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mTOR Complex1–S6K1 signaling: at the crossroads of obesity, diabetes and cancer

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Recent studies demonstrate that the mammalian target of rapamycin (mTOR) and its effector, S6 kinase 1 (S6K1), lie at the crossroads of a nutrient–hormonal signaling network that is involved in specific pathological responses, including obesity, diabetes and cancer. mTOR exists in two complexes: mTOR Complex1, which is rapamycin-sensitive and phosphorylates S6K1 and initiation factor 4E binding proteins (4E-BPs), and mTOR Complex2, which is rapamycin-insensitive and phosphorylates protein kinase B (PKB, also known as Akt). Both mTOR complexes are stimulated by mitogens, but only mTOR Complex1 is under the control of nutrient and energy inputs. Thus, to orchestrate the control of homeostatic responses, mTOR Complex1 must integrate signals from distinct cues. Here, we review recent findings concerning the regulation and pathophysiology associated with mTOR Complex1 and S6K1.

Introduction

The coordinated control of cell growth to produce a genetically predetermined cell size, organ shape or body plan is greatly influenced by mammalian target of rapamycin (mTOR) and its downstream effector S6 kinase 1 (S6K1), as first revealed by studies in mice and *Drosophila* [1]. In this context, S6K1 has emerged as a crucial effector of mTOR signaling. The ability of mTOR to phosphorylate and activate S6K1 depends on three associated proteins, the rapamycin-sensitive adaptor protein of mTOR (raptor), the G protein β-subunit-like protein (GβL) [2] and the proline-rich protein kinase B (PKB, also known as Akt) substrate 40 kDa (PRAS40) [3], which constitute mTOR Complex1 [2,4] (Box 1). However, although GβL was thought to be required for mTOR Complex1 signaling [2], this view has recently been questioned [5]. mTOR Complex1 interacts with downstream substrates through raptor, which recognizes mTOR substrates by their TOR signaling (TOS) motifs [6]. The anti-fungal macrolide rapamycin blocks mTOR Complex1 function by forming a gain-of-function inhibitory complex with the immunophilin FK506 binding protein 1A (FKBP12) [7]. This inhibitory complex binds to mTOR, thereby altering its ability to phosphorylate downstream substrates, including S6K1 [8]. Recent studies have shown that mTOR also occurs in a second complex, mTOR Complex2, which includes GβL, the adaptor protein rapamycin-insensitive companion of mTOR (rictor) and mammalian stress-activated protein kinase (SAPK)-interacting protein-1 (mSIN1) [9–11]. mTOR Complex2 is the kinase responsible for phosphorylation of S473, one of the two key residues required for full activation of PKB/Akt. This second complex is largely insensitive to the rapamycin–FKBP12 complex in an acute setting, but in some conditions it might be inhibited by chronic exposure to rapamycin because nascent mTOR kinase binds to rapamycin before its assembly into mature mTOR Complex2 [12]. The importance of mTOR Complex1 in nutrient signaling has been underscored by observations of mice in which the gene that encodes mTOR has been deleted. Such mice die shortly after implantation due to impaired trophoblast differentiation and failure of embryonic stem cells to proliferate [13,14]. Exposure of early mouse embryos to rapamycin also arrests trophoblast differentiation and embryonic stem cell proliferation, indicating that it is the rapamycin-sensitive mTOR Complex1 function that is essential during this stage of development [1]. Consistent with this hypothesis, the effects of rapamycin on early development can be recapitulated by the withdrawal of amino acids [1], which specifically blocks mTOR Complex1 function, as judged by S6K1 T389 phosphorylation. Moreover, it has been recently shown that raptor-deficient mice are also embryonic lethal at day E5.5, whereas either rictor- or GβL-deficient mice live up to day E10.5 [5].

In contrast to $mTOR^{-/-}$ mice, $S6K1^{-/-}$ mice are viable, although they are retarded in development [i.e. they are 20% smaller at birth than wild-type (WT) mice] [1]. Such mice are also hypoinsulinemic, mildly glucose intolerant and have reduced β-cell size [1]. The pronounced reduction of β-cell size was suggested to account for the hypoinsulinemic phenotype of these mice [1]. Moreover, in recent studies it was demonstrated that $S6K1^{-/-}$ mice also exhibit increased energy expenditure and lipolysis, and a reduction in adipose tissue mass, which could be accounted for by an apparent decrease in adipocyte-cell size [15]. It was also demonstrated that such mice remain exquisitely insulin sensitive on a high-fat diet (HFD), despite high levels of circulating free fatty acids [15]. This maintenance of insulin sensitivity was traced to the loss of a negative-feedback loop from S6K1 to the insulin receptor substrate 1 (IRS1) (see below). These phenotypes have underscored the

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Box 1. Mechanism of S6K1 activation

In mammals, there are two isoforms of S6K1, which are produced from the same transcript by alternative initiation translation start sites [1]. The larger isoform, S6K1L, contains an N-terminal 23-amino-acid extension [1], which seems to direct S6K1L to the nucleus. By contrast, the shorter form, S6K1S, seems to reside mainly within the cytoplasm [1]. Findings to date suggest that S6K1L and S6K1S are regulated in a similar manner; these are here referred to as S6K1. The important residues for S6K1 activation were initially mapped to T229 in the activation loop and T389 in a conserved hydrophobic motif, and later to S371 within the conserved linker domain [1]. Moreover, a set of serine/proline (SP) sites within the autoinhibitory domain were shown to facilitate activation of S6K1 through interaction with its N-terminal TOR signaling (TOS) motif [1]. In addition to the initial identification of mitogen-induced phosphorylation site, S6K1 T389 was found to be the principle rapamycin-sensitive site, leading to the subsequent finding that mTOR directly mediates T389 phosphorylation [1]. It was also revealed that the motif surrounding this site is conserved in all members of the A, G and C family of serine/threonine protein kinases, including PKB/Akt, and that it is responsible for mediating T229 phosphorylation by phosphoinositide-dependent kinase 1 (PDK1) [1].

importance of S6K1 as a downstream effector of mTOR Complex1 in several cellular processes, including transcription, translation, autophagy, insulin resistance, and tumorigenesis in regulating cell growth, metabolism and the oncogenic phenotype [8]. Thus, systemically, specific signals, including those of growth factors, hormones, nutrients and energy, converge at the level of mTOR Complex1–S6K1.

Growth factors and hormones

Growth factors and hormones, such as insulin, regulate mTOR Complex1 through the generic class I phosphatidylinositide 3-kinase (PI3K) signaling pathway [1]. Stimulation of class I PI3K initiates several selective signaling cascades that lead to increased growth and proliferation, a phenomenon conserved throughout metazoans [16]. The interaction of insulin with its cognate tyrosine kinase receptor results in intermolecular phosphorylation of the receptor, creating docking sites for the recruitment of IRS1 and IRS2 to the cell membrane [1]. In turn, specific phosphorylated residues in IRS1 and/or IRS2 serve as recognition motifs for the binding of key signaling molecules, such as class I PI3K. At the membrane, class I PI3K produces the second messenger phosphatidylinositol(3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] [1]. PtdIns(3,4,5)P_3 binds to the pleckstrin-homology (PH) domain of target proteins, including PKB/Akt [8] and phosphoinositide-dependent kinase 1 (PDK1). PtdIns(3,4,5)P_3 binding to the PH domain of PDK1 does not seem to be required for S6K1 T229 phosphorylation [17]. Binding of PtdIns(3,4,5)P_3 to the PH domain of PKB/Akt recruits this kinase to the cell membrane where it is activated through concerted phosphorylation by PDK1 at T308 and mTOR Complex2 at S473 [1]. The major negative regulator of this step in the signaling pathway seems to be the lipid phosphatase, phosphatase and tensin homolog (PTEN). PTEN converts PtdIns(3,4,5)P_3 into PtdIns(4,5)P_2, leading to a reduced recruitment of PKB/Akt to the cell membrane. In turn, activated PKB/Akt has several downstream substrates, including glycogen synthase kinase-3 (GSK3), forkhead box, sub-group O (FOXO) transcription factors and tuberous sclerosis protein 2 (TSC2) of the TSC1–TSC2 complex, which acts as a tumor suppressor complex. The phosphorylation of TSC2 results in its dissociation and degradation of TSC1–TSC2 complex, which releases the small GTPase RAS homologue enriched in brain (Rheb) from the inhibitory GTPase-activating protein activity of TSC2, driving Rheb into the GTP-bound active state [1]. It is believed that GTP-bound Rheb enables mTOR Complex1 signaling to downstream substrates, such as S6K1 (Box 1), by directly altering mTOR Complex1 activity or targeting it to a unique cellular compartment [1].

Nutrients

The insulin-induced class I PI3K–PKB signaling pathway is also activated by other growth factors, such as epidermal growth factor (EGF), insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF) [18]. Once activated through these pathways, PKB/Akt can mediate the phosphorylation of several specific substrates, including those described above, in addition to caspase 9 and the BCL-2-antagonist of cell death (BAD), culminating in a prosurvival response [19]. Although the activation of mTOR Complex1 is involved in this response, the kinase activity of mTOR Complex1 cannot be triggered by class I PI3K in the absence of nutrients or cellular energy. Recent studies that analyzed the role of nutrients, specifically amino acids, revealed the existence of a novel signaling cascade that activates mTOR Complex1 [20,21]. The notion that amino acids are involved in mTOR Complex1 signaling first arose in a study of cultured hepatocytes in which amino acids inhibited the progression of macroautophagy [22]. In this study [22], it was shown that this inhibitory effect on macroautophagy is paralleled by increased phosphorylation of 40S ribosomal subunit protein S6. Subsequently, several research groups demonstrated that induction of S6K1 and 4E-BP1 phosphorylation by amino acids, especially the branched-chain amino acids and particularly leucine, depends on mTOR Complex1 [22]. Later studies, performed in several insulin-responsive cell lines, showed that amino-acid withdrawal results in the rapid dephosphorylation of S6K1 and 4E-BP1, whereas addition of amino acids rescues this response in a rapamycin-sensitive manner [23]. In contrast to initial findings [24], recent studies suggested that TSC1–TSC2 complex is not required to transduce the amino-acid signal to mTOR Complex1 [20,25]. Indeed, these studies demonstrated that S6K1 phosphorylation is elevated and completely resistant to insulin in cells in which either TSC1 or TSC2 levels had been reduced or completely depleted. By contrast, S6K1 activity is still regulated by amino acids in the absence of the TSC1–TSC2 complex inhibitory signal. Moreover, although amino-acid-induced mTOR Complex1 signaling requires Rheb, amino-acid withdrawal from either TSC1- or TSC2-deficient cells has no effect on high Rheb–GTP levels; however, such treatment leads to a rapid S6K1 dephosphorylation [20]. Thus, endogenous Rheb–GTP is required for this response, but is insufficient to activate mTOR Complex1 signaling in the absence of amino acids. These results indicate that amino-acid input to mTOR Complex1 might occur through a parallel signaling

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pathway. Earlier studies from the late 1990s demonstrated that amino-acid stimulation of mTOR Complex1 signaling to S6K1 and 4E-BP1 is wortmannin-sensitive, despite the fact that amino acids do not induce PKB/Akt activation [22]. Indeed, small interfering (si)RNA-mediated depletion of class I PI3K almost completely blocks insulin-induced PKB/Akt S473 and S6K1 T389 phosphorylation, but has no effect on the ability of amino acids to induce S6K1 activation [20]. Thus, the wortmannin-sensitive target of amino-acid-induced mTOR Complex1 signaling might reside on a pathway that is distinct from the class I PI3K–PKB/Akt signaling pathway. By using several pharmacological, biochemical and molecular approaches, it was shown that the activity of the class III PI3K human vacuolar protein sorting-34 (hVps34) is modulated by amino-acid availability, and that the activation of S6K1 by amino-acid stimulation through mTOR Complex1 requires hVps34 [20,21] (Figure 1 and Box 2).

Energy

The mechanisms regulating mTOR Complex1 signaling through cellular energy are not as well defined as those for growth factors and nutrients. Studies have largely relied on the role of either acute (minutes) or chronic (hours) energy depletion on mTOR Complex1 signaling. It was shown that mTOR Complex1 signaling to S6K1 is sensitive to small changes of intracellular ATP levels and does not depend on alterations of amino-acid levels [26]. Dennis *et al.* [26] showed that acute treatment with the mitochondrial Complex1 inhibitor rotenone results in a small reduction of intracellular ATP levels and insulin-induced S6K1 activation [26]. Consistent with the possibility that homeostatic levels of ATP directly regulate mTOR signaling [26], the apparent K_m of mTOR for ATP is similar to cellular ATP concentrations [27]. However, subsequent studies suggested that acute energy depletion by treatment with oligomycin, an inhibitor of oxidative phosphorylation and respiration, inhibits mTOR Complex1 signaling through AMP-activated kinase (AMPK) phosphorylation and activation of TSC2 [28–30] (Figure 1). In agreement with this finding, activated variants of AMPK inhibit mTOR signaling [28]. However, Smith *et al.* [25] have recently reported that acute treatment of TSC2-deficient cells with 2-deoxyglucose, an energy depleting agent, leads to inactivation of S6K1. The *Drosophila* ortholog of TSC2, dTSC2, does not have

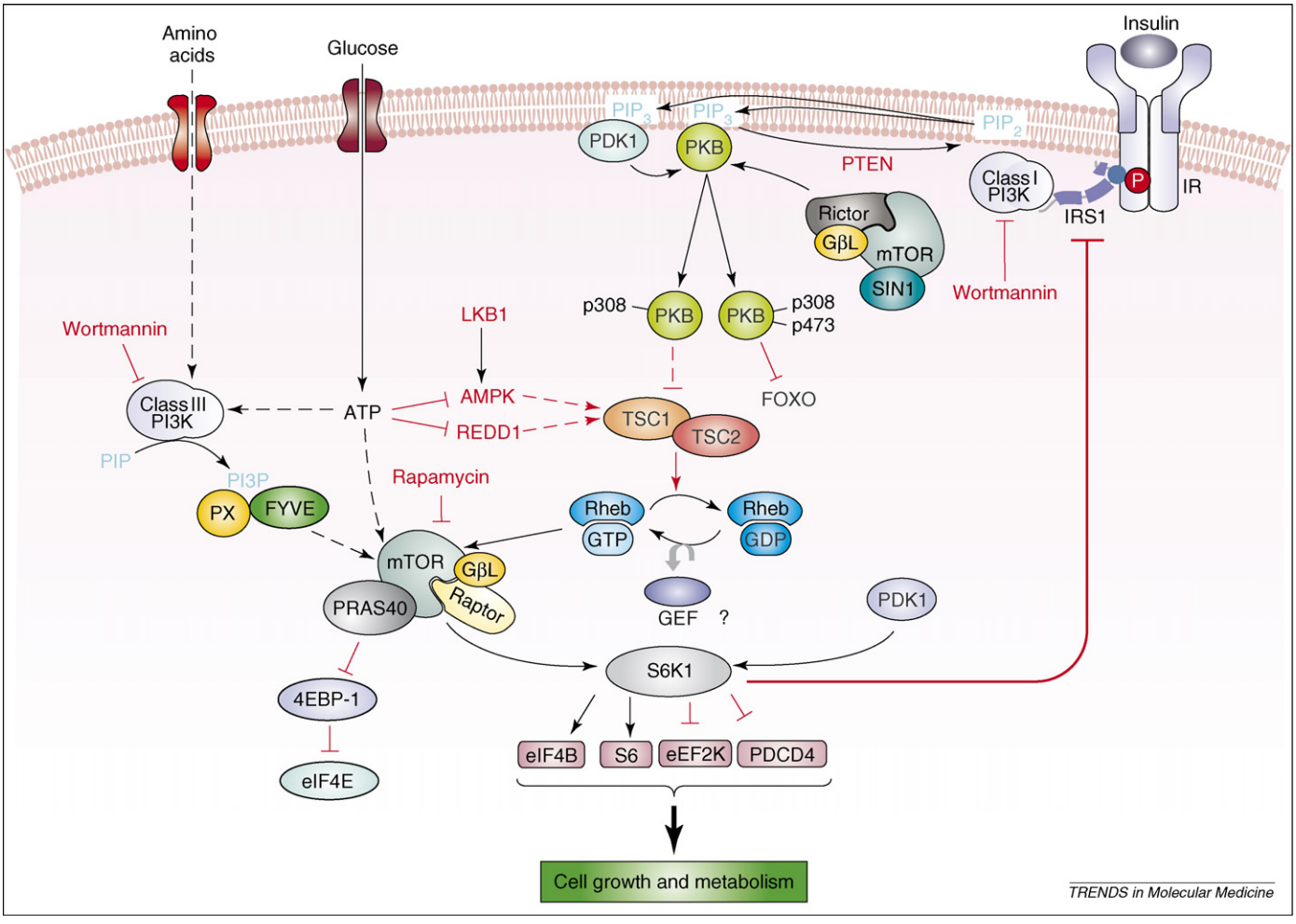

Figure 1. mTOR Complex1–S6K1 pathway. mTOR exists in two structurally distinct complexes. mTOR Complex1, which contains raptor, is regulated by inputs including amino acids, ATP and insulin, which initiate diverse signaling cascades (broken arrows indicate the uncertainty of the mechanisms involved). Recent studies indicate that mTOR Complex2 is not upstream of mTOR Complex1 [5,10]. Phosphorylation of S6K1 by mTOR Complex1 enables PDK1 to phosphorylate and activate S6K1. Abbreviations: eEF2K, eukaryotic elongation factor 2K; eIF4B, eukaryotic initiation factor 4B; FYVE, Fab1/YOTB/2K632.12/Vac1/EEA1 domain; GEF, guanosine nucleotide exchange factor; PDCD4, programmed cell death protein 4; PX, Phox homology domain.

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Box 2. Outstanding questions

- How does hVps34 signal as a positive effector of autophagy in the absence of nutrients and a positive effector of mTOR Complex1 in the presence of nutrients?
- What is the target of AMPK in mTOR Complex1–S6K1 signaling, in the absence of TSC1 and TSC2?
- Does the activation of AMPK by metformin result in inhibition of mTOR and S6K1?
- Are the effects of EGF-receptor inhibition on fasting glucose levels due to reduced PKB/Akt activity, leaving it more sensitive to the effects of circulating insulin?
- Would combination of current diabetic therapies with an inhibitor of mTOR result in a more dramatic affects on insulin resistance?
- Can dietary or lifestyle modifications increase the efficacy of current cancer therapeutics?

conserved AMPK phosphorylation sites despite dAMPK activation in *Drosophila* KC167 cells in response to energy depletion [31]. Thus, there might be additional, AMPK-independent pathways involved in acute energy depletion response. The hypoxia-inducible gene, regulated in development and damage responses 1 (REDD1), is induced by chronic energy depletion, and this in turn leads to inhibition of mTOR Complex1 signaling to S6K1, independent of AMPK [32] (Figure 1). In support of this notion, absence of REDD1 does not alter AMPK activation or its ability to phosphorylate TSC2; however, it ablates the inhibitory effects of chronic energy depletion on mTOR Complex1 signaling to S6K1 [32]. Interestingly, recent studies have linked the negative effects of glucocorticoids on mTOR Complex1 signaling to the induction of REDD1 [33]. The mechanism by which REDD1 mediates the effects of energy depletion on mTOR Complex1 signaling to S6K1 is unknown, but it seems to require TSC2 [32]. Taken together, these data suggest that there are multiple mechanisms by which energy levels impact mTOR Complex1 signaling and much more still needs to be learned (Box 2).

mTOR Complex1–S6K1 signaling in obesity and diabetes

As discussed before, mTOR Complex1–S6K1 integrates various extrinsic signals that regulate cell growth and metabolism. Activation of mTOR Complex1–S6K1 signaling by nutrients has received broad attention because of its implication in obesity and insulin resistance [1,34]. Nutrient overload by increased carbohydrate, fat and/or protein intake leads to obesity, which is characterized by increased adipocyte mass and number. Early experiments with rapamycin provided a link between mTOR Complex1–S6K1 and adipogenesis. In these studies, rapamycin inhibited both clonal expansion and adipocyte differentiation [1]. Recent studies have shown that S6K1-deficient mice exhibit increased lipolysis and reduced adipose tissue mass (Figure 2). They also demonstrated that S6K1-deficient mice are protected from diet- and age-induced obesity [15]. The decreased level of adipogenesis in these mice might be due to a cell-autonomous defect – that is, the failure in transduction of signals induced by adipogenic stimuli such as insulin or by amino acid availability. Another possibility, however, is that the adipogenesis defect in S6K1-deficient mice is not cell autonomous but caused by impaired humoral effects or secondary effects due to loss of S6K1 in early development. These possibilities might be addressed *in vitro* by using S6K1-deficient mouse embryonic fibroblasts (MEFs) and testing their potential to differentiate into adipocytes and *in vivo* by using adipocyte-conditional S6K1 knockout mice.

Interestingly, Cota *et al.* [35] recently demonstrated that leptin is involved in the control of feeding behavior in rats by activating mTOR Complex1 in the hypothalamus. These authors showed that intracerebroventricular administration of leucine results in an increase of mTOR activity and a subsequent decrease in food intake and body weight. Administration of leptin leads to a similar anorectic response, which is blunted by rapamycin [35]. However, it is still not known whether lack of S6K1 in the hypothalamus can elicit similar effects and whether this would affect adipogenesis.

It is well-known that morbidly obese patients are often affected by increased insulin resistance, resulting in the development of type 2 diabetes. Recent studies have shown that insulin resistance can be regulated by mTOR Complex1 activation of S6K1 through a negative-feedback loop. Initial observations found that amino-acid stimulation inhibits insulin-induced class I PI3K signaling, whereas subsequent studies showed that this inhibition is reversed by rapamycin treatment [36]. Studies in *Drosophila* revealed that the S6K1 and S6K2 ortholog, dS6K, is a negative effector of dPKB/dAkt activation, suggesting for the first time that S6K1 or S6K2 regulates PKB/Akt [37,38] In agreement with these findings, insulin receptors desensitize in S6K1-deficient mice maintained on a HFD, but the mice remain exquisitely insulin-sensitive, as does PKB/Akt activation [29]. This suggests that S6K1 elicits a selective inhibitory effect on PKB/Akt activation at a point that is downstream of the insulin receptor (IR). Phosphorylation of IRS1 at sites S307 and S632, which is known

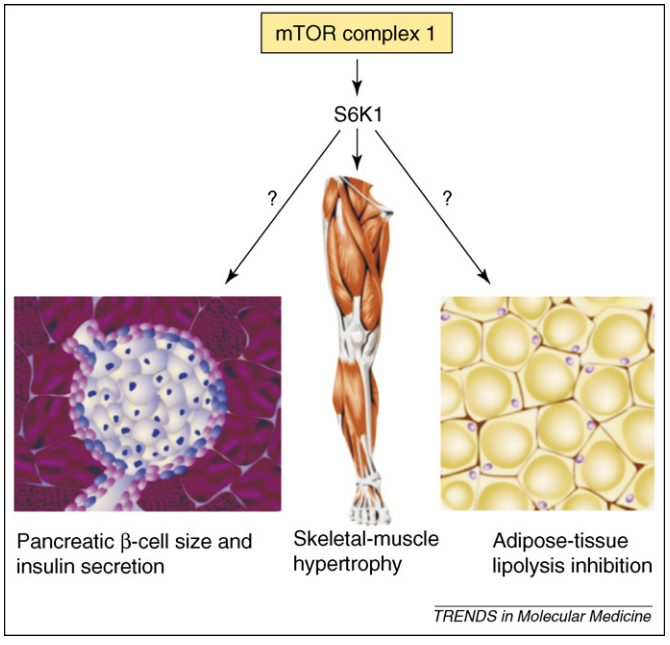

Figure 2. S6K1 function in different tissues. S6K1 has a crucial role in insulin- and nutrient-sensitive tissues in promoting anabolic processes. Although S6K1 promotes skeletal muscle hypertrophy via a cell-autonomous mechanism, there is still uncertainty (?) over cell autonomous versus humoral effects in pancreatic and adipose tissue.

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Table 1. Tumour suppressors that affect the mTOR Complex1–S6K1 pathway

Gene	Syndrome	Molecular function	Refs
TSC1 and *TSC2*	Tuberous sclerosis complex and lymphangioleiomyomatosis	Negative regulator of Rheb	[81]
PTEN	Cowden syndrome and prostate carcinoma	Negative regulator of class I PI3K	[82]
LKB1	Peutz–Jeghers syndrome	Negative regulator of mTOR Complex1 via AMPK	[30]
NF1	Neurofibromatosis 1	Negative regulator of RAS and PI3K–PKB/Akt	[83]
PML	Promyelocytic leukemia	Negative regulator of mTOR Complex1	[84]

to antagonize IRS1 signaling [39], is elevated in animal models of obesity and in muscle from type 2 diabetic patients [40]. The phosphorylation of these sites in S6K1-deficient mice on a HFD and in S6K1 siRNA-treated cells is strongly reduced, suggesting that S6K1 suppresses insulin signaling by mediating IRS1 phosphorylation [15]. In line with this interpretation, PKB/Akt activation is suppressed in WT animals fed a HFD and in two genetic mouse models of obesity, whereas S6K1 activity and the phosphorylation of IRS1 S307 and S632 remains high [15]. Recent studies suggest that S6K1 phosphorylation at a distinct set of sites mediates the phosphorylation of IRS1 S307 and S632 (see below). These findings imply that S6K1 might have a major role in insulin resistance under conditions of nutrient overload [1] (Figure 2).

An analysis of $TSC2^{-/-}$ MEFs, in which Rheb is relieved of inhibition by the TSC1–TSC2 complex and S6K1 is constitutively active, showed that IRS1 is hyperphosphorylated, resulting in its degradation [41–43]. These studies revealed that IRS1 S302, which is proximal to the IRS1 phosphotyrosine-binding (PTB) domain and contains an S6K1-recognition motif, is phosphorylated by S6K1 [41,43]. Phosphorylation of S302 disrupts the ability of the PTB domain to interact with activated IR, leading to decreased insulin signaling [41]. These findings support the model that S6K1 mediates IRS1 serine phosphorylation, disrupting its interaction with IR and leading to its degradation. Recently, Shah and Hunter [42] showed that several sites in IRS1 are direct targets of S6K1 and regulate the ability of an unknown rapamycin-resistant kinase to mediate phosphorylation of serine/proline (SP) sites that are known to be implicated in insulin resistance. It will be of interest to determine whether phosphorylation of the S6K1 sites is abrogated in S6K1-deficient mice and whether phosphorylation at these sites is involved in a predisposition for insulin resistance *in vivo*. Furthermore, the groups led by Lamb [41] and Hunter [43] demonstrated that IRS1 mRNA levels in $TSC2^{-/-}$ cells are reduced and restored by rapamycin treatment, the latter effect being blocked by actinomycin D. In addition, Lamb's group [41] has shown that suppression of S6K1 mimics the effect of rapamycin treatment and restores IRS1 mRNA levels. This raises the question of whether mTOR Complex1–S6K1 signaling contributes to the negative-feedback loop that downregulates PKB/Akt through IRS1 alone or multiple targets. Shah *et al.* [43] have shown that IRS2 protein levels are also reduced in $TSC2^{-/-}$ cells. Thus, in understanding the role of S6K1 in the regulation of insulin signaling, it is necessary to establish the extent to which IRS1 and IRS2 contribute to this response.

mTOR Complex1–S6K1 signaling and cancer

Because of the key role of mTOR Complex1 and S6K1 in cell growth and metabolism, it is reasonable to predict an association between mTOR Complex1 activity and aberrant forms of growth, including cancer. In fact, several of the upstream and downstream components of the mTOR Complex1 pathway are altered in cancer (Table 1 and Table 2). Upregulation and/or mutation of class I PI3K and PKB/Akt, loss of PTEN, mutation of the TSC genes, and upregulation of S6K1 and eIF4E have all been identified in specific cancers [44]. Not surprisingly, rapamycin and its derivatives have been developed and are being pursued in several clinical settings, either as monotherapies or in combination with other anti-cancer agents, with promising results reported in Phase II trials for breast cancer [45] and renal cell carcinoma [46]. In specific settings, such as tuberous sclerosis complex, rapamycin and its derivatives have a pronounced effect [47]. However, given that in nutrient-replete conditions rapamycin and its derivatives are largely cytostatic and not cytotoxic, the highest clinical potential for rapamycin derivatives might be in combination therapy. Consistent with this hypothesis, in a preclinical cell-based assay, the Novartis rapamycin derivative, RAD001, sensitized tumor cells to DNA-damage-induced apoptosis through inhibition of p21 translation [48], providing the basis for testing this combination in upcoming Phase II clinical trials. Similarly, the recent completion of a Phase II clinical trial in which breast-cancer patients were treated with either the aromatase inhibitor letrozole alone or in combination with rapamycin revealed a better progression-free survival rate in the combination therapy [49]. A recent study reported that resistance to the tyrosine kinase inhibitor imatinib (Gleevec, Novartis), which is caused by secondary mutations in

Table 2. Proto-oncogenes that affect the mTOR Complex1–S6K1 pathway

Gene	Syndrome	Molecular function	Refs
PI3K	Ovarian and gastrointestinal cancer	Activates PKB/Akt via PIP3	[85]
PKB/Akt	Breast and ovarian cancer	Activates mTOR Complex1	[86]
RAS	Pancreatic and colon cancer	Activates PI3K	[87]

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the breakpoint cluster region/Abelson proto-oncogene (BCR/ABL) fusion kinase in chronic myelogenous leukemia (CML) patients, seems to be in part mediated by activation of PI3K and mTOR [50]. Therefore, rapamycin treatment of such patients might be useful in helping to resolve tyrosine-kinase-inhibitor resistance.

Consistent with these results, OSI Pharmaceuticals, the developer of the EGF-receptor inhibitor Erolotinib (Tarceva), recently published a synergistic effect of Erlotinib and rapamycin on the activation of PKB/Akt and S6K1 in various tumor-derived cell lines [51]. Similarly, Wang *et al.* [52] have shown synergism between rapamycin and herceptin in slowing the growth of breast cancer cells with high expression of ERBB2 and in reducing tumorigenicity in xenograft models. Recently, Shokat's group [53] has developed a panel of PI3K and PI3K-like inhibitors with the hopes of moving them into the clinic. One of these, a dual class I PI3K and mTOR inhibitor, arrests growth of glioma-xenografted tumors with little-to-no toxic effects [54].

A commonly overlooked aspect of tumor development is the ability of malignant cells to survive in nutrient-deprived settings. Amino-acid and glucose transporters are commonly upregulated in specific tumors [55]. In many primary tumors, the mRNA for the amino-acid transporter large neutral amino acids transporter 1 [LAT1, also known as solute carrier family 7, member 5 (SLC7A5)] is overexpressed [56]. LAT1 represents the light chain of a heterodimer, together with the heavy chain solute carrier family A3, member 2 (SLC3A2, commonly known as CD98), and is selectively involved in the transport of the bulky branched-chain amino acids [57]. Although SLC3A2 forms heterodimers with other proteins that are implicated in cancer, such as integrins, Shishido *et al.* [58] reported that transformation and tumorigenicity of BALB3T3 cells requires overexpression of both SLC3A2 and LAT1. Moreover, Campbell and Thompson [59] have correlated the overexpression of LAT1, observed in hepatocarcinogenesis, with the ability of exogenous LAT1 alone to increase amino-acid transport in primary mouse hepatocytes, but not in fibroblasts. Recently, LAT1 expression has been correlated with poor survival in patients with glioblastomas, and expression of LAT1 was a strong predictor of outcome, independent of other variables [60]. It was also demonstrated in glioma C6 cells and in a xenograft model that the LAT1 inhibitor and leucine analog, 2-aminobicyclo (2.2.1) heptane 2-carboxylic acid (BCH), dose-dependently reduced cell growth. The increased intracellular flux of nutrients in a tumor cell would serve as fuel for the mTOR Complex1–S6K1 pathway, which would drive ribosome biogenesis, cell growth and suppress autophagy [22].

Diabetes, obesity and cancer

Although the link between obesity and diabetes is well established, that of metabolic disease and aberrant cell growth has received less attention. However, recent studies have suggested that obesity is not only a risk factor for diabetes, but also for many cancers [61,62]. In particular, endometrial and colon cancer risk have been positively correlated to the body mass index (BMI) [61]. Similarly, an inverse relationship exists between physical activity and incidence of endometrial, colon and breast cancer [61]. Using a cohort of 900 000 subjects from the USA, Calle *et al.* [63] showed that the heaviest individuals (those with a BMI >40) had cancer death rates 52% (men) and 62% (women) higher than their healthy weight counterparts. Furthermore, the same study attributed up to 14% and 20% of all cancer deaths in man and women, respectively, in the USA to the overweight and obese state. Similar trends have been recorded for large cohorts in Europe and Japan [64,65]. Diabetes itself has been shown to be a risk factor for hepatocellular carcinoma, endometrial, colorectal and breast cancer [66–69]. It is also well-known that, in insulin-resistant states of obesity, there is not only a rise in plasma concentrations of insulin and glucose but also in amino acids, particularly branched-chain amino acids [70–72]. These latter findings are associated with the increased consumption of processed meat over the past 50 years and with high-protein diets with glucose intolerance, insulin resistance and increased incidence of type 2 diabetes [1]. Thus, the diabetic tissues that express insulin receptors and glucose and amino-acid transporters might exist in a proto-oncogenic state. Therefore, a cell predisposed to malignancy in a diabetic would have access to a saturating growth-factor signal (e.g. insulin) and a saturating nutrient environment (e.g. glucose and amino acids). Although insulin receptors might desensitize over time, leading to insulin resistance, glucose and amino acids would still be taken up by peripheral tissues expressing passive nutrient transporters. In addition, in insulin-responsive tissues, the uptake of amino acids, especially the branched-chained amino acids, and glucose are stimulated by insulin [73].

If obesity and diabetes are linked to cancer through the PKB/Akt–mTOR pathway, is it possible to treat one condition using a drug that has been produced for the other? Recently, Shaw *et al.* [74] showed that the common therapeutic agent for diabetes, metformin, activates AMPK through an LKB1-dependent mechanism. Similarly, Zakikhani *et al.* [75] showed activation of AMPK, resulting in downregulation of S6K1 and general translation in several cancer cell types that have been treated with metformin. The activation of AMPK by metformin leads to diminished gluconeogenesis in the liver and enhanced glucose uptake in peripheral tissues. In addition to the effect on LKB1 activity, metformin also seems to inhibit the mitochondrial respiratory chain complex 1, resulting in a high cellular AMP:ATP ratio, which is a potent stimulator of AMPK activity. Although it is known that metformin alleviates diabetic symptoms, the link to the LKB1 tumor suppressor and AMPK suggests that it might also have a therapeutic benefit in a cancer setting. A recent case-control study from record-linkage databases suggested not only that metformin reduces the risk of cancer in diabetic patients but also that protection might be dose-dependent [76,77]. Conversely, there is some indication that treatment of diabetic patients with cancer therapeutics might be beneficial. One study described improved fasting glucose levels in diabetic patients with CML after treatment with imatinib [78]. Similarly, a recent independent case study described regression of type 2 diabetes in a woman with CML also undergoing treatment with imatinib [79]. Finally, a lung cancer patient with type 2 diabetes treated with a Erlotinib

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also experienced improvement in her diabetic condition [80]. It will be of interest to follow future clinical trials that target this pathway in cancer, also focusing on metabolic parameters.

Concluding remarks

It has become clear that the mTOR–S6K1 signaling pathway has a major role in cell growth by integrating growth factor and nutrient cascades. Using various model systems, it was shown that this pathway is essential for cellular homeostasis and that aberrant modulation of this pathway can contribute to obesity, diabetes and cancer. Strong epidemiological links exist between metabolic disorders and cancer, and these links are recapitulated by drug activity observed *in vivo* and *in vitro*. How does the activation of AMPK by metformin result in inhibition of mTOR and S6K1? Are the effects of EGF-receptor inhibition on fasting glucose levels due to reduced PKB/Akt activity, thereby making glucose levels more sensitive to the effects of circulating insulin? Would combination therapy with an inhibitor of mTOR have more dramatic effects on insulin resistance? Further clinical and biochemical investigation is needed to provide answers to these questions (Box 2). Considering the circumstantial biochemical and epidemiological links among obesity, diabetes and cancer, and the mTOR Complex1–S6K1 pathway, these questions will require careful consideration in the future.

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