Ringel, M. D. *et al.* Overexpression and overactivation of Akt in thyroid carcinoma. *Cancer Res.* **61**, 6105–6111 (2001).
150. Nakahara, Y. *et al.* Mutational analysis of the *PTEN*/*MMAC1* gene in non-Hodgkin's lymphoma. *Leukemia* **12**, 1277–1280 (1998).
151. Sakai, A., Thieblemont, C., Wellmann, A., Jaffe, E. S. & Raffeld, M. *PTEN* gene alterations in lymphoid neoplasms. *Blood* **92**, 3410–3415 (1998).
152. Fruman, D. A. *et al.* Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85α. *Science* **283**, 393–397 (1999).
153. Terauchi, Y. *et al.* Increased insulin sensitivity and hypoglycaemia in mice lacking the p85α subunit of phosphoinositide 3-kinase. *Nature Genet.* **21**, 230–235 (1999).
154. Hirsch, E. *et al.* Central role for G protein-coupled phosphoinositide 3-kinase-γ in inflammation. *Science* **287**, 1049–1053 (2000).
155. Li, Z. *et al.* Roles of PLC-β2 and -β3 and PI3Kγ in chemoattractant-mediated signal transduction. *Science* **287**, 1046–1049 (2000).
156. Sasaki, T. *et al.* Function of PI3Kγ in thymocyte development, T cell activation, and neutrophil migration. *Science* **287**, 1040–1046 (2000).

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