AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

PHARMACOLOGY OF MAMMALIAN TARGET OF RAPAMYCIN (mTOR)

Patel KN.[1*], Chorawala MR.[1], Shah GB.[1]

1. K. B. Institute of Pharmaceutical Education and Research, Gh-6, Sector-23, Gandhinagar-382023, Gujarat.

ABSTRACT

Mammalian target of rapamycin (mTOR) is an evolutionally conserved serine/threonine kinase that integrates both intracellular and extracellular signals and serves as a central regulator of cell metabolism, growth, proliferation and survival. Interactions of mTOR with regulatory associated protein of TOR or rapamycin insensitive companion of mTOR result in two mTOR complexes, mTOR complex-1 and mTOR complex-2. Here we review several upstream and downstream regulators of mTOR complex-1 and mTOR complex-2. Upstream elements of the mTOR signaling pathway include Ras-homolog enriched in brain, and tuberous sclerosis complex 1 and 2, with tuberous sclerosis complex 2 as the linker between phosphatidylinositol 3-kinase/protein kinase B or Ras-Raf-mitogen-activated protein kinase extracellular signal-regulated protein kinase pathways and the mTOR pathway. Ribosomal protein S6 protein kinase 1 and eukaryotic initiation factor 4E binding protein 1 are currently the 2 best-known downstream effectors of mTOR complex-1 signaling; whereas serum and glucocorticoid induced protein kinase (SGK1) and serine threonine kinase (Akt) are downstream effectors of mTOR complex-2 signaling. Along with this we also discuss role of mTOR in various disease conditions like diabetes, obesity and cancer.

Key words: mammalian target of rapamycin (mTOR), mTORC1, mTORC2, ribosomal S6 kinase 1 (S6K1)

*Corresponding Author Email: kaushalpatel2010@yahoo.in

Received 1 January 2012, Accepted 7 January 2012

Please cite this article in press as: Patel KN *et al.*, Pharmacology of Mammalian Target of Rapamycin (mTOR). American Journal of PharmTech Research 2012.

INTRODUCTION:

In yeast, during a screen for resistance to the drug sirolimus, the target of rapamycin genes (TOR 1 and TOR 2) were first discovered[1]. This discovery was quickly followed by the identification and characterization of mammalian target of rapamycin (mTOR), also known as FKBP12-rapamycin associated protein (FRAP), or rapamycin and FKBP12 target RAFT. mTOR is a ubiquitous atypical serine/threonine kinase that belongs to phospho-inositide 3-kinase (PI3K)-related kinase family, which integrates signals from nutrients, growth factors, hormones (Insulin, Insulin like growth factor, leptin), cellular energy stores, oxygen level, and other cues to control a variety of critical cellular processes, including growth, proliferation, differentiation, transcription, cytoskeletal organization, autophagy and mRNA translation.

Structural Domains and Motifs in mTOR

mTOR is made up of 2549 amino acids organized in several structural domains. The N-terminal portion of mTOR contains multiple HEAT (huntington, elongation factor 3, A subunit of type 2A protein phosphatase (PP2A), and TOR). FAT [FRAP-FKBP 12 rapamycin associated protein, ATM-ataxia telangiectasia mutated, and transformation=transcription domain associated protein (TRRAP)]. FRB- the FKBP12-rapamycin-binding domain binds the rapamycin /FKBP12 complex or phosphatidic acid [2, 3]. FAT c-terminal domains flank the kinase domain. These all are required for kinase activity and appear to cooperatively bind certain mTOR-interacting proteins [4]. Highly conserved and homologous to that in ATM and ATR, the c-terminal kinase domain (PI3-PI4-kinase) contains the serine=threonine kinase active site. (Figure 1)

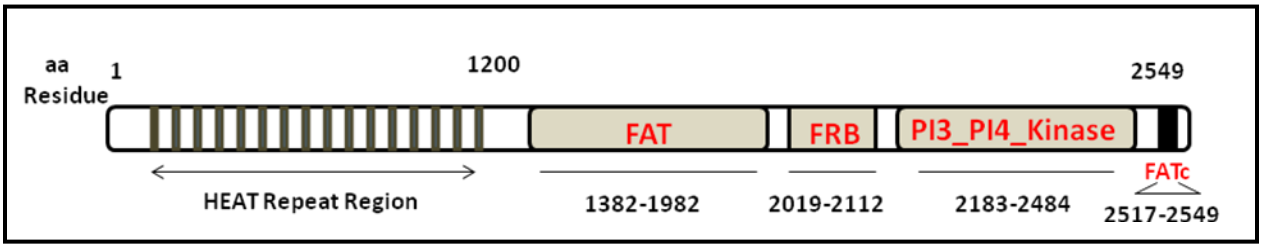

Figure 1: structural domains and motifs in mTOR.

HEAT = Huntington, elongation factor 3, A subunit of type 2A protein phosphatase (PP2A), and TOR; FAT = FRAP-FKBP 12 rapamycin associated protein, ATM-ataxia telangiectasia mutated, and transformation=transcription domain associated protein (TRRAP); FRB = FKBP12-rapamycin-binding domain binds the rapamycin /FKBP12 complex or phosphatidic acid; PI3-PI4-kinase = phosphoinositol 3-phosphoinositol 4 kinase; FATc = FAT c terminal domain.

Two mTOR containing complexes exist in mammalian cells were reviewed by Guertin [5]. Details of same are as follows.

mTORC1 and mTORC2

mTORC1

mTORC1 is composed of five components and is regulated by growth factors and nutrients. mTOR, which is the catalytic subunit of complex, Regulatory associated protein of mTOR (Raptor), Mammalian lethal with sec 13 protein 8 (mLST 8, also known as GβL-G protein β subunit like protein), Proline rich AKT substrate 40 Kda (PRAS 40), DEP-domain containing mTOR-interacting protein (Deptor) [6].The exact function of most of mTOR-interacting proteins in mTORC1 still remains to be disclosed. Raptor acts as an adaptor protein by recruiting the mTOR substrates eukaryotic initiation factor 4E (eIF4E)-binding proteins 1(4E-BP1), Ribosomal S6 kinase (S6K) and PRAS 40 through a Tor signaling (TOS) motif[1]. mTOR mediated phosphorylation of both S6K and 4E-BP1 require raptor and mutation within the TOS motif of 4E-BP1 cancel this phosphorylation [1]. mLST8 or GβL binds to the kinase domain of mTOR and by stabilizing the mTOR receptor interaction, it strongly enhances mTOR signaling. PRAS 40 and Deptor have been reported as distinct negative regulators of mTORC1 [6, 7,8] It was proposed that PRAS 40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding [9]. Activation of mTORC1 directly phosphorylates PRAS 40 and deptor, which reduces their physical interaction with mTORC1 and further activates mTORC1 signaling[6, 9]. The antifungal compound rapamycin, via interaction with the immunophilin FK506 binding protein of (FKBP 12), inhibits mTORC1 function[5]. Mutation of conserved serine (aa 2035 in human mTOR) in the FRB domain confers resistance to rapamycin[2]. Deregulation of mTOR signaling has been associated with various pathological conditions such as diabetes, obesity and cancer.

mTORC2

mTORC2 consists of six different proteins. Several of which are common to mTORC1 and mTORC2. mTOR, rapamycin insensitive companion of mTOR (Rictor), mammalian stress activated protein kinase interacting protein (mS1N1), protein observed with rictor-1 (Protor-1), mLST8 or GβL and Deptor. Rictor and mS1N1 stabilize each other, establishing structural foundation of mTORC2 [10, 11]. Rictor also interacts with protor-1, but the physiological function of this interaction is not clear [12, 13]. Like in mTORC1 deptor negatively regulates mTORC2 activity [6]. In contrast to mTORC1, rapamycin can not physically interact with or acutely inhibit mTORC2 [14,15]. So, mTORC2 has been characterized as rapamycin insensitive complexes. mTORC2 regulates cytoskeletal organization, cell survival, metabolism and proliferation and is insensitive to nutrients.

mTOR; Its Interacting Proteins and Phosphorylation Sites

mTOR interacts with Tel 2 (also known as TELO 2 and hcLK 2) and FXBW7. Tel 2 deletion results in destabilization of mTOR, where as FXBW7 targets mTOR for ubiquitin-proteasome-dependent deregulation [16, 17]. Interaction of mTOR with raptor occurs through binding to the N-terminal HEAT domain. Rictor, a specific component of mTORC2 may compete with raptor in binding to mTOR through the HEAT domain and thereby disrupts mTOR-Raptor interaction. Deptor, an inhibitory protein for mTOR binds through the FAT domain of mTOR, and over expression of deptor results in the suppression of S6K by inhibiting Akt, where as loss of deptor activates S6K and Akt. (Figure 2)

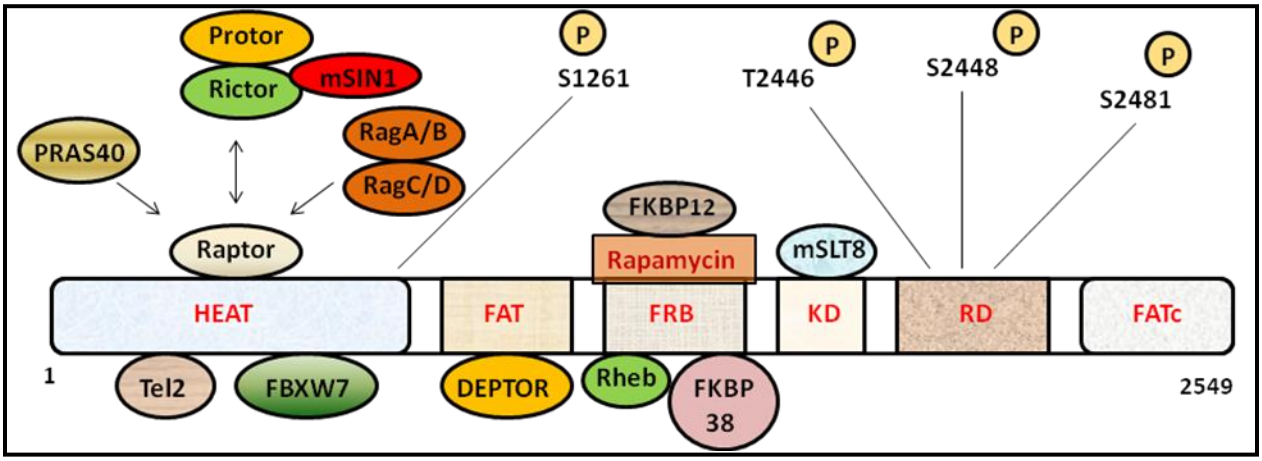

Figure 2: mTOR, its interacting proteins and phosphorylation sites

FBXW7 = F box and WD repeat domain containing 7 (also known as hcdc 4, FBW7 and hAGO); Protor = protein observed with rictor-1; KD = kinase domain; Rag = ras related GTP-binding protein; RD = regulatory domain; Raptor = Regulatory associated protein of mTOR; Rictor = rapamycin insensitive companion of mTOR; PRAS 40 = Proline rich AKT substrate 40 Kda; mLST 8 = Mammalian lethal with sec 13 protein 8; Rheb = Ras homolog enriched in brain; Tel2 = telangectasia 2; S1261, T2446, S2448, and S2481 are phosphorylation sites of mTOR.

FKBP 38, a mitochondrial membrane protein is an endogenous inhibitor of mTOR. It inhibits mTOR through binding to its FRB domain. FKBP 38 interaction with mTOR is increased by amino acid or serum starvation, leading to inhibition of mTOR activity. The Rheb GTPase protein also binds to the FRB domain of mTOR and is involved in mTOR activation by amino acids. 4 members of Rag subfamily of Ras small GTPase-Rag A, Rag B, Rag C and Rag D-binds to raptor directly upon amino acid stimulation. This Rag-bound mTORC1 translocates to Rab 7 (a GTPase required for transporter degradation) –positive perinuclear vesicular structures, where Rheb localizes. This relocalization enables mTORC1 kinase activation. Regulator recruits Rag family and mTORC1 to lysosomes to activate mTORC1[18]. mLST8 knockout studies

demonstrated that it is required to maintain the Rictor-mTOR, but not the Raptor-mTOR interaction despite of the fact that both mTORC1 and mTORC2 contain mLST8, indicating the mTORC2 specific role of mLST8[19].

Phosphorylation of mTOR

There are four characterized phosphorylation sites in mTOR. T2446 is regulated by nutrient availability and is probably phosphorylated by adenosine monophosphate kinase (AMPK). S2448 is phosphorylated by S6K, which directly reflects amino acid and nutrient status. S2481 is known as a rapamycin insensitive autophosphorylation site. mTORC1 contains mainly mTOR phosphorylated at S2448, whereas mTORC2 contains predominantly mTOR phosphorylated at S2481 [20]. There is some evidence that mTORC1 also contains mTOR phosphorylated on S2481 in the same cell line and that the phosphorylation on S2481 in both mTORC1 and mTORC2 is sensitive to wartmannin, a phosphatidylinositol 3 kinase inhibitor, indicates that insulin signals via phosphatidylinositol 3 kinase to promote mTORC1 and mTORC2 associated mTOR autophosphorylation on S2481 [21]. S1261 is phosphorylated by insulin/phosphatidylinositol 3 kinase in an amino acid dependent, rapamycin insensitive and autophosphorylation independent manner.S1261 phosphorylation stimulates the phosphorylation of S6K and 4E-BP1, and mutation on S1261 attenuates phosphorylation on S2481.

REGULATION OF mTOR ACTIVITY

Upstream regulation of mTORC1

The presence of extracellular (or systemic) and intracellular nutrients are sensed by mTORC1 and stimulates its activation. One of the most important sensors involved in mTORC1 activity is the tuberous sclerosis complex (TSC), which is a heterodimer that comprises TSC 1 (also known as hamartin) and TSC 2 (also known as tuberin). TSC 1\2 functions as a GTPase activation protein (GAP) for the small Ras related GTPase Rheb (Ras homolog enriched in brain). GTP bound form of Rheb directly interacts with mTORC1 and stimulates its activity [22, 7]. The exact mechanism by which Rheb activates mTORC1 still remains to be determined. TSC 1\2 converts Rheb into its inactive GDP-bound state and thus negatively regulates mTORC1 signaling [23, 24]. Loss of heterozygosity or inactivating mutation of TSC 1\2 give rise to tuberous sclerosis, a disease associated with presence of numerous benign tumors that are composed of enlarged and disorganized cells [25].

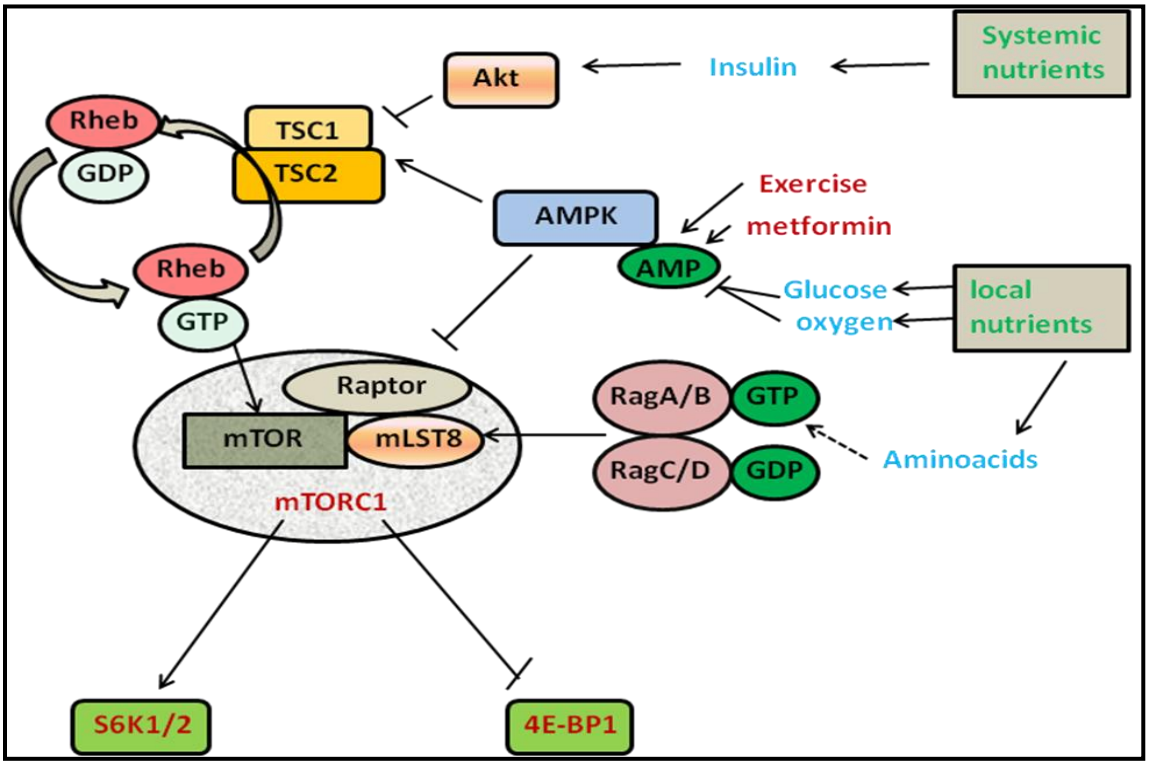

Figure 3: Nutrient sensing and upstream regulation of mTORC1.

Lines with end arrows indicate activation, whereas those with perpendicular bars at the end indicate inhibition. Rheb = Ras homolog enriched in brain; TSC1 = tuberous sclerosis complex-1; TSC2 = tuberous sclerosis complex-2; Rag = ras related GTP-binding protein; mLST 8 = Mammalian lethal with sec 13 protein 8; S6K1 = ribosomal S6 kinase; 4EBP1 = eukaryotic initiation factor 4E (eIF4E)-binding proteins 1.

Growth factors stimulate mTORC1 through the activation of the canonical insulin and Ras signaling pathways. The stimulation of these pathways increases the phosphorylation of TSC2 by Akt, by extracellular signal regulated kinase 1\2 (ERK1\2), and by p90 ribosomal S6 kinase 1 (RSK1), and leads to the inactivation of TSC1\2, and hence activation of mTORC1[26, 27, 28, 29]. The binding of insulin to its cell surface receptor results in starting of number of events, including recruitment of insulin receptor substrate 1 (IRS 1), production of phosphatidylinositol (3,4,5)-triphosphate [Ptd Ins (3,4,5) P3] through the activation of PI3K, and the recruitment and activation of Akt at the plasma membrane. Akt causes phosphorylation of TSC 2 and inhibits it activation. So, accumulation of Rheb-GTP occurs, which in turn activates mTORC1 signaling. Additionally, activation of Akt by growth factors can activate mTORC1 in a TSC 1\2 independent manner by promoting the phosphorylation and dissociation of PRAS 40 from mTORC1 [7, 8, 9].

Intracellular level of AMP is affected by energy status of cell, which in turn influenced by nutrients and other factors. AMP functions as an activation of AMPK, a master sensor of intracellular energy status signaled to mTORC1 about energy status of the cell. Upon low energy status (low ATP:ADP ratio), AMPK is activated in LKB 1 (also known as serine-threonine kinase 11) dependent manner and phosphorylates TSC 2 on sites distinct from Akt, which in turn increases the GAP activity of TSC 2 towards Rheb and diminish mTORC1 activation [30]. AMPK, upon energy depletion also phosphorylate Raptor (at S792) of mTORC1, hence reduce its activity.

mTORC1 activity is also depends on oxygen levels. AMPK is activated in mild hypoxic conditions, which in turn inhibits mTORC1 signaling as described above. Lower oxygen levels can also activate TSC 1\2 via transcriptional regulation of DNA damage response 1 (REDD1) [31, 32]. REDD1 release TSC 2 from its growth factor induced association with 14-3-3 proteins and hence blocks mTORC1 signaling [33]. This ability of REDD1 has probably evolved to limit energy consuming processes when oxygen, but not growth factors, is rare. Low oxygen levels can also activate promyelocytic leukemia (PML) tumor suppressor and BCL 2/adenovirus E1B 19 Kda protein interacting protein 3 (BNIP3), which reduce mTORC1 signaling by disrupting the interaction between mTOR and its positive regulator Rheb [34].

Proinflammatory cytokines, such as TNF-α activates IKB kinase-b (IKKb), which in turn phosphorylate and inactivates TSC 1, leading to activation of mTORC1 [35]. So inflammation mediated mTORC1 activation is thought to be important in tumor angiogenesis and in the development of insulin resistance [36]. Glycogen synthase kinase 3 (GSK 3) causes phophorylation of TSC 2 and hence, activates TSC 1\2 activity. GSK3 is inhibited upon stimulation of wnt pathway. So, GSK mediated phosphorylation of TSC 2 is block and hence TSC 1\2 activity. Consequently there is an increase mTORC1 activity[37]. Another activator of mTORC1 is phosphatidic acid (PA). Upon growth factor stimulation, the intracellular level of PA increases via phospholipase D activity (PLD 1 and PLD 2). It was shown that this activation is mediated by PLD 1, which is activated by growth factors via the small GTPase, cdc42 [38]. PA binds to FRB domain and by facilitating the assembly of mTOR complexes or stabilizing the complexes, activates mTOR signaling [39, 40].

The intracellular cascades through which amino acid (AA) signal to mTORC1 differ from those controlled by insulin [41]. It was demonstrated that AA regulation of mTORC1 signaling occurs via interactions with its kinase domain, but not the FRB domain used for binding by rapamycin

[42]. Thus, direct inhibition by rapamycin may have little effect on AA-related regulations on metabolism and growth mediated by mTOR signaling. Four mechanisms of AA stimulation, as shown in Figure 4 are now described. First, AA can stimulate the TSC1-TSC2 complex directly or indirectly with the reduced TSC2 inhibition of Rheb, leading to increased phosphorylation of mTORC1 [43, 44]. Second, it also was demonstrated that class 3 PI3K, also called Vps34, the oldest member of the PI3K family, can act through FYVE (abbreviation for Fab1p, YOTB, Vac1p, and early endosome antigen 1 domain containing proteins) and phox homology domain-containing proteins, and FYVE or phox homology domain-containing proteins can then directly or indirectly activate mTORC1 signaling [45].

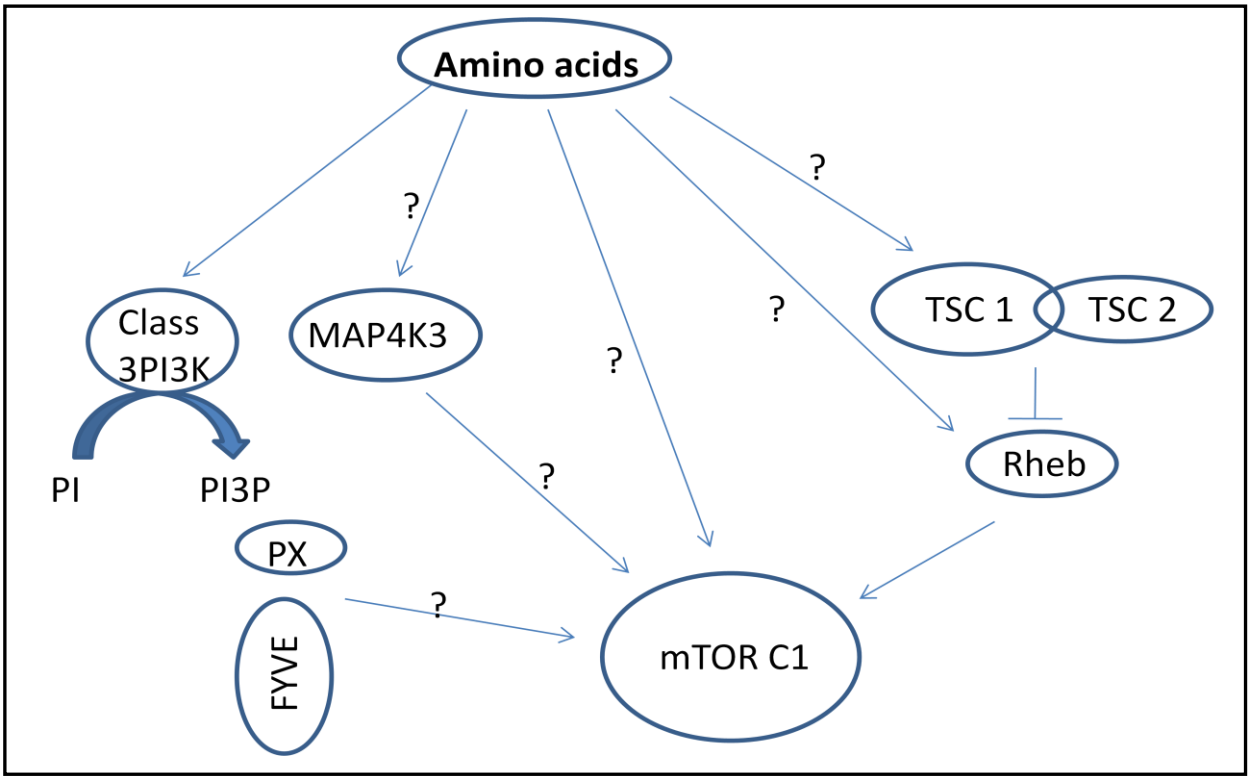

Figure 4: The proposed mechanisms of AA availability in regulating the mammalian target of rapamycin (mTOR)-signaling pathway for mediating metabolism and growth [45-47].

Lines with end arrows indicate activation, whereas those with perpendicular bars at the end indicate inhibition. Question marks imply that the steps are unclear or poorly defined. FYVE = Fab1p, YOTB, Vac1p, and EEA1 (early endosome antigen 1) domain-containing proteins; MAP4K3 = MAP (mitogen-activated protein) kinase 3, a sterile 20-protein family protein kinase (also referred to as germinal center-like kinase, GLK); PI = phosphatidylinositol; PI3P = phosphatidylinositol-3-phosphate; PX = phox homology domain-containing proteins; Rheb = Ras homolog enriched in brain; TSC1 = tuberous sclerosis complex 1; and TSC2 = tuberous sclerosis complex 2.

Third, tRNA aminoacylation and eIF4G (eukaryotic initiation factor 4G) phosphorylation are also proposed as being involved in AA regulation of mTOR signaling [48, 49]. Fourth, a recent

study by Findlay demonstrated that the protein kinase mitogen-activated protein kinase 3 (MAP4K3), also known as germinal center-like kinase, is an upstream AA-sensitive regulator of mTORC1 signaling [47]. However, how MAP4K3 or germinal center-like kinase activates mTORC1 is not clear at this time.

There are also some other negative regulator of mTOR signaling pathway such as GADD34 (growth arrest and DNA damage 34) is induced by almost all cellular stresses and binds and dephosphorylates TSC2. GADD342/2 cells are more sensitive to glucose starvation and virus infection than WT cells, resulting in apoptosis due to inability to suppress the mTOR pathway [50]. Treatment of rapamycin indeed suppresses apoptosis in GADD342/2 cells, suggesting that stress stimuli inhibit the mTOR pathway through the GADD34-TSC2 axis. Recently, an acetylation-mediated TSC2 regulation was reported [51]. ARD1 (arrest defective protein 1), an acetyl transferase and a putative tumor suppressor, binds, acetylates, and stabilizes TSC2, leading to inhibition of the mTOR pathway. The expression of ARD1 correlates with that of TSC2 in multiple tumor types, and loss of heterozygosity at the ARD1 locus was observed in human breast, lung, pancreatic, and ovarian cancer samples. KSR2, a regulator of extracellular signal-regulated kinase 1/2 (a mitogen-activated protein kinase), binds and modulates the activity of AMPK. KSR2 regulates AMPK-dependent glucose uptake and fatty acid oxidation, and KSR2 (kinase suppressor of Ras 2) knockout mice show decreased fatty acid oxidation and thermogenesis resulting in obesity [52].

Three sestrin family proteins inhibit the mTOR pathway through the AMPK-TSC2 axis. p53 target genes Sestrin1 and Sestrin2 are induced on oxidative stress and DNA damage and bind and activate AMPK, resulting in TSC2-dependent inhibition of the mTOR pathway [53]. The expression of mammalian Sestrin3 is regulated by Akt in a FOXO3a-dependent manner and activates AMPK and regulates cellular reactive oxygen species accumulation [54, 55]. Increased reactive oxygen species caused by accelerated oxygen consumption in Akt1/2 knockout cells was reduced by knockdown of Sestrin3, indicating that Sesn3 plays an important role in the regulation of cellular reactive oxygen species mediated by Akt and FOXO [55]. Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD1)–dependent deacetylase that has been implicated in regulation of the mTOR pathway. Although SIRT1 deacetylates LKB1 for the LKB1-AMPK activation, AMPK may regulate SIRT1 activity by increasing intracellular NAD1. AMPK kinase activity is required to trigger SIRT1-dependent response to exercise and fasting, but it remains unknown whether AMPK is required for fasting-induced activation of SIRT1 and deacetylation

of its targets [56]. Further studies will be required to determine the interdependence of AMPK and SIRT1 on the mTOR pathway.

Downstream regulation of mTORC1

In mammals, two main classes of mTORC1 substrates have emerged [57]. The ribosomal S6 kinases (S6K1 and S6K2) and the eukaryotic initiation factor 4E (eIF4E)-binding proteins (4EBP1 and 4EBP2). mTORC1 phosphorylates the hydrophobic motif on the S6Ks (T389 on the 70-KDa isoform of S6K1), which is essential for subsequent activating phosphorylation events. Activation of S6K1 leads to increases in mRNA biogenesis, cap dependent translation and elongation, and the translation of ribosomal proteins through regulation of the activity of many proteins, such as S6K1 aly/REF-like target (SKAR), programmed cell death 4 (PDCD4), eukaryotic elongation factor 2 kinase (eEF2K) and ribosomal protein S6. Also 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. mTORC1 has been shown to promote ribosome biogenesis by stimulating the transcription of ribosomal RNA through a process involving the protein phosphatase 2A(PP2A) and the transcription initiation factor IA (TIF-IA) [58]. mTORC1 exerts a more direct control on translation through its regulation of the 4EBPs, which bind to eIF4E at the 5'-cap of mRNAs, and block translation initiation. mTORC1 phosphorylates the 4EBPs on multiple residues, triggering their release from eIF4E, enabling eIF4E to promote cap dependent translation [59]. (Figure 5)

mTORC1 have predominant role in controlling autophagy. mTORC1 inhibition increases autophagy, whereas stimulation of mTORC1 reduces autophagy [60]. Recently, it is discovered that mTORC1 controls autophagy through the regulation of a protein complex composed of unc-51-like kinase 1 (ULK 1), autophagy related gene 13 (ATG13) and focal adhesion kinase family interacting protein of 200 KDa (FIP 200) [61]. These studies have revealed that mTORC1 represses autophagy by phosphorylating and thereby repressing ULK 1 and ATG 13.

mTORC1 regulates lipid synthesis by positively regulates the activity of sterol regulatory element binding protein 1 (SREBP1) and of peroxisome proliferator activated receptor-γ (PPAR-γ), two transcription factors that control the expression of genes encoding proteins involved in lipid and cholesterol homeostasis [62,63]. The molecular mechanism of SREBP1 activation by mTORC1 is unknown. Additionally, rapamycin reduces the phosphorylation of lipin-1, a phosphatidic acid (PA) phosphatase that is involved in glycerolipid synthesis and in the

coactivation of many transcription factors linked to lipid metabolism, including PPAR γ, PPAR α and PGC1α (PPARγ coactivator 1α) [64]. The precise impact of lipin-1 phosphorylation on lipid synthesis remains to be established.

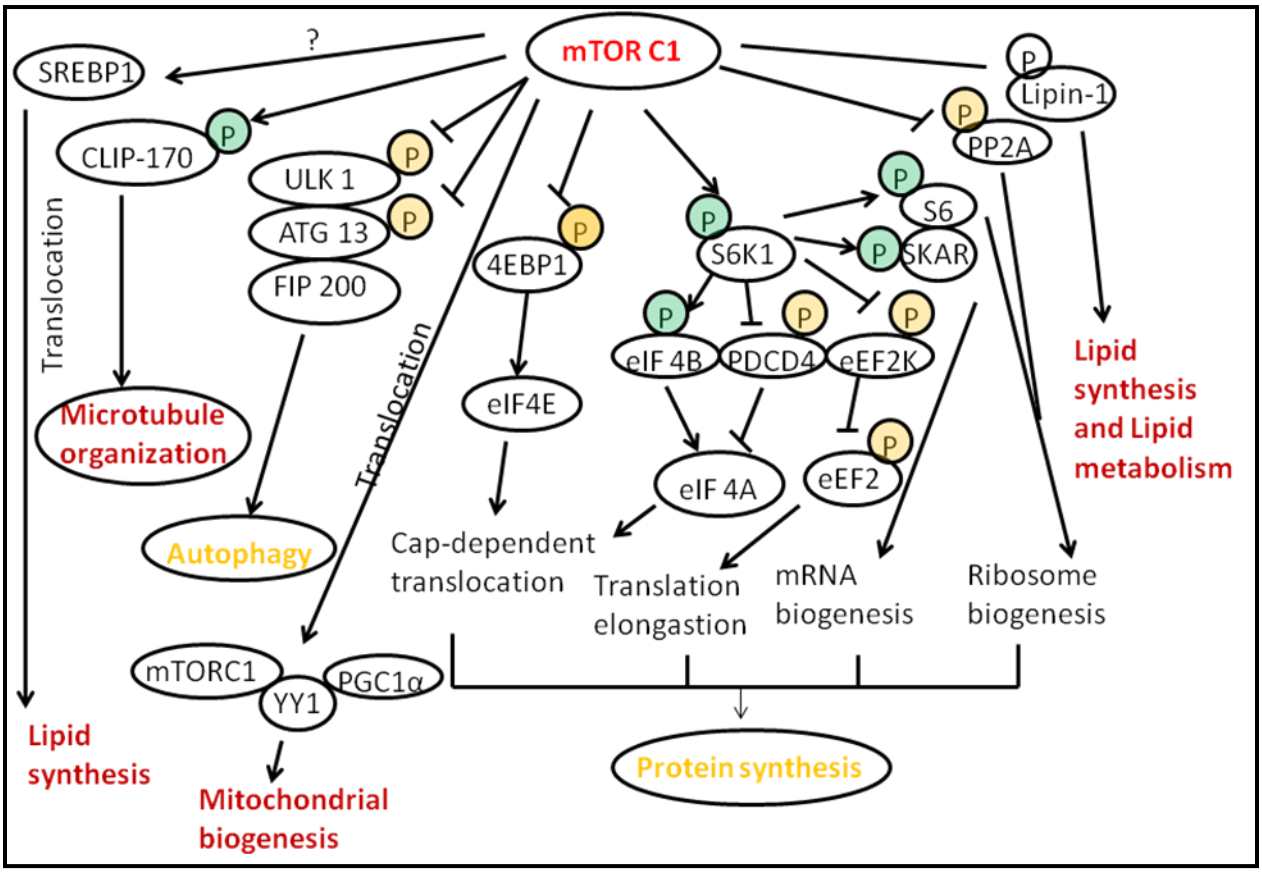

Figure 5: mTORC1 downstream signaling.

Lines with end arrows indicate activation, lines with perpendicular bars at the end indicate inhibition, whereas simple line indicate unknown function and Question mark imply that the step is unclear or poorly defined. ULK1 = unc-51-likekinase 1; ATG 13 = autophagy related gene 13; FIP 2000 = focal adhesion kinase family interacting protein of 200 KDa; SREBP1 = sterol regulatory element binding protein 1; CLIP-17 = CAP-GLY domain containing linker protein 1; YY1 = yin-yang 1; PGC1α = PPARγ coactivator 1α; 4EBP1 = eukaryotic initiation factor 4E (eIF4E)-binding proteins 1; eIF4E = eukaryotic initiation factor 4E; S6K1 = ribosomal S6 kinase 1; PDCD4 = programmed cell death 4; eEF2 = eukaryotic elongation factor 2; eEF2K = eEF 2 kinase; SKAR = S6K1 aly/REF-like target; PP2A = protein phosphatase 2A.

Mitochondrial metabolism and biogenesis are both regulated by mTORC1. Rapamycin lowers mitochondrial membrane potential, oxygen consumption and cellular ATP levels, and preferentially alters the mitochondrial phosphoproteome through inhibition of mTORC1 [65]. Mitochondrial DNA copy number accompanied with proteins involved in oxidative metabolism are reduced by rapamycin and increase by mutation that activate mTORC1 signaling [66, 67].

mTORC1 by directly altering physical interaction of PGC 1α with yin-yang 1 (YY1), an another transcription factor controls mitochondrial biogenesis and oxidative metabolism [67].

mTORC1 signaling also enhances the translation of hypoxia-inducible factor (HIF1a), which can transcriptionally activate genes encoding glucose transporters and glycolytic enzymes [68,69,70,71,72]. In this way mTORC1 activation can promote glucose uptake and flux through glycosis [73].

Upstream regulation of mTORC2

With growth-factor stimulation, AKT is phosphorylated at the cell membrane through the binding of PtdIns(3,4,5)P3 to its pleckstrin homology (PH) domain. Under these conditions, PDK1(phosphoinositide dependent kinase 1) is also recruited to the membrane through its PH domain and phosphorylates AKT at Ser308 [74]. Interestingly, the mTORC2 component mSIN1 possesses a PH domain at its C-terminus, suggesting that mSIN1 can promote the translocation of mTORC2 to the membrane and the phosphorylation of AKT at Ser473. Additional work is needed to support this model and to identify other cellular signals that play a role in the regulation of mTORC2. Growth factors could signal to mTORC2 via TSC1-TSC2 complex. TSC 1-TSC 2 complex- a GTPase activating protein (GAP) that lies upstream of and negatively regulates mTORC1, also regulates mTORC2 function by directly binding mTORC2[75]. In contrast to the negative regulation of mTORC1 by TSC 1-TSC2, TSC 1-TSC2 is thought to positively regulate mTORC2 activity in a GAP-independent manner. The GTPase Rheb, which lies directly downstream of TSC1-TSC2 and activates mTORC1, does not appear to lie upstream of mTORC2 [76]. The observation that TSC1-TSC2 GAP activity is not required for mTORC2 activation suggests that activation does not occur via mTORC1 and the negative feedback loop (a hallmark of activated mTORC1 signaling). However, the way in which TSC1 –TSC2 binding regulates mTORC2 activity, as well as potential GAP-independent activities for TSC1– TSC2, remain poorly understood.

Syndecan-4, which is a single-pass transmembrane proteoglycan, recruits PKCα to the plasma membrane and thereby regulates activity. This, in turn, is required for appropriate mTORC2 localization to the rafts and subsequent AKT activation. However, the mechanism by which PKCα regulates mTORC2 recruitment is unknown, and discovery efforts are complicated by the fact that PKCα is known to be a downstream target of mTORC2. Although there will be some insights into how mTORC2 might be regulated, it will be a major breakthrough to identify upstream regulators of the TORC2 signaling branch, in yeast and mammals.

Downstream regulation of mTORC2

mTORC2 enhances phosphorylation of PKCα (protein kinase Cα), phosphorylates paxillin and its relocalization to focal adhesions, and GTP loading of RhoA and Rac1. These all are responsible for controlling actin cytoskeleton, and hence control cytoskeletal organization. Deletion of mTORC2 results in alteration of actin polymerization and cell morphology[77, 78]. Molecular mechanism by which mTORC2 regulates these processes has remain to be determined.

Cell survival, metabolism and proliferation are highly dependent on activation status of Akt, an upstream positive regulator of mTORC1 [79]. Phosphorylation of Akt at ser 308 and ser 473 by phosphjoinositide dependent kinase 1 (PDK1) and mTORC2 must be required for its full activation respectively [80]. Reduction in the phosphorylation status of forkhead box o1 (FOXO1) and FOXO3a transcription factor following mTORC2 depletions suggests its role in Akt activation. These above mentioned transcription factors control the expression of genes involved in stress resistance, metabolism, cell cycle arrest and apoptosis [81]. Now, on other hand the phosphorylation state of TSC2 and GSK3 is unaffected by mTORC2 inactivation. Recently,

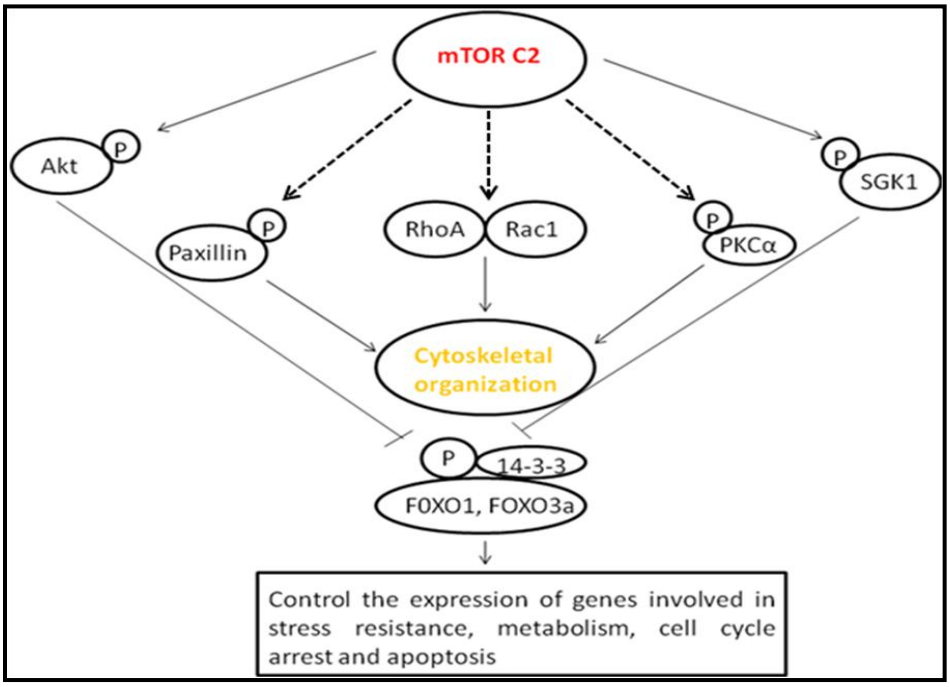

Figure 6: Downstream of mTORC2.

Lines with end arrows indicate activation, lines with perpendicular bars at the end indicate inhibition, doted lines indicate indirect evidence. SGK1 = serum and glucocorticoid induced protein kinase 1; FOXO1 = forkhead box o1; FOXO3a = forkhead box o1/3a.

serum and glucocorticoid induced protein kinase 1 (SGK1), which shares homology with Akt has been shown to be regulated by mTORC2 [82]. In contrast to Akt, SGK1 activity is totally lost upon

mTORC2 inhibition. SGK1 and Akt phosphorylate FOXO1 and FOXO3a on same sites. So in mTORC2 deficient cells it is possible that the lack of SGK1 activity is responsible for the inhibition of phosphorylation of FOXO1 and FOXO3a. (Figure 6)

mTORC1 SIGNALING IN VARIOUS DISORDERS

As discussed earlier, mTORC1–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 [83, 84]. Nutrient overload by increased carbohydrate, fat and protein intake leads to obesity, which is characterized by increased adipocyte mass and number. Early experiments with rapamycin provided a link between mTORC1–S6K1 and adipogenesis. In these studies, rapamycin inhibited both clonal expansion and adipocyte differentiation [83]. Recent studies have shown that S6K1-deficient mice exhibit increased lipolysis and reduced adipose tissue mass.

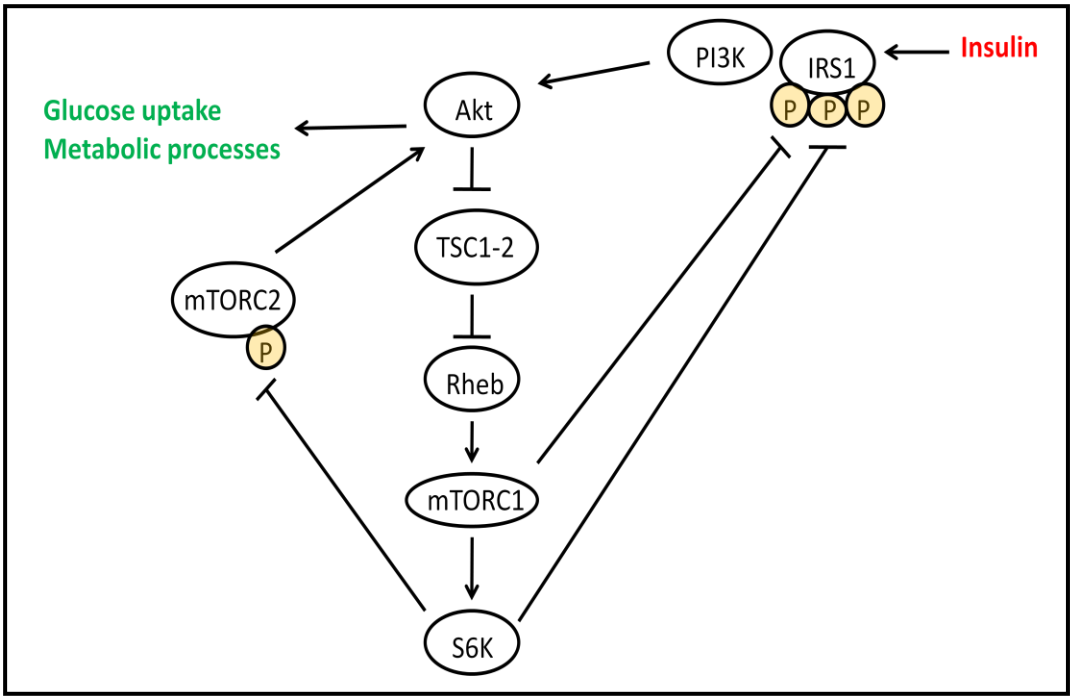

Figure 7: mTORC1 signaling attenuates Akt activation through negative feedback mechanism.

Lines with end arrows indicate activation, lines with perpendicular bars at the end indicate inhibition. Akt = Serine threonine kinase; TSC1-2 = tuberous sclerosis complex 1-2; Rheb = Ras homologue enrich in brain; S6K = Ribosomal S6 kinase; PI3K = Phosphatidyl inositol 3 kinase; IRS1 = Insulin receptor substrate 1.

They also demonstrated that S6K1-deficient mice are protected from diet and age induced obesity [85]. 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, it is recently established that leptin is involved in the control of feeding behavior in rats by activating mTOR C1 in the hypothalamus [86]. These authors showed that intra-cerebroventricular 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 [86]. 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 [87]. 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 [88]. 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 [89]. 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 to antagonize IRS1 signaling, is elevated in animal models of obesity and in muscle from type 2 diabetic patients [90, 91]. 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 [85]. 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 [85]. Recent studies suggest that S6K1 phosphorylation at a distinct set of sites mediates the phosphorylation of IRS1 S307 and S632. These findings imply that S6K1 might have a major role in insulin resistance

under conditions of nutrient overload [83]. 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 [92, 93]. 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. Phosphorylation of S302 disrupts the ability of the PTB domain to interact with activated IR, leading to decreased insulin signaling [92]. These findings support the model that S6K1 mediates IRS1 serine phosphorylation, disrupting its interaction with IR and leading to its degradation. Recently, it is observed 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 [94]. 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, Lamb and Hunter demonstrated that IRS1 mRNA levels in TSC2$^{-/-}$ cells are reduced and restored by rapamycin treatment, the latter effect being blocked by actinomycin D [92, 93]. In addition, Lamb's group has shown that suppression of S6K1 mimics the effect of rapamycin treatment and restores IRS1 mRNA levels. This raises the question of whether mTOR C1–S6K1 signaling contributes to the negative-feedback loop that down regulates PKB/Akt through IRS1 alone or multiple targets. It has shown that IRS2 protein levels are also reduced in TSC2$^{-/-}$ cells [93]. 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.

Because of the key role of mTORC1 and S6K1 in cell growth and metabolism, it is reasonable to predict an association between mTORC1 activity and aberrant forms of growth, including cancer. In fact, several of the upstream and downstream components of the mTORC1 pathway are altered in cancer. Up regulation and/or mutation of class I PI3K and PKB/Akt, losses of PTEN, mutation of the TSC genes, and up regulation of S6K1 and eIF4E have all been identified in specific cancers. (Table 1)

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 anticancer agents, with promising results reported in Phase II trials for breast cancer and renal cell carcinoma [95, 96]. In specific settings, such as tuberous sclerosis complex, rapamycin and its derivatives have a pronounced effect [97]. 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 (Everolimus), sensitized tumor cells to DNA-damage-induced apoptosis through inhibition of p21 translation, providing the basis for testing this combination in upcoming Phase II clinical trials [98].

Table 1: Regulators of mTOR signaling and translation factors implicated in human cancers [1].

Protein	Cellular function	Genetic alteration	Clinical outcome
PI3K P85/p110	Positive regulator of Akt/mTOR signaling	Over-expression Over-activation	Head and neck, ovarian cancer
PTEN	Negative regulator of PI3K signaling	Loss/Mutation	Glioblastoma, breast, prostate, endometrial cancer, cowden syndrome
Akt	Positive regulator of mTOR	Over-expression Over-activation	Breast, ovarian cancer
TSC1/TSC2	Negative regulator of mTOR	Loss/mutation	Tuberous sclerosis complex: Hamartomas, renal cancer
S6K	Positive regulator of translation	Over-expression	Breast cancer
STK11 (LKB1)	Regulator of AMPK	Loss/mutation	Peutz-jeghers syndrome: colorectal polyps, breast, testicular cancer
eIF4E	Translation factor	Over-expression	Colon, breast, bladder cancer
eIF4G	Translation factor	Over-expression	Lung cancer
eIF4A	Translation factor	Over-expression	Melanoma
eIF4E-BP1	Repressor of eIF4E	Increase in phosphorylation	Ovarian, breast, prostate cancer

Similarly, the 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 [99]. A recent study reported that resistance to the tyrosine kinase inhibitor imatinib (Gleevec, Novartis), which is caused by secondary mutations in 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 [100]. 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 [101]. Similarly, Wang 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 [102]. Recently, Shokat's group 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 [103].

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 [104]. 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 over-expressed [105]. 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 [106]. Although SLC3A2 forms heterodimers with other proteins that are implicated in cancer, such as integrins. Shishido reported that transformation and tumorigenicity of BALB3T3 cells requires over expression of both SLC3A2 and LAT1 [107]. Moreover, Campbell has correlated the over expression 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 [108]. 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 [109]. It was also demonstrated in glioma C6 cells and in a xenograft model that the LAT1 inhibitor and leucine analog, 2-aminobicyclo 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 mTORC1–S6K1 pathway, which would drive ribosome biogenesis, cell growth and suppress autophagy [110].

Temsirolimus (CCI-779) and deforolimus (AP23573) are the novel derivatives of sirolimus. Temsirolimus treated cancer in phase 1, phase 2 and phase 3 clinical trial are renal, breast, lung; breast, endometrial, glioblastoma, lung, mental cell lymphoma; renal cell carcinoma respectively [1]. Temsirolimus is approved by FDA in the treatment of advanced breast cancer. Deforolimus treated cancer in phase 1 and phase 2 clinical trials are solid tumors, glioblastoma, renal cell, sarcoma; advanced sarcoma, hematological malignancies [1].

FUTURE ASPECTS

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 such as, endometrial and colon cancer [111,112]. 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, it has been showed that the common therapeutic agent for diabetes, metformin, activates AMPK through an LKB1-dependent mechanism [113]. Similarly, it has been shown that activation of AMPK, resulting in downregulation of S6K1 and general translation in several cancer cell types that have been treated with metformin [114]. 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 [115,116]. 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 [117]. Similarly, a recent independent case study described regression of type 2 diabetes in a woman with CML also undergoing treatment with imatinib [118]. Finally, a lung cancer patient with type 2 diabetes treated with a Erlotinib also experienced improvement in her diabetic condition [119]. It will be of interest to follow future clinical trials that target this pathway in cancer, also focusing on metabolic parameters.

REFERENCES:

1. Dowling R, Pollak M, Sonberg N. Current status and challenges associated with targeting mTOR for cancer therapy. Biodrugs 2009; 23(2):77-91.
2. Chen J, Zheng XF, Brown EJ, Schreiber SL. Identification of an 11-kDa FKBP12-rapamycin binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. Proc Natl Acad Sci USA 1995; 92:4947–51.

3. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science 1996; 273:239–42.
4. Jacinto E, Lorberg A. TOR regulation of AGC kinases in yeast and mammals. Biochem J 2008; 410:19–37.
5. Guertin DA, Sabatini DM. Defining the Role of mTOR in Cancer. Cancer Cell 2007; 12: 9-22.
6. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, et al. Deptor is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell 2009; 137:873-86.
7. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, et al. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell 2007; 25: 903-915.
8. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ. Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol 2007; 9:316-23.
9. Wang L, Harris TE, Roth RA, Lawrence JC. PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. J Biol Chem 2007; 282:20036-44.
10. Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, et al. mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. Curr Biol 2006; 16:1865-70.
11. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, et al. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 2006; 127:125-37.
12. Thedieck K, Polak P, Kim ML, Molle KD, Cohen A, Jeno P, et al. PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. PLoS ONE 2007; 2(11): e1217.
13. Woo SY, Kim DH, Jun CB, Kim YM, Haar EV, Lee SI, et al. PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor beta expression and signaling. J Biol Chem 2007; 282:25604-612.
14. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol 2004; 6:1122-28.

15. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin insensitive and raptor independent pathway that regulates the cytoskeleton. Curr Biol 2004; 14: 1296-1302.\
16. Takai H, Wang RC, Takai KK, Yang H, Delange T. Tel2 regulates the stability of PI3K-related protein kinases. Cell 2007; 131:1248–59.
17. Mao JH, Kim IJ, Wu D, Climent J, Kang HC, Delrosario R, Balmain A. FBXW 7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. Science 2008; 321:1499–1502.
18. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 2010; 141:290–303.
19. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCαlpha, but not S6K1. Dev Cell 2006; 11(6): 859–71.
20. Copp J, Manning G, Hunter T. TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser2 481 is a marker for intact mTOR signaling complex 2. Cancer Res 2009; 69:1821–27.
21. Soliman GA, Acosta-Jaquez HA, Dunlop EA, Ekim B, Maj NE, Tee AZ**.** mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action. J Biol Chem 2010; 285:7866–79.
22. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. Rheb binds and regulates the mTOR kinase. Curr Biol 2005; 15:702-13.
23. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell 2003; 115:577-90.
24. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol 2003; 13:1259-68.
25. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. N Engl J Med 2006; 355:1345-56.
26. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 2002; 4:648-57.

27. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. Nat Cell Biol 2002; 4:658-65.
28. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. Cell 2005; 121:179-93.
29. Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. Proc. Natl Acad Sci USA 2004; 101:13489-494.
30. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 2009; 10:307–18.
31. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, et al. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Dev Jr 2004; 18:2893-2904.
32. Reiling JH, Hafen E. The hypoxia induced paralogs Scylla and Charybdis inhibit growth by down regulating S6K activity upstream of TSC in Drosophila. Genes Dev 2004; 18: 2879-92.
33. DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. Genes Dev 2008; 22:239-51.
34. Bernardi R, Guernah I, Jin D, Grisendi S, Alimonti A, Teruya-Feldstein J, et al. PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. Nature 2006; 442:779-85.
35. Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, Wei Y, et al. IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. Cell 2007; 130:440-55.
36. Lee DF, Kuo HP, Chen CT, Wei Y, Chou CK, Hung JY, Yen CJ, Hung MC. IKKbeta suppression of TSC1 function links the mTOR pathway with insulin resistance. Int J Mol Med 2008; 22:633-38.
37. Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell 2006; 126:955-68.

38. Fang Y, Park IH, Wu AL, Du G, Huang P, Frohman MA, et al. PLD1 regulates mtor signaling and mediates cdc42 activation of S6K1. Curr Biol 2003; 13:2037-44.
39. Fang Y, Vilella-Bach M, Bachmann R, Flanigan A and Chen J. Phosphatidic acid mediated mitogenic activation of mTOR signaling. Science 2001; 294:1942-45.
40. Toschi A, Lee E, Xu L, Garcia A, Gadir N, Foster DA. Regulation of mTORC1 and mTORC2 complex assembly by phosphatidic acid: competition with rapamycin. Mol Cell Biol 2009; 29:1411-20.
41. Phamp TT, Heydrick SJ, Fox HL, Kimball SR, Jefferson LS, Lynch CJ. Assessment of cell-signaling pathways in the regulation of mammalian target of rapamycin (mTOR) by amino acids in rat adipocytes. J Cell Biochem 2000; 79:427–41.
42. Edinger AL, Linardic CM, Chiang GG, Thompson CB, Abraham RT. Differential effects of rapamycin on mammalian target of rapamycin signaling functions in mammalian cells. Cancer Res 2003; 63:8451–60.
43. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr 2006; 136:227S–31S.
44. Long X, Ortiz-Vega S, Lin Y, Avruch. Rheb binding to mammalian target of rapamycin (mTOR) is regulated by amino acid sufficiency. J Biol Chem 2005; 280:23433–436.
45. Nobukuni T, Kozma SC, Thomas G. hvps34, an ancient player, enters a growing game: mTOR Complex1/S6K1 signaling. Curr Opin Cell Biol 2007; 19:135–41.
46. Kimball SR. Interaction between the AMP-activated protein kinase and mTOR signaling pathways. Med Sci Sports Exerc 2006; 38:1958–64.
47. Findlay GM, Yan L, Procter J, Mieulet V, Lamb RF. A MAP4 kinase related to Ste20 is a nutrient-sensitive regulator of mTOR signalling. Biochem J 2007; 403:13–20.
48. Iiboshi Y, Papst PJ, Kawasome H, Hosoi H, Abraham RT, Houghton PJ, et al. Terada N. Amino acid-dependent control of p70s6k: Involvement of tRNA aminoacylation in the regulation. J Biol Chem 1999; 274:1092–99.
49. Bolster DR, Vary TC, Kimball SR, Jefferson LS. Leucine regulates translation initiation in rat skeletal muscle via enhanced eIF4G phosphorylation. J Nutr 2004; 134:1704–10.
50. Minami K, Tambe Y, Watanabe R, Isono T, Haneda M Isobe K, et al. Suppression of viral replication by stress-inducible GADD34 protein via the mammalian serine/threonine protein kinase mTOR pathway. J Virol 2007; 81(20):11106–115.

51. Kuo HP, Lee DF, Chen CT, Liu M, Chao-Kai C, Lee HJ, et al. ARD1 stabilization of TSC2 suppresses tumorigenesis through the mTOR signaling pathway. Sci Signal 2010; 3(108):ra9.
52. Costanzo-Garvey DL, Pfluger PT, Dougherty MK, Stock JL, Boehm M, Chaika O, et al. KSR2 is an essential regulator of AMP kinase, energy expenditure, and insulin sensitivity. Cell Metab. 2009; 10(5):366–78.
53. Budanov AV, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell 2008; 134:451–60.
54. Chen CC, Jeon SM, Prashanth TB, Nogueira V, Deepa S, Tonic I, et al. FoxOs inhibit mTORC1 and activate Akt by inducing the expression of Sestrin3 and Rictor. Dev Cell 2010; 18(4):592–604.
55. Nogueira V, Park Y, Chen CC, Xu PZ, Chen ML, Tonic I, et al. Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. Cancer Cell 2008; 14(6):458–70.
56. Canto C, Lake QJ, Deshmukh AS, Mataki C, Agnes C, Marie L, et al. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab 2010; 11(3):213–19.
57. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 2009; 10:307–18.
58. Mayer C, Zhao J, Yuan X, Grummt I. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. Genes Dev 2004; 18:423-34.
59. Richter JD, Sonenberg N. Regulation of cap-dependent translation by eIF4E inhibitory proteins. Nature 2005; 433:477-80.
60. Codogno P, Meijer AJ. Autophagy and signaling: their role in cell survival and cell death. Cell Death Differ 12 Suppl 2005; 2:1509-18.
61. Ganley IG, Lamdu H, Wang J, Ding X, Chen S, Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 2009; 284:12297-305.
62. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab 2008; 8:224-36.

63. Kim JE, Chen J. Regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. Diabetes 2004; 53: 2748-2756.
64. Huffman TA, Mothe-Satney I, Lawrence JC. Insulin-stimulated phosphorylation of lipin mediated by the mammalian target of rapamycin. Proc Natl Acad Sci USA 2002; 99: 1047-52.
65. Schieke SM, Phillips D, McCoy JP, Jr Aponte AM., Shen RF, Balaban RS, et al. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. J Biol Chem 2006; 281:27643-652.
66. Chen C, Liu Y, Liu R, Ikenoue T, Guan KL, Liu Y, et al. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J Exp Med 2008; 205:2397-2408.
67. Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. Nature 2007; 450:736-40.
68. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, et al. Modulation of hypoxia-inducible factor 1 expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000; 60(6):1541– 45.
69. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) Signaling increases the rate of hypoxia inducible factor 1{alpha} (HIF-1{alpha}) synthesis: novel mechanism for hif-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001; 21(12):3995–4004.
70. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, et al. Regulation of hypoxia-inducible factor 1{alpha} expression and function by the mammalian target of rapamycin . Mol Cell Biol 2002; 22(20):7004–14.
71. Semenza GL, Roth PH, Fang HF, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 1994; 269: 23757–763.

72. Hu CJ, Wang LY, Chodosh LA, Keith B, Celeste MS. Differential roles of hypoxia-inducible factor 1{alpha} (HIF-1{alpha}) and HIF-2{alph a} in hypoxic gene regulation. Mol Cell Biol 2003; 23:9361–74.
73. Duvel K, Yecies JL, Menon S, Pichai R, Lipovsky AI, Souza AL, et al. Activation of a metabolic gene regulatory network downstream of mTOR Complex 1. Mol Cell 2010; 39(2):171–83.
74. Lawlor MA, Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J. Cell Sci 2001; 114:2903-10.
75. Huang J, Dibble CC, Matsuzaki M, Manning BD. The TSC1-TSC2 complex is required for proper activation of mTOR complex 2. Mol Cell Biol 2001; 28(12):4104–15.
76. Manning BD, Cantley LC. Rheb fills a GAP between TSC and TOR. Trends Biochem Sci 2003; 28:573–76.
77. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, et al. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 2006; 127:125-37.
78. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 2004; 14:1296-1302.
79. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell 2007; 129: 1261-74.
80. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005; 307:1098-1101.
81. Calnan DR, Brunet A. The FoxO code. Oncogene 2008; 27:2276-88.
82. Garcia-Martinez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum and glucocorticoid-induced protein kinase 1 (SGK1). Biochem J 2008; 416:375-85.
83. Um SH, D'Alessio D, George T. Nutrient overloads insulin resistance and ribosomal protein S6 kinase 1. Cell Metabol 2006; 3(6):393–402.
84. Patti ME. Kahn BB. Nutrient sensor links obesity with diabetes risk. Nat Med 2004; 10:1049–50.

85. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, et al. Absence of S6K1 protects against age and diet induced obesity while enhancing insulin sensitivity. Nature 2004; 431:200–05.
86. Cota D, Proulx K, Blakesmith KA, Kozma SC, George T, Seeley R, et al. Hypothalamic mTOR signaling regulates food intake. Science 2006; 312:927–30.
87. Nobukuni T, Kozma SC, George T. Hvps34, an ancient player, enters a growing game: mTOR Comp lex1/S6K1 signaling. Curr Opin Cell Biol 2007; 19:135–41.
88. Radimerski T, Montagne J, Rintelen F, Stocker H, Van-der-kaay J, George T, et al. dS6K-regulated cell growth is dPK B/ dPI(3)K-independent, but requires dPDK1. Nat Cell Biol 2002; 4:251–55.
89. Corradetti MN, Inoki K, Bardeesy N, Depinho RA, Guan KL. Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz–J eghers syndrome. Genes Dev 2004; 18(13):1533–38.
90. Zick Y. Uncoupling insulin signaling by serine/threonine phosphorylation: a molecular basis for insulin resistance. Biochem. Soc Trans 2004; 32:812–16.
91. Bouzakri K, Roques M, Gual P, Espinosa S, Guebre-Egziabher F, Jean-Paul R, et al. Reduced activation of phosphatidylinositol- 3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of skeletal muscle cells from patients with type 2 diabetes. Diabetes 2003; 52:1319–25.
92. Harrington LS, et al. The TSC 1-2 tumor suppressor controls insulin–PI3K signaling via regulation of IRS proteins. J Cell Biol 2004; 166:213–23.
93. Shah OJ, Wang J, Hunter T. Inappropriate activation of the TSC/Rhe b/ mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr Biol 2004; 14(18):1650–56.
94. Shah OJ, Hunter T. Turnover of the active fraction of IRS1 involves raptor–mTOR- and S6K1-dependent serine phosphorylation in cell culture models of tuberous sclerosis. Mol Cell Biol 2006; 26:6425–34.
95. Chan S, Scheulen ME, Johnston S, Mross K, Cardoso F, Dittrich C, et al. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR , in heavily pretreated patients with locally advanced or metastatic breast cancer. J Clin Oncol 2005; 23:5314–22.
96. Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, et al. Randomized phase II study of multiple dose levels of CCI -779, a novel mammalian

target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol 2004; 22(5):431–32.

97. Franz DN, Leonard J, Tudor C, Chuck G, Care M, Sethuraman G, et al. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. Ann Neurol 2006; 59(3):490–98.
98. Beuvink I, Boulay A, Fumagalli S, Ziblbermann F, Ruetz S, Reilly TO, et al. The mTOR inhibitor RAD001 sensitizes tumor cells to DNA- damaged induced apoptosis through inhibition of p21 translation. Cell 2005; 120(6):747–59.
99. Chollet P, Abrial C, Tacca O, Durando X, Cure H, Leheurteur M, et al. Mammalian target of rapamycin inhibitors in combination with letrozole in breast cancer. Clin Breast Cancer 2006; 7(4):336–38.
100. Burchert A, Wang Y, Cai D, von Bubnoff N, Paschka P, Müller-Brüsselbach S, et al. Compensatory PI3-kinase/Akt/mtor activation regulates imatinib resistance development. Leukemia 2005; 19:1774–82.
101. Buck E, Eyzaquirre A, Brown E, Petti F, Mccormack S, Haley JD, et al. Rapamycin synergizes with the epidermal growth factor receptor inhibitor erlotinib in non-small-cell lung, pancreatic, colon, and breast tumors. Mol Cancer Ther 2006; 5(11):2676–84.
102. Wang LH, Chan JL, Li W. Rapamycin together with herceptin significantly increased anti-tumor efficacy compared to either alone in ErbB2 over expressing breast cancer cells. Int J Cancer 2007; 121(1):157-64.
103. Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, stokoe D, et al. A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. Cancer Cell 2006; 9(5): 341–49.
104. Macheda ML Rogers S, Best JD. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J Cell Physiol 2005; 202(3):654–62.
105. Fuchs BC, Bode BP. Amino acid transporters ASCT2 and LAT1 in cancer: partner sin crime? Semin. Cancer Biol 2005; 15:254–66.
106. Mastroberardino L, Spinder B, Pfeiffer R, Skelly PJ, Loffing J, Shoemaker CB, et al. Amino-acid transport by heterodimers of 4F2hc/CD 98 and members of a permease family. Nature 1998; 395:288–91.
107. Shishido T, Uno S, Kamohara M, Tsuneoka-Suzuki T, Hashimoto Y, Enomoto T, et al. Transformation of BALB3T3 cells caused by over-expression of rat CD98 heavy chain

(HC) requires its association with light chain: missense mutation in a cysteine residue of CD98HC eliminates its transforming activity. Int J Cancer 2000; 87(3):311–16.

108. Campbell WA, Thompson NL. Over expression of LAT1/ CD98 light chain is sufficient to increase system L-amino acid transport activity in mouse hepatocytes but not fibroblasts. J Biol Chem 2000; 276:16877–884.
109. Nawashiro H, Otani N, Shinomiya N, Fukui S, Ooigawa H, Shima K, et al. L-type amino acid transporter 1 as a potential molecular target in hum an astrocytic tumors. Int J Cancer 2006; 119:484–92.
110. Dann SG, Thomas G. The amino acid sensitive TOR pathway from yeast to mammals. FEBS Lett 2006; 580:2821–29.
111. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004; 4:579–91.
112. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004; 127:S27–S34.
113. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 2005; 310:1642–46.
114. Zakikhani M, Dowling RJ, Fantus IG, Pollak M, Sonberg N. Metformin inhibitis mammalian target of rapamycin dependent translation initiation in breast cancer cells. Cancer Res 2007; 67:10804–812.
115. Evans JM, Ogston SA, Emslie-Smith A, Morris AD. Risk of mortality and adverse cardiovascular outcomes in type 2 diabetes: a comparison of patients treated with sulfonylureas and metformin. Diabetologia 2006; 49:930–36.
116. Evans JM, Louise AD, Alistair ME, Dario RA, Andrew DM. Metformin and reduced risk of cancer in diabetic patients. Bio Med J 2005; 330:1304–05.
117. Breccia M, Muscaritoli M, Aversa Z, Mandelli F, Allimena G. Imatinib mesylate may improve fasting blood glucose in diabetic Ph+ chronic myelogenous leukemia patients responsive to treatment . J Clin Oncol 2004; 22:4653–55.
118. Veneri D, Massimo F, Enzo B. Imatinib and regression of type 2 diabetes. N Engl J Med 2005; 352:1049–50.
119. Costa DB, Huberman MS. Improvement of type 2 diabetes in a lung cancer patient treated with Erlotinib. Diabetes Care 2006; 29:1711.