Rapamycin, a valuable drug with diverse clinical applications, inhibits mTOR (mammalian target of rapamycin), which is a protein kinase that controls cell growth by regulating many cellular processes, including protein synthesis and autophagy. The sensitivity of select tumor cells to rapamycin has ignited considerable excitement over its potential as an anti-cancer therapeutic. Recent findings identified a rapamycin-insensitive function of mTOR in regulating a cell-survival pathway that is hyperactive in many cancers, particularly those with elevated PtdIns3K signaling or harboring mutations in the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10). These new findings suggest that targeting this function of mTOR might have broader applications in cancer therapy. In this article, we re-evaluate mTOR signaling, suggesting a more central role for mTOR in cancers with defective PtdIns3K–PTEN signaling and conceptually discuss these implications in the context of drug discovery.

New horizons for mTOR signaling

Advances in our understanding of cancer have made clear that even tumors of the same pathological classification can have markedly different signaling-pathway profiles and gene-expression patterns. Effective treatments will probably require the use of molecular markers to decipher these signatures on a patient-to-patient basis and the custom design of appropriate treatment strategies. Biomarkers indicate that the mTOR (mammalian target of rapamycin) growth pathway is hyperactive in certain cancers, particularly those with elevated PtdIns3K signaling or harboring mutations in the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10). These new findings suggest that targeting this function of mTOR might have broader applications in cancer therapy. In this article, we re-evaluate mTOR signaling, suggesting a more central role for mTOR in cancers with defective PtdIns3K–PTEN signaling and conceptually discuss these implications in the context of drug discovery.

An expanding role for mTOR in cancer

David A. Guertin¹ and David M. Sabatini¹,²

¹Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02141, USA
²Massachusetts Institute of Technology, Department of Biology, Cambridge, MA 02141, USA

Here, we discuss evidence suggesting that the inhibition of mTOR by rapamycin is a promising anti-cancer strategy. The prevailing view is that mTOR lies downstream of and is activated by AKT. This model predicts that the aberrant activation of AKT upregulates mTOR growth signaling, sensitizing transformed cells to rapamycin treatment. Although this model is still controversial, adding to the debate is new evidence implicating mTOR as a direct AKT activator, placing mTOR on both sides of the AKT signaling hub. These differential functions of mTOR can be explained by the existence of at least two distinct mTOR complexes containing unique interacting proteins. We discuss each mTOR complex with respect to pathway specificity and conclude with a comment on the value of mTOR inhibitors for the treatment of cancer.

mTOR signaling in cancer

The best-described genetic deficiencies commonly found in human cancer and impinging upon mTOR signaling are mutations in the PTEN gene [4]. PTEN mutations are associated with a spectrum of cancers, including prostate, breast, lung, bladder, melanoma, endometrial, thyroid, brain, renal carcinomas and others, making it one of the most frequently mutated tumor-suppressor genes (Table 1) [5–7]. PTEN, a lipid phosphatase, counteracts the lipid-kinase activity of class I PtdIns3Ks (Figure 1). Following growth-factor stimulation, activated receptor tyrosine kinases, or intermediaries such as the insulin-receptor substrates IRS-1 and IRS-2, recruit PtdIns3K to the membrane. PtdIns3K phosphorylates phosphatidylinositol (4,5) bisphosphate [PtdIns(4,5)P₂] at the membrane to generate PtdIns(3,4,5)P₃. PtdIns(3,4,5)P₃ serves as a docking site for effector proteins with pleckstrin-homology (PH) domains, including the AKT and 3-phosphoinositide-dependent protein kinase 1 (PKD1) protein kinases. In addition to PTEN deletions, amplifications of both PtdIns3K and AKT occur in several cancers [4,8]. Transforming events not affecting the PtdIns3K, PTEN or AKT genes directly, such as the BCR–ABL translocation and HER-2/neu or epidermal growth-factor receptor (EGFR) amplification, also activate PtdIns3K signaling [8]. Considering the prevalence of PTEN mutations and aberrantly activated PtdIns3K–AKT signaling in cancer, identifying the associated downstream signaling events that are crucial for tumor progression is an area of intense investigation.

AKT belongs to the cAMP-dependent, cGMP-dependent protein kinase C (AGC) family of protein kinases that includes ribosomal S6 kinases (S6K1 and S6K2), serum-
glucocorticoid protein kinase (SGK) and protein kinase C (PKC) [9]. Two essential phosphorylation sites characterize AGC kinases: one in the activation loop and one in a C-terminal hydrophobic motif. At the membrane, phosphorylation on Thr308 in the activation loop and Ser473 in the hydrophobic motif activates AKT. In mice and humans, three AKT isoforms exist (Akt1 or PKBα, Akt2 or PKBβ and Akt3 or PKBγ, respectively, for the mouse and human forms), which are the products of distinct genes. Mouse knockout models indicate that, in spite of the physiological significance of these interactions is still under investigation [19]. AKT activation probably promotes cellular transformation and resistance to apoptosis by collectively promoting growth, proliferation and survival while inhibiting apoptotic pathways. Elevated AKT signaling might additionally cause cells to adopt a more glycolytic metabolism [8]. Such a glycolytic shift could help to sustain cellular metabolism in the harsh nutrient-limiting environment of a tumor and discourage apoptosis by promoting mitochondrial membrane potential [8,20,21]. Thus, inhibitors of AKT signaling are sought-after drugs and current strategies target both the kinase and PH-domain functions of AKT directly, in addition to its upstream activators [5,22,23].

**mTOR–raptor, a regulator of growth**

Because hyperactive AKT signaling is associated with elevated mTOR signaling in some cancers, the mTOR inhibitor rapamycin could have promise as an anti-cancer therapeutic [1,2,8,24,25]. mTOR is a highly conserved protein belonging to the PtdIns3K-related kinase family (PIKK family) of serine/threonine protein kinases that includes ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PKcs). In cells, mTOR exists in at least two distinct complexes: a rapamycin-sensitive complex defined by its interaction with the accessory protein raptor (regulatory-associated protein of mTOR) and a rapamycin-insensitive complex defined by its

Table 1. mTOR signaling in disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Linked genetic mutation and clinical pathology</th>
<th>Predicted functional link to mTOR signaling</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-prone syndromes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSC (tuberous sclerosis complex)</td>
<td>TSC1 or TSC2; harmatomas in multiple organs</td>
<td>TSC1 and 2 negatively regulate Rheb</td>
<td>[60,61]</td>
</tr>
<tr>
<td>LAM (lymphangioleiomyomatosis)</td>
<td>TSC2; abnormal proliferation of smooth-muscle-like cells in the lung</td>
<td>TSC1 and 2 negatively regulate Rheb</td>
<td>[100]</td>
</tr>
<tr>
<td>Cowden’s disease</td>
<td>PTEN; harmatomatous tumor syndrome</td>
<td>Might promote AKT-dependent inhibition of TSC2 and mTOR phosphorylation</td>
<td>[62]</td>
</tr>
<tr>
<td>Proteus syndrome</td>
<td>PTEN; harmatomatous tumor syndrome</td>
<td>Might promote AKT-dependent inhibition of TSC2 and mTOR phosphorylation</td>
<td>[62]</td>
</tr>
<tr>
<td>Lhermitte–Duclos disease</td>
<td>PTEN; harmatomatous tumor syndrome</td>
<td>Might promote AKT-dependent inhibition of TSC2 and mTOR phosphorylation</td>
<td>[62,101]</td>
</tr>
<tr>
<td>JJS (Peutz–Jeghers syndrome)</td>
<td>STK11/LKB1; gastrointestinal hamartoma tumor syndrome</td>
<td>STK11 activates AMPK, a positive regulator TSC2</td>
<td>[52–54]</td>
</tr>
<tr>
<td>HCM (familial hypertrophic cardiomyopathy)</td>
<td>AMPK; myocardial hypertrophy</td>
<td>AMPK promotes TSC2 function</td>
<td>[102]</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>PTEN</td>
<td>PTEN loss promotes AKT activation</td>
<td>[103,104]</td>
</tr>
<tr>
<td>Breast</td>
<td>PTEN; PtdIns3K, AKT or Her2/ neu amplification or hyperactivation</td>
<td>PTEN loss or gene amplifications promote AKT activation</td>
<td>[4,104–106]</td>
</tr>
<tr>
<td>Lung</td>
<td>PTEN; HER amplification</td>
<td>PTEN loss or gene amplifications promote AKT activation</td>
<td>[4,107]</td>
</tr>
<tr>
<td>Bladder</td>
<td>PTEN</td>
<td>Promotes AKT activation</td>
<td>[108]</td>
</tr>
<tr>
<td>Melanoma</td>
<td>PTEN</td>
<td>Promotes AKT activation</td>
<td>[4]</td>
</tr>
<tr>
<td>Renal-cell carcinoma</td>
<td>PTEN</td>
<td>Promotes AKT activation</td>
<td>[4]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>PTEN; PtdIns3K, AKT or Her2/ neu amplification or hyperactivation</td>
<td>PTEN loss or gene amplifications promote AKT activation</td>
<td>[4,105,106,109]</td>
</tr>
<tr>
<td>Endometrial</td>
<td>PTEN</td>
<td>Promotes AKT activation</td>
<td>[4]</td>
</tr>
<tr>
<td>Thyroid</td>
<td>PTEN; PtdIns3K, AKT or Her2/ neu amplification or hyperactivation</td>
<td>PTEN loss or gene amplifications promote AKT activation</td>
<td>[4]</td>
</tr>
<tr>
<td>Brain (glioblastoma)</td>
<td>PTEN</td>
<td>Promotes AKT activation</td>
<td>[4,104,110]</td>
</tr>
<tr>
<td>CML (chronic myeloid leukemia)</td>
<td>BCR–ABL translocation</td>
<td>Promotes AKT activation</td>
<td>[8]</td>
</tr>
</tbody>
</table>

*List is not comprehensive, but rather attempts to indicate diseases commonly linked to genes involved in mTOR signaling.

*Listed only are the linked mutations that might affect mTOR signaling.

*Best prediction based on existing data.
phenotype observed in certain mouse cells that are null for mTOR with rapamycin suppresses the enhanced growth
esis after balloon angioplasty [25]. The inhibition of insulin or insulin-like growth factors. At the cell membrane, PtdIns3K phosphorylates PtdIns(4,5)P2 to generate PtdIns(3,4,5)P3 (PIP3). The tumor suppressor PTEN, a lipid receptor tyrosine kinase (RTK) for non-insulin-like growth factors], this triggers PtdIns3K (PI3K) membrane recruitment and activation directly, or through IRS proteins for insulin or insulin-like growth factors. At the cell membrane, PtdIns3K phosphorylates PtdIns(4,5)P2 to generate PtdIns(3,4,5)P3 (PIP3). The tumor suppressor PTEN, a lipid phosphatase, counteracts PtdIns3K lipid-kinase activity. PIP3 serves as a membrane-docking site for the PH-domain-containing proteins AKT and PDK1. Active PDK1 phosphorylates the activation loop of S6K1 and AKT, two AGC family kinases that are key effectors of cell growth and cell-survival pathways, respectively. Growth-factor signaling, along with nutrient availability (particularly amino acids and glucose), regulates cell growth by controlling the activity of the mTOR–raptor complex. Most available evidence suggests the TSC complex (TSC1 and TSC2) integrates these diverse inputs along with inputs from energy status, stress response and hypoxia pathways to mTOR–raptor through a small GTPase called Rheb. Energy status is communicated to TSC2 through direct phosphorylation by AMPK, whereas the mediators of hypoxia, stress and nutrient availability are unknown. It remains possible that nutrients and growth factors, by unknown mechanisms, could also signal to mTOR independently of the TSC complex. mTOR–raptor controls cellular growth in part by phosphorylating the hydrophobic motif of S6K1 and activating the kinase. mTOR–raptor also phosphorylates and inhibits 4E-BPs, which themselves inhibit the eIF4E-dependent translation of capped mRNAs. mTOR–raptor has less defined but important roles in regulating transcription and autophagy. Rapamycin (when bound to FKBP12) specifically inhibits the nutrient-sensitive mTOR–raptor complex. Compelling evidence suggests that a negative feedback loop enables the nutrient-sensitive mTOR–raptor pathway, through S6K1, to desensitize insulin signaling. S6K1 mediates the feedback by phosphorylating and inactivating IRS1. Recent findings indicate that mTOR, when bound to rictor instead of raptor, activates AKT by phosphorylating its hydrophobic motif in a manner sensitive to growth factors. AKT is a crucial regulator of cell proliferation and survival pathways and is hyperactivated in many cancers. mTOR is also phosphorylated on a C-terminal site (not shown), but which mTOR complex is phosphorylated and why is unknown and thus we have excluded that information from the figure. Evidence also suggests that AKT phosphorylates and inhibits TSC2, thus activating the mTOR–raptor growth pathway. Other studies have questioned the significance of this interaction, so although it is an attractive model to explain why some tumors with hyperactive AKT signaling are sensitive to rapamycin, debate on its integrity is ongoing. The main pathways are depicted with black arrows, whereas feedback loops are in red. The physiological relevance of these feedbacks for modulating normal mTOR signaling is still under investigation but, regardless, it appears that considerable cross-talk exists. We have indicated selected direct phosphorylation sites with a ‘P’, but only for sites discussed in the main text. Broken lines and question marks indicate poorly defined molecular connections.

interaction with rictor (rapamycin-insensitive companion of mTOR) [26–30]. Both complexes additionally contain the small WD-repeat protein GβL and possibly other unidentified proteins [28,31].

Upon entering cells, rapamycin binds to its intracellular receptor, FKBP12, and the complex binds to and specifically inhibits mTOR [19,32]. This small lipophilic macrolide antibiotic is already a clinically valuable drug used as an immunosuppressant and in preventing restenosis after balloon angioplasty [25]. The inhibition of mTOR with rapamycin suppresses the enhanced growth phenotype observed in certain mouse cells that are null for PTEN or expressing constitutively activated AKT [33,34]. Similar effects occur in transformed chicken embryo fibroblasts, multiple myeloma cells and other transformed cells [35,36]. Although the reason why rapamycin is particularly effective against some transformed cells and not others remains unknown, such studies provide the rationale for exploring mTOR inhibition as a treatment for cancers with PTEN inactivation.

Why might rapamycin be an effective drug against tumor cells with PTEN loss of function? One hypothesis is that the loss of PTEN activates a rapamycin-sensitive mTOR growth pathway, sensitizing such
transformed cells to the drug. Evaluating this hypothesis requires a brief discussion of current thought as to how the mTOR growth pathway is regulated.

The rapamycin-sensitive mTOR–raptor complex controls cellular growth (the accumulation of cellular mass) by regulating protein synthesis and probably also by suppressing autophagy. In doing so, mTOR–raptor coordinates growth-promoting signals from nutrient and energy availability, particularly from amino acids, and relays them to downstream targets [19,32]. One target is the translational regulator S6K1, which mTOR–raptor phosphorylates in coordination with the PDK1 protein kinase (Figure 1). Through its interaction with S6K1, raptor directs mTOR to phosphorylate Thr389 in the hydrophobic motif of S6K1 in a rapamycin-sensitive manner, whereas the PDK1 kinase phosphorylates Thr229 in the activation loop [37]. A second S6 kinase (S6K2), which is >80% identical to S6K1, is also phosphorylated in a rapamycin-sensitive manner [38,39]. Considerably less is known about S6K2, but genetic models suggest S6K1 and S6K2 have redundant and distinct functions [38,40–42]. Although more attention should be given to S6K2, we will not discuss it further because S6K1 appears to be more crucial for growth control. mTOR–raptor can also phosphorylate and inhibit the eIF4E-binding proteins (4E-BPs), which are a family of negative regulators of eukaryotic translation-initiation factor 4E (eIF4E)-dependent translation [19]. Studies implicate both S6K1 and eIF4E in cellular transformation [43–45].

The normal function of S6K1 and 4E-BP1 in protein synthesis has been extensively described elsewhere [19,32,37]. In brief, S6K1 is believed to drive translation in part by phosphorylating the ribosomal protein S6, but the function of this event is still being studied. Thus, conclusive evidence as to the role of S6K1 (and S6K2) in growth remains elusive. Two recent reports further suggest that S6K1 can phosphorylate mTOR on a C-terminal site previously thought to be an AKT-phosphorylation site, but the functional significance of this event is also unknown [19,46,47]. 4E-BP1 inhibits eIF4E, which, in complex with other translational regulators, binds to the 5′ m7GpppN cap of nuclear-transcribed mRNA. This association recruits the cap-dependent translational activator eIF4G and the 40S ribosomal subunit. Hypophosphorylated 4E-BP1 competes with eIF4E-binding proteins (4E-BPs), which are a family of negative regulators of eukaryotic translation-initiation factor 4E (eIF4E)-dependent translation [19]. Studies implicate both S6K1 and eIF4E in cellular transformation [43–45].

The TSC connection
The tuberous sclerosis complex (TSC) is a heterodimer of two proteins, hamartin (TSC1) and tuberin (TSC2), which is predicted to integrate signals derived from nutrient availability, cellular energy status and hypoxia into a common growth regulatory signal to the mTOR–raptor complex (Figure 1) [19]. The uncharacterized nutrient-derived growth signal feeds to mTOR–raptor through TSC2, a GAP (GTPase-activating protein)-domain-containing protein that negatively regulates a small GTPase called Rheb [19]. Adding credence to this model are recent biochemical data showing that GTP-bound Rheb can bind to and activate mTOR in an amino-acid-sensitive manner [48–50].

By phosphorylating TSC2, hyperactive AKT might promote mTOR–raptor-dependent cell growth (Figure 1). Evidence indicates that AKT-dependent phosphorylation of TSC2 inactivates the TSC complex, leading to mTOR–raptor activation and the hyperphosphorylation of S6K1 and 4E-BP1 [19]. By activating mTOR–raptor, tumors might gain a growth advantage, suggesting one explanation as to why rapamycin is an effective cytostatic agent against cancer cells with hyperactive AKT signaling. Importantly, the relationship between PTEN inactivation, which presumably hyperactivates AKT, and rapamycin sensitivity is not overly compelling and predicting rapamycin sensitivity based on PTEN status alone is not particularly effective.

The downregulation of mTOR–raptor activity also occurs through the TSC complex. In times of energy crisis, AMP-activated kinase (AMPK) promotes TSC inhibition of mTOR–raptor by phosphorylating unique sites on TSC2 [51,52]. Because AMPK responds to increasing levels of AMP, it links growth to the cellular ATP:AMP ratio [52]. By phosphorylating AMPK, the LKB1 kinase (also called STK11) contributes to AMPK activation and, accordingly, LKB1-null mouse-embryo fibroblasts exhibit elevated mTOR–raptor signaling [53–56]. By a mechanism that differs from AMPK, hypoxia promotes the TSC-dependent inhibition of mTOR through the hypoxia-inducible factor (HIF)-1-inducible genes REDD1 (DDIT4) and REDD2 (DDIT4L) [57,58]. HIF-1α is a transcription factor that also promotes angiogenesis in response to low oxygen [59]. Moreover, HIF-1α expression is sensitive to rapamycin [60,61], thus a complex interaction between mTOR and oxygen sensing exists.

The clinical value of inhibiting the mTOR–raptor pathway extends beyond cancer (Table 1). Mutations in the TSC1 or TSC2 genes result in the hyperphosphorylation of mTOR–raptor effectors and are linked to the hamartomatous syndrome tuberous sclerosis [62,63]. Related hamartoma syndromes such as lymphangioleiomyomatosis (LAM), which is linked to tuberous sclerosis, Peutz–Jeghers syndrome (PJS), which is caused by mutations in LKB1, and Cowden’s disease, Lhermitte–Duclos disease and Bannayan–Riley–Ruvalcaba syndrome, which are linked to PTEN inactivation, might all be ameliorated by the inhibition of mTOR–raptor [62–64]. Mutations in AMPK are associated with familial cardiac hypertrophy, which also appears to be manifested through mTOR–raptor [62]. Clinical trials with rapamycin are underway in patients with some of these diseases.

Feedback inhibition of IRS1
The hamartoma syndromes described above rarely progress to metastatic cancer, raising the obvious question of why not? Perhaps part of the answer lies in the recent confirmation of a negative-feedback loop to the PtdIns3K pathway. Compelling evidence shows that S6K1
phosphorylates and inhibits the insulin-receptor substrate IRS1, an important mediator of insulin-receptor-dependent activation of PtdIns3K (Figure 1) [65–68]. Chronic insulin stimulation leads to the phosphorylation and degradation of IRS1 protein in a rapamycin-sensitive manner. Interestingly, the deletion of S6K1 hypersensitizes mice to insulin, protecting them from age- and diet-induced obesity [41]. These data predict that S6K1 activation promotes growth, but by desensitizing insulin-receptor signaling through IRS1, S6K1 indirectly deactivates PtdIns3K–AKT signaling. Whether this negative feedback exclusively targets IRS1 remains to be seen. In fact, AKT phosphorylation in response to platelet-derived growth factor (PDGF) is reduced in some TSC-null cells as a result of reduced expression of the PDGF receptor, which could be analogous to IRS1 downregulation [69].

Biologically, this feedback might be important to control the insulin-dependent influx of nutrients to cells, balancing intake with expenditure. Pathologically, TSC-deficient cells, which have elevated mTOR–raptor activity, fail to activate AKT following insulin stimulation [65]. Amino acids, which also activate mTOR–raptor activity, can similarly attenuate PtdIns3K signaling following insulin stimulation [70,71]. Thus, activating mTOR–raptor could inhibit the AKT-dependent pathways that contribute to cellular transformation, which might explain the benign nature of tuberous sclerosis. Importantly, rapamycin treatment of TSC2-null cells enables the stimulation of AKT phosphorylation in response to insulin and insulin growth factor (IGF)-1; thus, inhibiting the mTOR–raptor pathway with rapamycin in some tumor cells could promote AKT-dependent survival pathways [66,67]. Unlike a TSC-null deficiency, PTEN-null cancers might exhibit cumulative tumorigenic signals that override any potential negative feedback to IRS1; alternatively, PtdIns3K could be activated independently of IRS1.

**mTOR–rictor, a missing link to AKT activation**

To this point, we have focused on targeting the mTOR–raptor growth pathway in cancers characterized by hyperactive AKT. However, AKT has key regulatory roles in cell-cycle control, apoptosis and metabolism, in addition to cell growth. In fact, the physiological significance of AKT-dependent phosphorylation of TSC2 has been challenged in *Drosophila* in a recent report showing that a nonphosphorylatable version of TSC2 completely rescues the lethality of TSC2-null flies [72]. Other AKT-dependent processes are likely to be crucial for tumor progression. Therefore, inhibitors of AKT or its upstream activators might have broader applications than mTOR–raptor inhibitors.

**mTOR–rictor: the elusive PDK2?**

AKT activation requires the phosphorylation of Thr308 in the activation loop and Ser473 in the hydrophobic motif [9]. Similar to S6K1, PDK1 phosphorylates AKT in its activation loop. The identity of the Ser473 kinase (often referred to as PDK2) is controversial. In a recent report, it was suggested that the mTOR–rictor complex fulfills the role of PDK2 in *in vitro* and *in vivo*; importantly, this role is conserved in cultured *Drosophila* cells [3].

Rictor was identified both in yeast (AVO3) and mammals as a novel mTOR-interacting protein defining a second, raptor-independent mTOR complex [28–30]. As its name implies, the mTOR–rictor complex, unlike its mTOR–raptor sibling, cannot bind to FKBP12–rapamycin and is insensitive to acute rapamycin treatment [28,29]. This discovery provided solid evidence that rapamycin treatment does not represent a complete inhibition of mTOR function, which had been speculated. The first reports on the mTOR–rictor complex suggest that one of its cellular functions is to regulate the cytoskeleton [29,30]. PKCα could mediate this function, because its phosphorylation state is sensitive to RNA-interference (RNAi)-mediated silencing of mTOR and rictor and, in yeast, AVO3p regulates the actin cytoskeleton through PKC1 [28,29]. PKC, interestingly, is an AGC-family kinase and its mTOR–rictor-dependent phosphorylation site (Ser657) shares homology with the hydrophobic motif sites of S6K1 and AKT [73].

By using cells particularly sensitive to RNAi (such as cultured *Drosophila* cells) and lentiviral short-hairpin expression systems in mammalian cells, it has been possible to achieve robust RNAi-mediated silencing of mTOR-pathway components. A clue that mTOR has a role in AKT phosphorylation was the observation that silencing raptor, but not mTOR or rictor, activates AKT on Ser473 (Ser505 in *Drosophila*), presumably by releasing the negative-feedback inhibition to the insulin–IGF pathway described above [3]. This suggested that mTOR has a positive role in regulating AKT phosphorylation that is not shared with raptor but possibly is with rictor. The realization that the Ser473 site in AKT is similar to the hydrophobic motif site in S6K1 and PKC prompted the discovery that in both *Drosophila* and mammalian cells, the phosphorylation of AKT Ser473 decreases following RNAi-mediated silencing of mTOR and rictor, but not raptor [3]. Biochemical experiments indicate that mTOR–rictor, but not mTOR–raptor, phosphorylates AKT Ser473 in *vitro* and that full mTOR–rictor activity requires growth factors, confirming the suspicion that mTOR, when bound to rictor, is a Ser473 kinase [3].

Still unknown is the mechanism by which mTOR–rictor is activated. Although mTOR–rictor activity is sensitive to growth factors, the fact that constitutively active PtdIns3K alone triggers AKT Ser473 phosphorylation [4,8] suggests that if mTOR–rictor is the sole Ser473 kinase, its activation lies downstream of PtdIns3K. No known component of either mTOR complex contains a PH domain or other known lipid-binding domain. Thus, membrane recruitment following lipid phosphorylation is not an obvious possibility. Furthermore, a lack of unambiguous cellular localization data for mTOR and its interacting proteins obscures attempts to identify subcellular compartments that might serve as mTOR signaling centers. Understanding how mTOR–rictor activity is modulated and what signals promote the interaction between mTOR–rictor and AKT is paramount to understanding its role in cancer. It would be interesting to determine whether elevated mTOR–rictor activity or overexpression is a hallmark of any particular cancer cell line.
Considering the importance of AKT signaling in disease, attempts to identify the crucial Ser473 kinase have been hotly pursued. Many other potential candidates have been identified, including PDK1, integrin-linked kinase (ILK), AKT itself and DNA-PKcs, but in each case either in vivo or in vitro data or conservation could not be confirmed [74–77]. Because the mTOR–rictor complex fulfills all three criteria, we suggest that it is a Ser473 kinase. One implication of this proposal, considering current models, is that mTOR–rictor lies upstream and could indirectly regulate mTOR–raptor through the AKT–TSC2–Rheb axis. As previously mentioned, the debate concerning the relevance of this interaction is ongoing. However, in the context of a transformed cell, hyperactive AKT could become promiscuous and phosphorylate off-target proteins. If this were the case for TSC2, then by hijacking the mTOR–raptor pathway in transformed cells, the mTOR–rictor sibling (through AKT) would exert its dominance in the pathogenesis of some cancers. Nevertheless, if the role of mTOR–rictor in AKT activation withstands future experimental challenges, then mTOR–rictor will attract considerable attention as a novel drug target. Determining whether mTOR–rictor is the crucial Ser473 kinase in tumors characterized by hyperactive AKT will be an essential step to this end.

With hindsight, there are clues in the literature about the important role of rictor and mTOR in AKT activation. For example, a loss-of-function allele of pianissimo, the Dictostylium orthologue of rictor [78], causes chemotaxis and cell-aggregation defects that are remarkably similar to those caused by loss of the Dictostylium orthologue of AKT [79]. In addition, the orthologues of mTOR in both Drosophila and Caenorhabditis elegans regulate the lifespan of the organism [80–82], a well-characterized role of AKT and its downstream effectors. mTOR was previously not considered an AKT kinase because Ser473 phosphorylation is not sensitive to acute rapamycin treatment. But, because the rapamycin–FKBP12 complex tightly binds to mTOR in the absence of associated proteins, it remains possible that chronic exposure to rapamycin might compromise the ability of newly synthesized mTOR protein to associate with rictor. Consistent with this, long-term rapamycin treatment decreases AKT phosphorylation in at least one cell type [83]. This idea provides an alternative hypothesis for why rapamycin is particularly effective at inhibiting the proliferation of certain PTEN-deficient cancer cells. Such a scenario would necessitate a review of current rapamycin treatment regimens.

Considering the structural similarity between S6Ks and AKTs, particularly within the hydrophobic motif, it makes sense in retrospect that mTOR is an AKT Ser473 kinase. Indirectly, this finding strengthens the argument that mTOR directly phosphorylates S6K1 rather than an intermediate. Challenging this assumption was the fact that deleting a C-terminal domain of S6K1 rendered the phosphorylation of its hydrophobic motif insensitive to rapamycin [84–86]. Interestingly, deleting this C-terminal domain from S6K1 makes its hydrophobic motif site accessible to phosphorylation by the rapamycin-insensitive mTOR–rictor complex [86]. In fact, the S6K1 C-terminal truncation mutant closely resembles the structure of AKT which, outside of its PH domain, is similar to S6K1 except that it lacks the C-terminal domain. Although the mechanism of inhibition imposed on mTOR–rictor by this inhibitory domain is unclear, this observation does explain how the S6K1 C-terminal truncation mutant can be rapamycin insensitive if mTOR–raptor is the physiological S6K1 hydrophobic-motif kinase. The ability of mTOR to phosphorylate similar motifs in different kinases provides an excellent example of how one kinase, through specific interactions with accessory proteins, can function in diverse cellular processes. It will be interesting to determine if mTOR phosphorylates the hydrophobic motifs of other AGC-family kinases and whether different accessory proteins are required.

Prospects for drug discovery

Three rapamycin analogs, CCI-779 (Wyeth: http://www.wyeth.com/), RAD001 (Novartis: http://www.novartis.com/) and AP23573 (Ariad Pharmaceuticals Inc: http://www.ariad.com/) are in clinical trials for the treatment of cancer [1,24,25]. Early reports indicate that the drugs show promise in several tumor types, such as renal-cell carcinoma, breast carcinomas and non-small-cell lung carcinomas, and have tolerable side effects, including skin reactions, mucositis and myelosuppression [24,25]. Preliminary evidence suggested that rapamycin was particularly effective against tumors with PTEN inactivation, but an important point from the clinical trials is that biomarkers are needed to predict tumor sensitivity to the drug. One study of breast cancer cell lines indicates that AKT phosphorylation and S6K1 overexpression, rather than PTEN status or S6K1 phosphorylation, are the best predictors of rapamycin sensitivity [87]. Moreover, in many instances, the disease remains progressive following rapamycin treatment, even in PTEN-deficient tumors. In fact, one of the most favorable responses to rapamycin was observed in mantle-cell lymphoma, which is characterized by cyclin-D1 overexpression [24]. The development and validation of biomarkers is crucial for determining which patients will benefit from rapamycin treatment.

Interestingly, rapamycin triggers apoptosis in some cell-culture systems, suggesting a role for the mTOR–raptor complex in cell survival [88–92]. Under serum-free conditions in rhabdomyosarcoma cells lacking p53, rapamycin has been suggested to trigger apoptosis by activating a stress response involving the apoptosis-signal-regulating kinase 1 (ASK1) and c-JUN [93]. However, studies implicating rapamycin-dependent activation of apoptosis in general rely on long-term rapamycin treatment, which might, in some cells, compromise AKT signaling by disrupting the mTOR–rictor complex, as suggested above. Renal tumors bearing TSC2 mutations are also sensitive to rapamycin-induced apoptosis [90]. Perhaps these tumors, by upregulating mTOR–raptor signaling in the absence of TSC2 function, activate the negative-feedback loop to IRS1, preventing the full activation of AKT-dependent survival mechanisms and rendering the cells more susceptible to apoptosis. Other
data indicate that in B-cell lymphoma, eIF4E overexpression can promote cell survival in a rapamycin-insensitive manner, probably because it lies downstream of mTOR–raptor [94,95]. In summary, mTOR–raptor signaling contributes to a cell-survival mechanism in some instances, but this is through apparently diverse means that are not necessarily sensitive to rapamycin treatment.

Clearly an mTOR–rictor inhibitor might be beneficial for treating tumors with elevated AKT phosphorylation. Theoretically, such an inhibitor might downregulate the growth, proliferation and survival effects that are associated with AKT activation and could be used in combination with other chemotherapeutic agents or rapamycin. If mTOR–rictor is a crucial activator of AKT-dependent survival processes, such a drug might promote apoptosis in tumor cells that have adapted to AKT-dependent regulatory mechanisms. The essential element will be to find an mTOR–rictor-specific inhibitor with a promising therapeutic window and acceptable toxicity. However, the lack of mTOR–rictor structural information might hinder such efforts. Could a hyperactivated mTOR–raptor pathway, by TSC2 inhibition or another activator, drive cells to deplete energy stores? And, in combination with a compromised AKT-survival pathway, perhaps with an mTOR–rictor inhibitor, might apoptosis be potently activated? At present, we can only speculate, but with the newly discovered multifunctional roles for mTOR, open debate on new drug discovery and therapeutic approaches should be stimulated.

Re-evaluating mTOR signaling

Considering these new findings, we feel the role of mTOR in growth and disease warrants re-evaluation. Growth is defined as an increase in size or mass. With respect to cellular growth, mTOR has been referred to as a ‘master regulator’ because nutrient sensing through mTOR is an ancient mechanism for determining individual cell mass. Extending the ‘master regulator’ role of mTOR to organ and organism growth had been complicated by the fact that most organs additionally rely on a complex balance between cell proliferation and cell death to determine final organ size. The discovery that mTOR is a regulator of AKT implies a direct role for mTOR in these biological processes. Mouse knockout models of mTOR exhibit early embryonic lethality with cell growth and proliferation defects, which supports this theory [96–98]. Moreover, although not a major focus of this article, metabolism and aging, which are tightly linked to AKT-dependent processes, are probably more wired to mTOR signaling than previously thought [18,99]. Many mutations that affect insulin–IGF-1 signaling extend lifespan, as do some mutations that decrease TOR activity in C. elegans and Drosophila [18,99]. Perhaps the extended life span of TOR mutants reflects in part the role of TOR in AKT phosphorylation. Considering that mTOR regulates cell proliferation and cell-survival pathways, in addition to growth, we suggest a more global role for mTOR in controlling organ and organism growth.

Rapamycin treatment for cancers with loss of PTEN function is a potential clinical strategy. However, the therapeutic potential of rapamycin has been considered mainly because the loss of PTEN is associated with hyperactive AKT signaling and existing evidence suggests that AKT regulates the TSC–mTOR–Raptor–S6K1 growth pathway. Because mTOR–rictor was recently shown to directly regulate AKT in a manner that was insensitive to acute rapamycin treatment, mTOR probably has a more central role in cancers with hyperactive PtdIns3K–AKT signaling. Designing new drugs that specifically inhibit mTOR–rictor might have great therapeutic value.

Concluding remarks

During the last few years, our understanding of mTOR signaling in many organisms has greatly advanced thanks to excellent reports on many fronts. Although these discoveries have led to considerable interest and evaluation of mTOR signaling, exemplified by the many reviews written on the subject, we eagerly anticipate learning the many secrets that this molecule still holds. In this article, we have addressed the prevailing views on the role of mTOR in cancer and the hype surrounding rapamycin treatment as a promising anti-cancer strategy. However, new evidence suggests that mTOR might have a more central rapamycin-insensitive role in the pathology of some cancers. This discovery begins a new chapter in the mTOR odyssey, importantly opening a new door in the search for promising anti-cancer drug targets.

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