Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis

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Abstract | The tumour-suppressor phosphatase with tensin homology (PTEN) is the most important negative regulator of the cell-survival signalling pathway initiated by phosphatidylinositol 3-kinase (PI3K). Although *PTEN* is mutated or deleted in many tumours, deregulation of the PI3K–PTEN network also occurs through other mechanisms. Crosstalk between the PI3K pathways and other tumorigenic signalling pathways, such as those that involve Ras, p53, TOR (target of rapamycin) or DJ1, can contribute to this deregulation. How does the PI3K pathway integrate signals from numerous sources, and how can this information be used in the rational design of cancer therapies?

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The tumour suppressor *PTEN* (phosphatase with tensin homology, which is deleted on chromosome 10) was originally identified as a gene that is mutated in multiple sporadic tumour types as well as in patients with cancer predisposition syndromes such as Cowden disease. PTEN is a lipid phosphatase that negatively regulates the phosphatidylinositol 3-kinase (PI3K) signalling pathway[1]. The PI3K pathway is an important driver of cell proliferation and cell survival, most notably in cells that are responding to growth-factor–receptor engagement. By opposing the effects of PI3K activation, PTEN functions as a tumour suppressor. So, the PI3K–PTEN signalling network functions as a crucial regulator of cell survival decisions. When *PTEN* is deleted, mutated or otherwise inactivated, activation of PI3K effectors — particularly the activation of the key survival kinase protein kinase B (PKB, also known as AKT) — can occur in the absence of any exogenous stimulus, and tumorigenesis can be initiated. Numerous types of tumours, both sporadic and those that arise as a component of a cancer predisposition syndrome, show alterations in *PTEN*[2].

PTEN mutations — the tip of the iceberg?

When PTEN was first discovered, it seemed likely that *PTEN* mutations would account for most of the cases of PI3K pathway deregulation observed in tumours[3–5]. However, 8 years of analysis have shown some intriguing discontinuities between *PTEN* mutations, the tumour spectrum of Cowden disease[4] and the activation of known downstream PI3K-pathway components such as PKB. For example, spontaneous forms of breast cancer rarely show loss of both *PTEN* alleles, but this tumour type is commonly observed in patients with germline *PTEN* mutations[2,4]. Immunohistochemical staining of breast tumour samples has shown that approximately half contain hyperactive PKB signalling, but as few as 3% contain identifiable *PTEN* mutations[2,4,6]. It has therefore become clear that there must be mechanisms in addition to direct mutation or deletion of *PTEN* by which the PI3K signalling pathway can become constitutively activated. Within the complex environment of an organism, cells are continually integrating a plethora of signals to determine cell fate, survival and proliferation. It is in this light that the PI3K–PTEN pathway can be considered as a central integrator of a tangled web of signalling networks with direct and indirect effects on each other. This integratory role casts the PI3K–PTEN pathway as an important arbiter of cell fate. Recent data supports the idea that crosstalk among signalling pathways contributes to a deregulation of PI3K–PTEN signalling that can lead to tumorigenesis.

Biochemistry of the PI3K pathway

Identifying the component elements of the PI3K–PTEN signalling network and determining how they are regulated will improve our understanding of cancer pathogenesis and lead to the rational development of novel therapeutics. The core PI3K pathway has been defined through both biochemical and genetic experiments (FIG. 1).

At a glance

- The phosphatidylinositol 3-kinase (PI3K)–phosphatase with tensin homology (PTEN) signalling pathway is one of the most commonly altered pathways in human tumours. However, mutations of the *PTEN* gene itself account for only a fraction of these molecular changes.
- The PI3K–PTEN pathway promotes cell survival and proliferation, increases in cell size and chemoresistance. Each of these biological outcomes results from the interaction of this pathway with other signalling networks.
- Ras and its downstream effectors can activate components of the PI3K–PTEN pathway through numerous mechanisms. Each mechanism might be restricted to a particular tumour type, allowing the design of a specific therapy that kills cancer cells but leaves normal tissue unharmed.
- Crosstalk between the PI3K–PTEN and p53 pathways occurs at multiple nodes in these pathways. When both PTEN and p53 are inactivated by mutations, malignancy is promoted in a synergistic manner.
- The Ras, PI3K–PTEN and p53 pathways all converge either directly or indirectly on the tumour suppressor TSC2, indicating a crucial role for this molecule in the integration of multiple signals.
- DJ1 is a novel regulator of the PI3K–PTEN pathway and is associated with breast and lung cancers.
- The multiple pathways that influence the PI3K–PTEN signalling network do so through a variety of mechanisms, providing numerous potential drug targets. Drugs that act on these targets could be formulated to work either synergistically with agents that act directly on PI3K or on elements that function downstream of mutated pathway components. These drugs might offer an attractive additional or alternative approach to combating PI3K-dependent tumours.

In its active form, PI3K consists of a regulatory p85 subunit and a catalytic p110 subunit. When activated by any one of a variety of mechanisms (BOX 1), PI3K activation results in the generation of the second messenger lipid phosphatidylinositol (3,4,5) triphosphate (PIP_3). PIP_3 in turn recruits both phosphatidylinositol-dependent kinase 1 (PDK1) and PKB to the membrane, where PDK1 phosphorylates and activates PKB. There are three highly homologous isoforms of PKB that are transcribed from independent genes and have overlapping but distinct functions[7]. In mice, PKBα (also known as AKT1) mediates signals downstream of PI3K activation that promote cell survival and proliferation. By contrast, PKBβ (also known as AKT2) activation is associated with insulin-mediated metabolic processes[8,9]. *Pkbγ*$^{-/-}$ (also known as *Akt3*) mice have reduced brain size and weight, which might be attributed to reduced cell size and cell number[10]. The net result of the activation of all isoforms of PKB is protection from apoptosis and increased proliferation — events that favour tumorigenesis.

Several direct substrates of PKB phosphorylation have crucial roles in cell-cycle regulation. These substrates include the cell-cycle inhibitor p27 (also known as KIP1), the forkhead box transcription factors (FOXO), glycogen synthase kinase 3 (GSK3), serum- and glucocorticoid-induced kinase 1 (SGK1) and tuberous sclerosis complex 2 (TSC2)[11–13] (FIG. 1). Phosphorylation of p27 by PKB results in p27 inactivation and thereby promotes cell cycle entry. In addition, p27 expression is subject to another level of regulation, which is mediated by FOXO3A. When unphosphorylated, FOXO3A functions as a selective transcription factor in the nucleus, inducing the transcription of the genes that encode p27, the cell-cycle-inhibitor cyclin G2 and the pro-apoptotic molecule BIM[14]. Phosphorylation of FOXO3A by PKB results in expulsion of FOXO3A from the nucleus and, therefore, decreased transcription of the gene that encodes p27. In addition, nuclear exclusion of FOXO3A increases cyclin D1 expression, as unphosphorylated FOXO3A functions as a transcriptional repressor for this gene (among several others)[15]. Interestingly, *Drosophila melanogaster* Foxo and mammalian FOXO1 have been found to transcriptionally regulate expression of the insulin receptor when the nutrient supply is limited. FOXO proteins can act as insulin sensors that allow the rapid activation of the insulin signalling pathway during times of low nutrient levels[16]. FOXO could therefore mediate a key feedback-control mechanism that regulates insulin signalling and metabolism.

Another key molecule inactivated by PKB phosphorylation is TSC2. When unphosphorylated, TSC2 heterodimerizes with TSC1 to promote the GTPase activity of RHEB, a Ras homologue that is highly expressed in brain tissue[17]. Active, GTP-bound RHEB promotes the activity of the kinase TOR (target of rapamycin). TOR functions as a nutrient sensor that integrates PI3K-mediated growth-factor signalling, glucose availability and amino-acid availability[18]. PKB activation inhibits the ability of the TSC1–TSC2 complex to act as a RHEB-GTPase activating protein (RHEB-GAP), which therefore increases the amount of RHEB-GTP present and consequently activates TOR.

Activated TOR exists in two complexes. The rapamycin-sensitive TOR complex contains raptor (regulatory associated protein of TOR) and GβL (G-protein β-subunit-like), and phosphorylates S6 kinase (S6K) and the EIF4E (eukaryotic translation-initiation factor 4E)-inhibitory binding protein 4EBP[19–22]. Phosphorylated S6K is active, and might affect protein translation and cell size, although the mechanisms remain controversial. In both mice and *D. melanogaster*, deficiency for *s6k* results in decreased cell size[23,24]. The second, rapamycin-insensitive TOR complex contains rictor (rapamycin-insensitive companion of TOR) and mediates signals to the cytoskeleton[25–27]. The rictor-containing TOR complex can also phosphorylate and activate PKB[28].

PI3K, PTEN and the TSC1–TSC2–TOR axis

The signalling axis that involves the TSC1–TSC2 complex and TOR (TSC1–TSC2–TOR) has become a focal point in studies of PI3K-mediated tumorigenesis for two reasons. First of all, mutations in either *TSC1* or *TSC2* lead to tuberous sclerosis, a hamartoma syndrome associated with a predisposition to malignancy[17]. Notably, tuberous sclerosis hamartomas generally display loss of heterozygosity (LOH) at the mutant locus[29]. Secondly, there is indirect evidence derived from studies of TOR inhibition that a decrease in TOR activity prevents tumour development in both humans and mice. For example, chemical TOR inhibitors such as the rapamycin derivatives CCI-779, RAD001 and AP23573 seem to have anti-tumour activity for a wide range of malignancies, including

Hamartoma
A benign growth.

Loss of heterozygosity
The loss of the remaining normal allele when one allele is already lost or mutated.

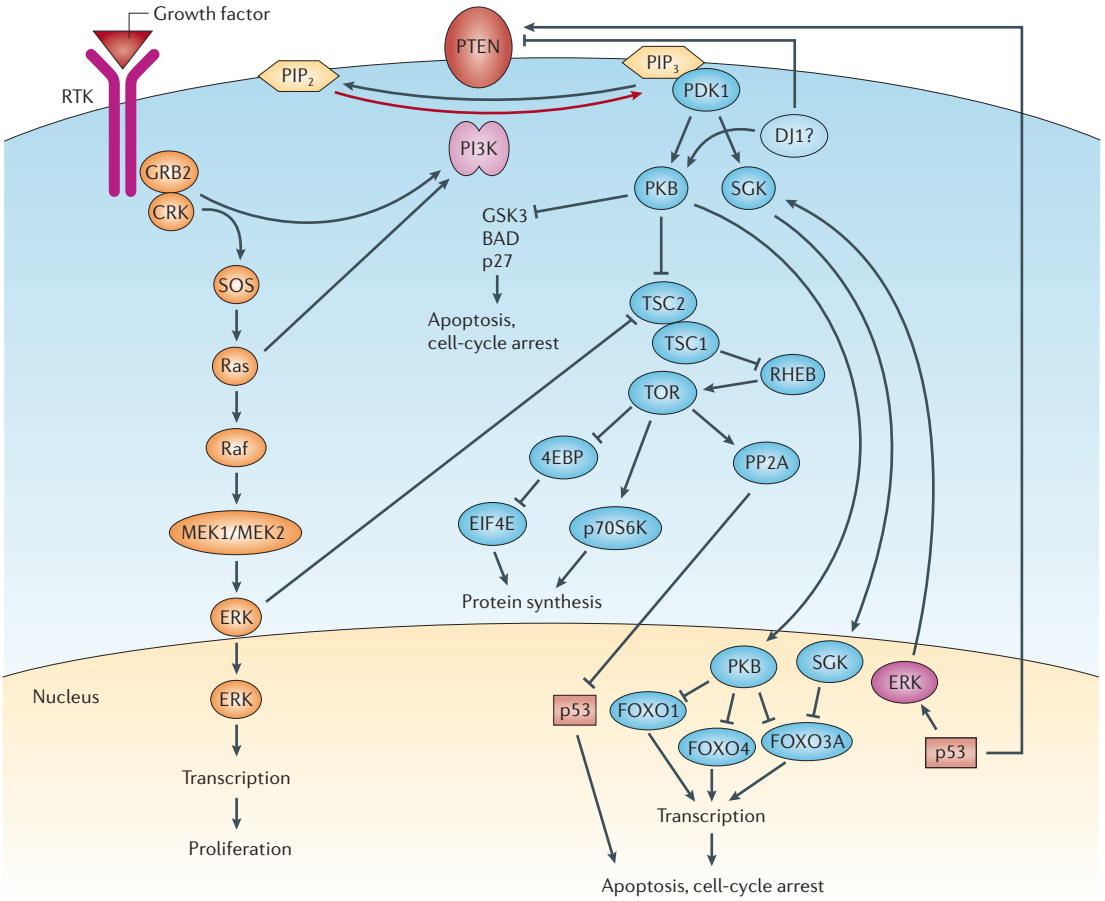

Figure 1 | **The PI3K–PTEN signalling network.** The core phosphatidylinositol 3-kinase (PI3K) signalling pathway (indicated by blue symbols) begins with PI3K activation (indicated by pink symbol) by receptor tyrosine kinases (RTKs) (BOX 1). PI3K activity phosphorylates and converts the lipid second messenger phosphatidylinositol (4,5) bisphosphate (PIP_2) into phosphatidylinositol (3,4,5) triphosphate (PIP_3), which recruits and activates phosphatidylinositol-dependent kinase 1 (PDK1). PDK1 in turn phosphorylates and activates protein kinase B (PKB, also known as AKT), which inhibits the activities of the forkhead (FOXO) transcription factors (which are mediators of apoptosis and cell-cycle arrest), resulting in cell proliferation and survival. The tumour-suppressor phosphatase with tensin homology (PTEN) negatively regulates PI3K signalling by dephosphorylating PIP_3, converting it back to PIP_2. The Ras signalling pathway (orange symbols) can be triggered by a set of RTKs that are activated by growth factors. Ras can activate PI3K both directly and indirectly, as described in BOX 1. The activation status of p53 can also affect the outcome of PI3K signalling by interacting with the PKB-regulated FOXO transcription factors and with extracellular-regulated kinase 1 (ERK1) and ERK2. Other members of the PI3K signalling pathway include SGK (serum- and glucocorticoid-induced kinase), TSC1/TSC2 (tuberous sclerosis 1 and 2), RHEB (Ras homologue enriched in brain), TOR (target of rapamycin), 4EBP (eukaryotic initiation factor 4E (EIF4E)-binding protein), p70S6K (ribosomal protein, S6 kinase 70kD), and PP2A (protein phosphatase 2A). Members of the RTK–Ras signalling pathway include GRB2 (growth factor receptor-bound protein 2), SOS, Ras, Raf, MEK (mitogen-activated ERK kinase) and ERK — activation of this pathway leads to cell proliferation.

non-small-cell lung cancer, breast cancer, renal-cell carcinoma, anaplastic astrocytoma, mesothelioma, soft-tissue sarcoma, cervical cancer and uterine cancer[30]. In particular, CCI-779 has proven to be of clinical benefit to patients with breast or renal carcinomas[31,32]. In *Pten*$^{+/-}$ mice, phaeochromocytomas and endometrial tumours are rendered cytostatic by CCI-779 treatment[33] (TABLE 1). Also, overexpression of PKB in a murine lymphoma model induces rapamycin-sensitive tumorigenesis and drug resistance that can be phenocopied by overexpression of EIF4E[34]. So, it seems that the maintenance of tumours that harbour hyperactive PI3K–PKB signalling requires intact TOR signalling.

The mechanism by which TOR activation drives carcinogenesis remains elusive. Because rapamycin can inhibit the formation of PKB-dependent cancers, targets of the TOR–raptor complex must mediate at least some of the observed tumorigenic effects. It is currently unclear precisely how the TOR–raptor-mediated phosphorylation of S6K and 4EBP promotes tumour formation. Although a correlation exists between increased translation and tumorigenesis, whether increased translation is either necessary or sufficient for increased cancer susceptibility has yet to be determined[35]. Correlative evidence also indicates that increased cell size might be involved in tumour formation, as brain lesions that arise in patients

Phaeochromocytoma
Adrenal gland tumour.

with tuberous sclerosis often contain abnormally large cells[36]. However, a definitive demonstration of causality here is also lacking. The generation and analysis of mice that are deficient in *S6k* or *Eif4ebp* and heterozygous for *Tsc1*, *Tsc2* or *Pten* would establish whether tumorigenic PI3K–TOR signalling acts through these molecules. Interestingly, the rapamycin analogues used in the tumour suppression studies should only affect the raptor-containing complex. The efficacy of TOR inhibitors might be substantially improved by the concomitant inhibition of PKB through pharmacological inhibition of either PKB itself, its upstream activators, or the TOR–rictor complex.

Between PI3K and TOR lies the TSC1–TSC2 complex on which numerous signalling pathways converge. TSC2 is phosphorylated not only by PKB, but also by extracellular-regulated kinase (ERK), both of which function downstream of Ras[37] (FIG. 1). ERK-mediated phosphorylation occurs at a site that is distinct from that phosphorylated by PKB. Nevertheless, TSC2 phosphorylation by either PKB or ERK disrupts the GAP activity of the TSC1–TSC2 complex. Interestingly, increased TOR activity is found in tumours from patients with loss-of-function mutations in the Ras-GAP NF1, and TOR is required to sustain proliferation in these cells[38]. Mutations in *NF1* increase both the magnitude and duration of Ras activation, and patients with germline *NF1* mutations have the tumour predisposition syndrome neurofibromatosis type I (REF. 39). These observations indicate that the PKB–TSC2–TOR pathway might have a crucial role not only in PI3K-dependent tumours, but also in Ras-dependent malignancies.

To what extent are tumours with mutations in Ras or PI3K dependent on TSC1–TSC2 signalling for their expansion and/or maintenance? Unexpectedly, expression of a mutated form of TSC2 that lacks all putative PKB phosphorylation sites (and therefore cannot be inhibited by PI3K signalling), or a mutant form of TSC2 that contains phospho-mimetic residues were both able to rescue the cell size and lethality defects of *D. melanogaster gigas* (the homologue of *TSC2*) mutants[40]. The contribution of the ERK phosphorylation site was not examined in this situation, and the role of this site in development remains undetermined. During tumorigenesis, however, the loss of one *Tsc2* allele promotes tumorigenesis in *Pten*$^{+/-}$ mice[41,42]. As is detailed below, Ras can activate PI3K (and therefore PKB) as well as ERK1/ERK2. So, abnormalities in the Ras signalling pathway could affect TSC2 regulation through both PKB and ERK1/ERK2. Experiments using knock-in mice in which different TSC2 phosphorylation residues are altered will help to dissect the *in vivo* contribution of deregulated PKB and/or ERK to TSC2-associated tumour formation.

Between TSC1–TSC2 and TOR lies the Ras family GTPase RHEB. Many other Ras family GTPases, including Ras, Rac and Rho, act on more than one downstream effector molecule. Accordingly, tumours that are characterized by abnormal Ras signalling often show the simultaneous activation of multiple pathways. The same might be true for RHEB, and the future discovery of RHEB effectors other than TOR might shed light on the mechanism by which aberrant RHEB activation causes tumours. Interestingly, neither mutations in TOR nor its downstream effectors

Box 1 | **Mechanisms of PI3K activation**

Phosphatidylinositol 3-kinase (PI3K) can become activated by at least three independent pathways, all of which start with binding of ligand to receptor tyrosine kinases (RTKs). This causes RTKs to dimerize and undergo autophosphorylation (P) at tyrosine residues, which allows them to interact with Src homology 2 (SH2)-domain-containing molecules[47,82]. Specificity in binding between each specific phosphotyrosine residue and its cognate SH2-domain-containing protein is achieved through residues that surround the phosphotyrosine[83]. In one PI3K activation pathway (see left side of diagram), the 85 kDa regulatory subunit of PI3K (p85) binds directly to phospho-YXXM motifs (in which X indicates any amino-acid) within the RTK[84], triggering activation of PI3K's 110 kDa catalytic subunit (p110). Other PI3K signalling pathways depend on the adaptor protein GRB2 (growth factor receptor-bound protein 2, red horseshoe-shaped symbol), which binds preferentially to phospho-YXN motifs of the RTK[85]. In the middle pathway of the diagram, GRB2 binds to the scaffolding protein GAB (GRB2-associated binding protein), which in turn can bind to p85. GRB2 also activates Ras (through the activation of SOS), and Ras activates p110 independently of p85. GRB2 can also exist in a large complex that contains both SOS, Ras, and GAB or other scaffolding proteins, bringing these activators into close proximity with p110 PI3K[86]. It is not clear precisely which of these pathways predominates in different physiological situations. There is evidence that Ras has to function in concert with phosphotyrosine-bound p85 to activate p110. For example, HRAS promotes the catalytic activity of PI3K only when p85 is bound to phosphorylated tyrosine residues[87]. So, Ras-mediated PI3K activation might require two steps: the phosphorylation of YXXM motifs, and the activation of small GTPases. Experiments that directly address the in vivo relevance of Ras–PI3K interactions will further elucidate the importance of Ras-mediated PI3K activation in normal cell signalling and tumorigenesis. PKB, protein kinase B.

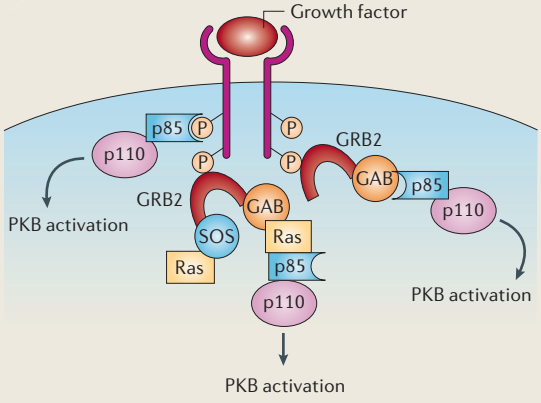

Table 1 | **Tumour development in *Pten*$^{+/-}$ mice**

Gene or drug	Tumour tissue site	Characterization of tumour phenotype	References
Pten$^{+/-}$ mice alone	Lymph nodes, endometrium, adrenal gland, prostate and breast	Tumours are associated with loss of heterozygosity of the *Pten* locus	94
CCI-779 (TOR inhibitor)	Adrenal gland and endometrium	Cytostatic, decreased proliferation	33
Cdkn1b$^{-/-}$	Prostate	*Cdkn1b*$^{-/-}$*Pten*$^{+/-}$ mice develop carcinomas with complete penetrance, whereas *Pten*$^{+/-}$ mice develop hyperplasias with ~50% penetrance	95
Cdkn2a$^{-/-}$	Adrenal gland, prostate and endometrium	Decreased latency; altered tumour spectrum — *Cdkn2a*$^{-/-}$*Pten*$^{+/-}$ mice develop melanomas and squamous cell carcinomas	96
Trp53$^{+/-}$	Lymphoma (spontaneous or radiation-induced)	Increased penetrance of tumour phenotype	97,98
Trp53$^{-/-}$	Prostate	Increased invasiveness of tumours	75
Nkx3.1$^{+/-}$	Prostate	Increased metastasis, high grade prostatic intraepithelial neoplasia	99
Truncated SV40 large-T-antigen transgenic	Astrocytes	Transgenic expression of SV40 large-T antigen inactivates pRB, p107 and p130 and induces astrocytomas; astrocytoma frequency is increased in *Pten*$^{+/-}$ mice	100
Tsc2$^{+/-}$	Prostate and lymph node	Increased invasion (prostate) and penetrance (lymph node hyperplasia)	41,42
Mlh1$^{-/-}$ (involved in DNA mismatch repair)	Endometrium	Accelerated tumour formation	101
Grb2$^{+/-}$	All	No change in tumour formation	102
TACC1 transgenic	Mammary gland	Accelerated tumour formation in *Pten*$^{+/-}$ mice and in a model of breast cancer based on a mutant form of polyoma middle-T antigen	103
Dimethylbenzoic acid	Skin carcinogenesis	Accelerated tumour formation; decreased frequency of Ras^{V12} mutations	55

Cdkn, cyclin-dependent kinase inhibitor; *Grb2*, growth factor receptor-bound protein 2; *Nkx3.1*, mouse homologue of NK homeobox, family 3, member A; *Pten*, phosphatase with tensin homology; *TACC1*, transforming, acidic coiled-coil containing protein 1; TOR, target of rapamycin; *Tsc2*, tuberous sclerosis 2.

S6K and 4EBP have been found in human cancers, which begs the question of whether TOR is the sole downstream effector of RHEB. If tumours that show hyperactivated PKB- or Ras-signalling are nevertheless dependent on *TOR*, *S6K* or *EIF4EBP* for survival or proliferation, the lack of mutations in these genes might provide a unique opportunity for inhibiting a crucial pathway without promoting drug resistance. Imatinib (Glivec), the relatively specific BCR–ABL kinase inhibitor that is currently used for the treatment of chronic myeloid leukaemia, tends to lose efficacy over the course of treatment as mutations accumulate in the tumorigenic *BCR–ABL* fusion gene[43]. If activating TOR mutations continue to be rare, even in the presence of TOR-specific inhibitors, these chemotherapeutic agents might have a prolonged term of efficacy and might be useful even in late-stage disease.

Similar to most other signalling pathways, the PI3K pathway contains mechanisms by which it can turn itself off. For example, a negative-feedback loop probably exists downstream of the TOR effector S6K because S6K activation results in both the transcriptional repression and the inhibitory phosphorylation of insulin receptor substrate (IRS) proteins[44–46]. IRS proteins are adaptor molecules that are phosphorylated in response to insulin or insulin-like growth factors (IGFs). Phosphorylated IRS proteins relay signals from receptors that bind these growth factors to both PI3K and Ras[47]. IRS protein activation might account for the different tumour spectra observed in patients with germline *PTEN* mutations versus those with germline *TSC1* and *TSC2* mutations. Mutations in TSC1 and TSC2 presumably activate the negative-feedback loop, thereby activating only the TOR pathway, whereas normal regulatory control of other PI3K-mediated pathways would still be in place. On the other hand, PTEN mutations presumably lead to the hyperactivation of all PI3K-mediated pathways. Accordingly, Cowden disease, which is associated with germline *PTEN* mutations and therefore with the activation of multiple PI3K-mediated signals, is characterized by a much higher cancer risk than is tuberous sclerosis (germline *TSC2* mutations and TOR activation)[29,48]. Furthermore, negative feedback from a hyperactivated PI3K pathway would be predicted to decrease the activity of other IRS-mediated responses to insulin or IGFs. Consistent with this hypothesis, IGF1 is unable to induce ERK phosphorylation in *Pten*$^{-/-}$ mouse embryonic fibroblasts (MEFs) (M.C. and T.W.M., unpublished observations). Pharmacological inhibition of the TOR pathway could remove this negative-feedback loop, thereby increasing the activity of other branches of the PI3K pathway as well as additional molecules activated by IRS proteins. TOR inhibitors might therefore be more effective as cancer therapeutics when used in combination with inhibitors that target either upstream molecules (such as growth factor receptors) or other pathways activated by IRS proteins, such as Ras or PI3K signalling pathways.

PI3K, PTEN and the Ras signalling pathwa y

Although PI3K is activated by the direct binding of its p85 regulatory subunit to a phosphorylated receptor tyrosine kinase (RTK), additional mechanisms of PI3K activation exist (BOX 1). The p110 (catalytic) subunit of PI3K can also be activated through interaction with Ras[49]. *In vivo* data support a functional link between the Ras and PI3K pathways. In endometrial tumours, as well as in cell lines derived from melanomas, mutation of the *RAS* and *PTEN* genes is mutually exclusive[50,51]. Whereas *RAS* mutations are common in pancreatic, lung and colon cancers, they are rarely found in glioblastomas; the opposite is true for *PTEN* mutations[52–54]. In mouse models of chemically-induced skin carcinogenesis, *Ras* mutations typically arise in $Pten^{+/+}$ mice. In $Pten^{+/-}$ mice, there is a decreased incidence of *Ras* mutations[55]. Furthermore, tumours that lack *Ras* mutations tend to lose the second (wild-type) *Pten* allele. These observations indicate that Ras activation and PTEN loss probably serve the same function during tumorigenesis[55].

In addition to Ras itself, there are a number of cytoplasmic molecules that can activate PI3K, including the IRS and GAB (growth factor receptor-bound protein 2 (GRB2)-associated binding protein) families of adaptor molecules. When RTKs engage ligand, IRS and GAB1/GAB2 become phosphorylated at tyrosine residues, resulting in PI3K activation. Activation of GAB2 is required for BCR–ABL-mediated leukaemogenesis in mice, and $Gab2^{-/-}$ cells are resistant to BCR–ABL-induced transformation[56]. Another adaptor molecule, GRB2, functions upstream of both Ras and GAB1/GAB2, and therefore has an important role in both Ras and GAB-mediated activation of PI3K. $Grb2^{+/-}$ mice are partially resistant to polyoma-middle-T-antigen-induced mammary tumorigenesis, a system in which both Ras and PI3K are activated[57]. Breast cancer cells that overexpress the epidermal growth factor (EGF) receptor ERBB2 depend on GRB2 activity for both proliferation and tumour formation[58].

The identification of these multiple mechanisms that can activate PI3K signalling might be of significant therapeutic value, as drugs that target each of these interactions could have differential effects, based on the activity of each signalling element in different tissues. For example, inhibition of GRB2 might be the most effective way to block the PI3K signalling pathway in mammary tumours, whereas inhibition of the Gab family proteins could be a therapeutic target in the treatment of leukaemia. Although protein–protein interactions are notoriously difficult to disrupt through chemical inhibition, the *in vivo* delivery of inhibitory RNA molecules could be a feasible approach to inhibiting this pathway[59]. By choosing targets far upstream in the PI3K pathway, it might be possible to change the activation status of numerous downstream effectors and therefore reduce their contributions to tumour formation.

PTEN and p53

The tumour suppressor p53 activates the transcription of both *PTEN* and *TSC2*, and therefore functions as a negative regulator of the entire PI3K signalling pathway[60–62]. The downregulation of PI3K signalling by p53 activation is further enforced through p53-mediated transcriptional repression of the gene encoding the p110 subunit of PI3K[63]. p53 is activated in response to a wide variety of cellular stress signals, including DNA damage, hypoxia, mitotic spindle damage, heat and cold shock, inflammation, nitric oxide production and oncogene activation[64–66]. These stresses all have the potential to decrease the fidelity of cell-cycle progression and DNA replication, and thereby increase mutation rates in cells. Transcriptional regulation of the PI3K pathway by p53 eliminates PI3K-mediated survival and proliferation signals, providing an additional level of protection against continued DNA replication in the presence of genotoxic stress.

Activation of p53 can also induce cellular senescence[65]. The role of senescence in blocking cancer formation has been vigorously debated for many years, but a series of recent publications indicate that senescence, induced by activation of either Ras or p53, can prevent tumour progression[67–70]. Most notably, prostate-specific disruption of *Pten* in mice results in prostate neoplasias that are associated with senescence markers. Additional deletion of *Trp53* in these mice prevents this senescence and results in the formation of malignant tumours[67].

p53 is one of the most commonly mutated genes in human cancers. However, despite our extensive knowledge of p53 function and regulation, significant difficulties in gene delivery systems have limited our ability to restore wild-type p53 expression to tumour cells. Inhibition of PI3K signalling might offer a means of treating patients who have tumours that carry p53 mutations. Because the p53 apoptotic response requires the downregulation of the PI3K pathway through the transcriptional activation of *PTEN*[60], simultaneous inhibition of the PI3K pathway and activation of apoptosis downstream of p53 might have synergistic effects. In addition, drugs that activate senescence pathways downstream of p53 or Ras could also synergistically increase the anti-tumour effects of drugs that target PI3K or TOR.

PI3K, p53 and FOXO

Another example of intermolecular crosstalk exists in the recently documented interaction between the tumour suppressors p53 and FOXO3A. The activation, inactivation and even the selection of genes to be transcribed or repressed by p53 or FOXO is accomplished in part by protein modifications. DNA damage triggers p53 activation, which in turn leads to the induction of SGK1 and the subsequent nuclear expulsion of FOXO3A[71]. In this way, p53 regulates the expulsion of FOXO3A from the nucleus in response to DNA damage. This seemingly counter-intutitive phenomenon could be part of a positive-feedback loop required for complete p53 activation, as nuclear FOXO3A can inactivate p53 transcriptional activity (H.Y. *et al.*, unpublished data). Translocation of FOXO3A into the cytoplasm therefore allows p53 to act in the nucleus. This reciprocal inhibition of p53 and FOXO3A is avoided under normal circumstances — as p53 concentrations increase in response to stress signals, the induction of SGK1 results in the nuclear exclusion of FOXO3A[71]. However, FOXO3A has either pro- or

Box 2 | **Potential side-effects of PI3K inhibition**

Because the phosphatidylinositol 3-kinase (PI3K) signalling pathway is an important regulator of numerous normal cell processes, drugs that target this enzyme could potentially have many side-effects. For example, PI3K activity is important for insulin signalling and metabolism, so inhibition of PI3K signalling in an effort to control tumour progression could lead to decreased insulin sensitivity and diabetes[8,88]. Regulation of PI3K is also required for normal brain function, as decreased PI3K signalling has been associated with schizophrenia[80]. Similarly, the successful treatment of bipolar disorder with lithium, a glycogen synthase kinase 3 (GSK3) inhibitor, implies that PI3K signalling cannot be reduced below a certain crucial level[89]. Parkinson disease might be another potential consequence of drug-induced PI3K deregulation. Homozygous mutations in the Parkinson-disease-associated gene *PARK7* (also known as *DJ1*) are associated with early-onset Parkinson disease[90]. Loss-of-function mutations in *Dj1* attenuated dopamine-dependent behaviours and increased sensitivity to dopaminergic neuron loss induced by oxidative stress in mice[91–93]. Increased expression levels of DJ1 have been reported in both lung and mammary tumours, and DJ1 suppresses cell death in a PTEN (phosphatase with tensin homology)-dependent manner in cultured mouse cells[74]. So, it is important to understand PI3K signalling in both normal and diseased tissues before undertaking PI3K inhibition as an anticancer strategy. Drugs that inhibit PI3K signalling for a short period of time in normal tissues might have reversible or treatable side-effects, but such agents might not be useful for the maintenance of stable or cytostatic disease. Nevertheless, because tumour cells are often dependent on a hyperactive PI3K pathway, restoring normal levels of PI3K signalling to these cells could be sufficient to slow tumour progression. Pharmacological agents that decrease PI3K signalling only when this pathway is hyperactive could therefore be of significant therapeutic value.

anti-apoptotic roles that are context-dependent[72], so the biological significance of this crosstalk is currently under investigation.

PI3K, PTEN and PARK7

Human breast and lung tumour cells rarely have *PTEN* mutations, but hyperactivation of PKB is observed in 25–75% of these tumours[73,74]. Several mechanisms have been proposed to drive this hyperactivation. For example, lung cancers commonly carry activating mutations in Ras, which would be expected to activate PKB through both PI3K and TOR. The inactivation of p53 — another frequent occurrence in these tumour types — should decrease PTEN expression and result in increased levels of phosphorylated PKB. A third mechanism arises from the results of a recent study in which expression of the Parkinson-disease-associated gene *PARK7* (also known as *DJ1*) was correlated with the presence of increased phosphorylated PKB in both breast and lung cancer samples. Moreover, increased DJ1 expression correlated with an increased rate of relapse[74]. In the lung, this correlation was strongest in early-stage tumours but weakest in late-stage tumours that carried Ras mutations. How DJ1 fits into the PI3K–PTEN signalling network and negatively regulates PKB activation remains unclear. DJ1 might inhibit PTEN or provide another type of PKB-activating signal in early-stage tumours. At later stages, genomic instability might result in mutations that alleviate the selective pressure for DJ1 overexpression. The precise function of DJ1 and its relationship to PI3K, PKB and PTEN remain under investigation.

Future directions

A new paradigm in cancer treatment is the rational development of anticancer drugs, such as Herceptin, that specifically target molecules that drive tumorigenesis. Although this new generation of therapeutics holds great promise, our currently incomplete understanding of the molecular mechanisms that control key pathways in both normal and cancer cells limits our ability to use these drugs efficiently. Several studies have demonstrated the difficulties in determining which subset of patients will benefit from treatment with a targeted agent[75–79]. These difficulties arise because we do not have biomarkers that can identify patients who are most likely to respond to therapy. Detailed dissection of tumorigenic pathways will allow us to develop 'molecular signatures' that can provide information on the activation status of the many individual pathways within a cell. Analysis of these signatures should aid us in predicting which drugs will be effective for which patients. Conventional chemotherapy often leads to the emergence of drug resistance, providing further evidence that the simultaneous targeting of multiple key pathways will be the most effective strategy for killing cancer cells.

Determining which factors to target requires a thorough understanding of the crosstalk among numerous signalling pathways, some or all of which could be activated within a given tumour. Blanket inhibition of multiple pathways is not an option because of the risk of side-effects. For example, the inappropriate inhibition of the PI3K pathway has been associated with diseases as diverse as diabetes and schizophrenia[80,81] (BOX 2). The efficacy and safety of these inhibitors will depend on our understanding of the role of PI3K in these disorders as well as during tumour formation. Gene expression profiling to select patients who are most likely to respond to a certain inhibitor could help us to avoid testing the efficacy of drugs in the wrong patient cohort. The selection of appropriate patients for a clinical trial is of paramount importance in judging a new drug's true merits. Pre-screening candidate patients by molecular signature can identify a suitable subset of subjects for each new drug and predict which drugs might have synergistic effects. The experimental answers derived from this type of work are only as good as the questions. By expanding our understanding of the PI3K signalling network we will not only be able to ask the right questions, but, more importantly, find the right treatment for each patient.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES

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