J Mol Med (2015) 93:1061–1073
DOI 10.1007/s00109-015-1326-7

JMolMed

MOLECULES IN MEDICINE

mTOR in health and in sickness

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Received: 25 May 2015 / Revised: 14 July 2015 / Accepted: 22 July 2015 / Published online: 22 September 2015

Abstract Target of rapamycin (TOR) is a highly conserved protein kinase that plays a key role in mediating cell growth and homeostasis. It is activated by nutrients, growth factors, and cellular energy levels to control a number of anabolic and catabolic processes. It is a validated drug target implicated in a variety of diseases. In this review, we describe the molecular mode of action of TOR in the context of cellular and organismal physiology. We focus on mammalian TOR (mTOR) signaling in cancer and neurological disease and discuss usage of TOR inhibitors in the clinic.

Keywords mTOR · Health · TOR · mTORC1 · mTORC2 · mTOR inhibitors · Rapamycin · Rapalogs · Disease

mTOR in physiology

mTOR and its complexes

Target of rapamycin (TOR) was discovered in 1991 in the yeast *Saccharomyces cerevisiae* using a genetic selection designed to find genes that confer resistance to rapamycin, a macrolide produced by a bacterium isolated from soil samples collected on Easter Island [1]. The selection identified three genes, *FPR1*, *TOR1*, and *TOR2* [1]. *FPR1* encodes FK506 binding protein 12 (FKBP12), a peptidylprolyl isomerase that acts as an intracellular "receptor" for rapamycin and is thus essential for rapamycin action. *TOR1* and *TOR2* encode the two yeast TOR proteins [2, 3]. TOR orthologues were subsequently identified in all other eukaryotic species which unlike yeast have only one TOR protein. Mammalian TOR (mTOR), identified in 1994, is a 290-kDa protein that is an atypical serine/threonine kinase belonging to the phosphoinositide kinase-related kinase (PIKK) family [4, 5]. mTOR forms two structurally and functionally distinct complexes, mammalian TOR complex 1 (mTORC1) and mammalian TOR complex 2 (mTORC2) [6–8] (Fig. 1). The core subunits of mTORC1 are regulatory-associated protein of mTOR (RAPTOR) and mammalian lethal with SEC13 protein 8 (mLST8). mTORC1 signaling is rapamycin sensitive. The core subunits of mTORC2 are rapamycin-insensitive companion of mTOR (RICTOR), mammalian stress-activated protein kinase interacting protein 1 (mSIN1), and mLST8. mTORC2 is insensitive to acute rapamycin treatment but is indirectly sensitive to long-term treatment [6, 9]. Non-essential components of the mTOR complexes include proline-rich AKT substrate (PRAS40), protein observed with RICTOR (PROTOR1/2), and DEP domain containing mTOR interacting protein (DEPTOR). PRAS40 associates with RAPTOR and appears to be a negative regulator of mTORC1 [10, 11]. It has been shown to be phosphorylated by AKT, as well as mTORC1, and released from mTORC1 upon insulin stimulation [10]. Consistent with PRAS40 being a negative regulator of mTORC1, liver of PRAS40 knockout mice displays increased S6K phosphorylation [12]. PROTOR1 and PROTOR2 associate with RICTOR to negatively regulate mTORC2 [13]. DEPTOR has been reported to bind mTOR in both mTORC1 and mTORC2 and thereby to negatively regulate both complexes [14]. DEPTOR is also phosphorylated and negatively regulated by mTOR [14].

Structurally mTOR protein is organized in domains conserved throughout the PIKK family members [15]. The N-terminal half of mTOR contains ~20 tandemly repeated

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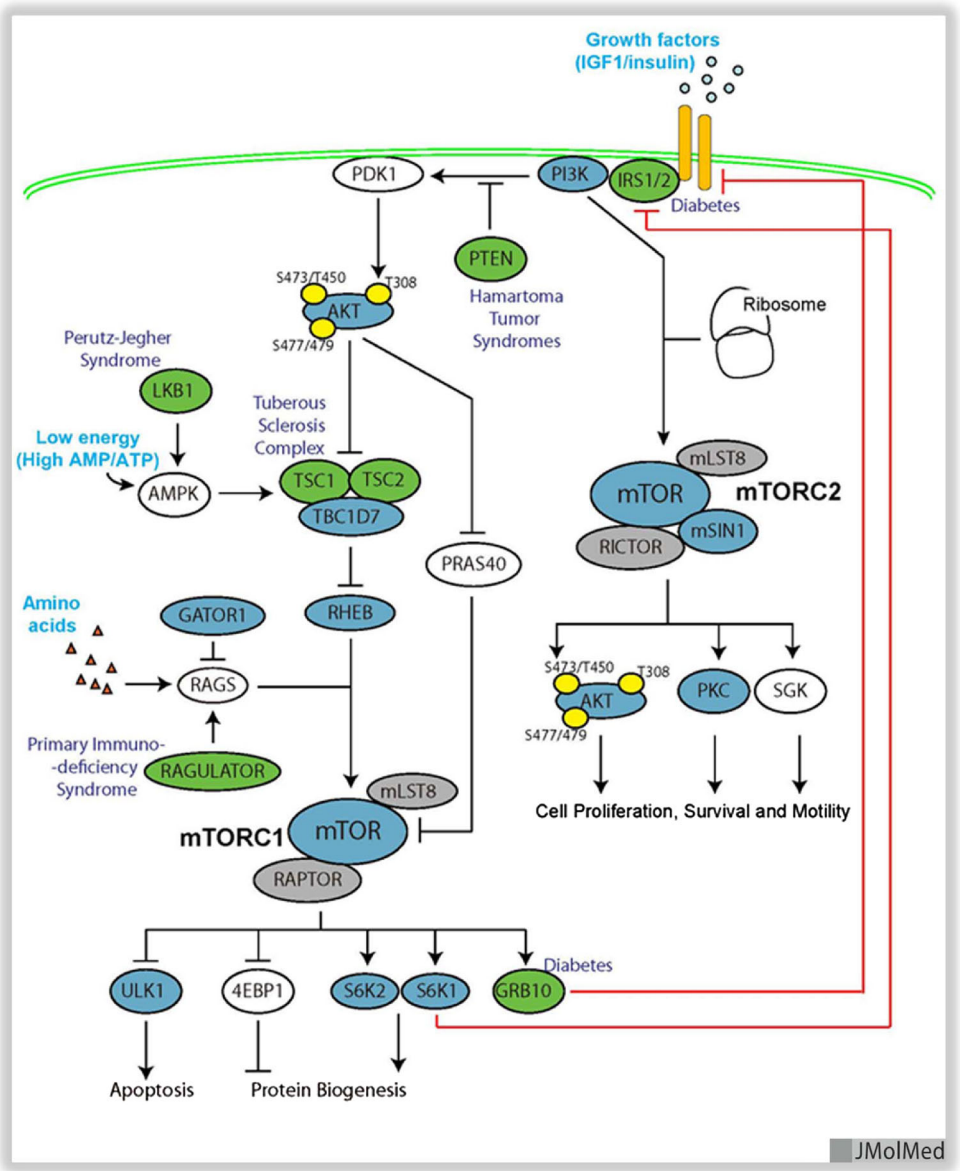

Fig. 1 mTOR signaling and disease. Mutated mTORC1 and mTORC2 signaling nodes involved in disease. For simplicity, only the core components of the mTOR complexes and major signaling nodes are depicted. *Yellow circles* on AKT display phosphorylation events: T308 by PDK1, T450/S473 by mTORC2, and S477/S479 by Cdk2-cyclin A [144–147]. The *green-colored* nodes represent proteins that upon loss-of-function give rise to disease. The syndromes associated with loss-of-function of each molecule are noted [148–150, 76, 95, 151, 152, 96, 153–155]. Loss-of-function of PTEN and LKB1 also gives rise to other types of tumors [96, 153]. The *blue-colored* nodes represent proteins that are mutated or amplified in cancer patients. [38, 156, 157, 84, 158–161, 154, 162–164]

Huntingtin, EF3, PP2A and TOR (HEAT) motifs that generally favor protein–protein interactions and are required for RAPTOR or RICTOR binding [16]. The HEAT repeats are followed by a FRAP, ATM, and TRRAP (FAT) domain and the kinase domain. The latter comprises the FKBP12-rapamycin binding (FRB) domain, the catalytic site that is structurally related to phosphoinositide kinases, and the FAT-C terminal (FATC) domain [16]. The crystal structure of the mTOR catalytic region, including the FRB, FAT, catalytic, and FATC domains, in complex with mLST8 was recently solved [17]. The mTOR kinase domain, like in other PIKKs, consists of two lobes that form a catalytic cleft. This cleft is flanked N-terminally by the FRB domain and C-terminally by the FATC domain and the mLST8 binding element (LBE). Unlike in other PIKKs, the catalytic domain in mTOR displays a constitutively active conformation where activity is dependent on substrate access. The active conformation is stabilized by the FAT domain which was modeled as a "half-donut" structure that girdles the backside of the catalytic cleft. Substrate access is directly affected by the positioning of the FRB domain and the interactions between FATC and LBE with the catalytic cleft [17].

The FKBP-rapamycin complex binds the FRB domain on the surface closest to the catalytic cleft, restricting access of certain substrates [18]. FKBP-rapamycin binds mTOR in mTORC1 but cannot bind mTOR in mTORC2. However, long-term rapamycin treatment could affect mTORC2 via sequestering free mTOR and thereby inhibiting mTORC2 assembly [19, 8]. A recent report attributed mTORC2 rapamycin (in)sensitivity to the cellular levels of FK binding proteins FKBP12 and FKBP51 [20]. A higher FKBP12/FKBP51 ratio appears to increase mTORC2 sensitivity

toward rapamycin. This finding could have consequences on the use of rapamycin in the clinic. Other mTOR inhibitors, such as Torin 1/2 or PP242, are ATP analogs that bind the ATP pocket to inhibit both mTORC1 and mTORC2 directly. Torin 1/2 and PP242 are selective for mTOR, as opposed to phosphoinositide 3-kinase (PI3K) and other PIKK members, as their key interactions are thought to involve non-conserved amino acids in the catalytic cleft [17].

Low-resolution 3D cryo-EM structures of mTORC1 and TORC2 in yeast have also been reported [21, 22]. These structures confirm that both complexes are dimers and show that they form a rhomboid shape with a central cavity. In mTORC1, each mTOR terminus interacts with a distinct RAPTOR molecule, forming the dimer interface [22]. RAPTOR binding at that C-terminus of mTOR, close to the kinase domain, is consistent with a role for RAPTOR in affecting substrate access. In this model, mLST8 forms contacts with only one mTOR molecule and is positioned away from the main RAPTOR-mTOR core [22]. In physiological conditions, mTORC1 is found almost exclusively as a dimer [23]. Dimerization of mTORC1 can be a regulated event, as for example upon energetic stress due to glucose and glutamine deprivation. However, overall amino acid levels or insulin stimulation does not affect dimerization [23, 24]. As TOR exerts its activity as a dimer, further elucidation of how mTOR complex dimerization can be controlled is of interest. The structure of yeast TORC2, while similar in shape to the structure of mTORC1, appears to be bigger with a bigger central cavity [21]. In the case of TORC2, the dimer interface appears to be smaller than that of mTORC1 and is thought to consist of interactions between Adheres VOraciously 1 (AVO1) (the yeast homologue of mSIN1) and the N-terminus of TOR2. Furthermore, unlike in mTORC1, LST8 is located close to the central core of TORC2, between AVO1 and the TOR2 kinase domain. Adheres VOraciously (AVO3) (the yeast homologue of RICTOR) is also found in the central core of TORC2, where it interacts with the FRB and the FAT domains of TOR2. Importantly, the C-terminus of AVO3 was shown to account for the rapamycin insensitivity of TORC2 as it forms contacts with the FRB domain of TOR2 thereby masking the FKBP12-rapamycin binding site in TORC2 [21]. It would be of interest to determine if a similar mechanism exists in higher eukaryotes.

The structures depicted above will certainly guide the development of more specific mTOR catalytic or allosteric inhibitors. A new class of allosteric inhibitors could target the FAT domain altering the stability of the kinase domain. Moreover, elucidation of the structure of mTORC1 and mTORC2 may enable the design of complex-specific inhibitors.

Physiological role of mTOR complexes

mTORC1 and mTORC2 mediate cell and organismal homeostasis by controlling several anabolic and catabolic processes [25–30]. mTORC1 integrates nutrients, growth factors, and the cellular AMP/ATP ratio to activate glycolysis, upregulate protein, lipid and nucleotide biosynthesis, and to inhibit autophagy [30]. All three inputs are necessary for mTORC1 to achieve maximal activity (Fig. 1). Growth factors, such as insulin, activate mTORC1 via the receptor tyrosine kinases (RTK)-PI3K-PDK1-AKT signaling pathway. AKT phosphorylates tuberous sclerosis complex 2 (TSC2) to inhibit the tuberous sclerosis complex (TSC) complex consisting of TSC1, TSC2, and TBC1D7 [31–33]. The TSC complex is a GTPase-activating protein and thereby a negative regulator of the small GTPase RAS homologue enriched in brain (RHEB). GTP-loaded RHEB directly binds and activates mTORC1 [34]. Other cell signaling pathways, such as the LKB1-AMPK and RAF-MEK-ERK pathways, also modulate mTORC1 via TSC2 phosphorylation [30]. Nutrients, in particular amino acids, in addition to growth factor(s), are required to fully activate mTORC1. Amino acids, of which leucine is the most effective, activate RAS-related small GTP binding protein (RAG) heterodimers which in turn recruit mTORC1 to the lysosomal surface [35]. On the lysosome, mTORC1 interacts with growth factor-activated RHEB to achieve full activity [35]. The mechanism(s) by which amino acids are sensed and activate mTORC1 is currently a very active and rapidly evolving topic (reviewed in [36, 37]). Two of the best characterized complexes that affect RAG activity, and are implicated in disease, are the positive regulator RAGULATOR and the negative regulator GATOR1 that control the GDP/GTP loading status of RAG heterodimers [38–40]. The best characterized mTORC1 substrates are UNC51 like autophagy activating kinase 1 (ULK1) that regulates autophagy and the eukaryotic initiation factor 4E (eIF4E) binding protein (4EBPs), 4EBP1/2/3, and S6 kinases (S6Ks), S6K1/2, that regulate protein synthesis [29]. 4EBPs are negative regulators of translation that bind eIF4E to prevent eIF4E-eIF4G interaction, which is necessary for cap-dependent translation initiation. mTORC1 phosphorylates 4EBP1 on multiple sites causing its dissociation from eIF4E; this allows eIF4E to interact with eIF4G leading to pre-initiation complex formation and translation initiation [41, 42]. The mTORC1-4EBP1 branch has been proposed to control mRNAs containing either a 5'polypyrimidine tract or a complex 5′ UTR [43–45]. However, recent evidence suggests that the control of these types of mRNAs by mTOR is more complex and does not rely entirely on mTOR-4EBP1 [46–48]. Phosphorylation of S6K by mTORC1 also affects protein translation. Both S6K1 and S6K2 phosphorylate ribosomal protein S6 (RPS6), a component of the 40S ribosomal subunit. The mTOR-S6K1 branch affects both translation initiation and elongation. S6K1 promotes translation initiation via phosphorylation and thereby recruitment of eIF4B to the pre-initiation complex. eIF4B potentiates eIF4A, an RNA helicase necessary for translation initiation, especially on mRNAs with structured 5′ UTRs

[49, 50]. S6K1 also phosphorylates and inhibits programmed cell death 4 (PDCD4), a negative regulator of eIF4A [51]. Additionally, S6K1 also enhances translation elongation via phosphorylation and inhibition of eukaryotic elongation factor 2 kinase (eEF2K), a negative regulator of eukaryotic elongation factor 2 (eEF2) [52]. Other targets of S6K1 include S6K1 Aly/Ref like target (SKAR) and nuclear cap binding protein (NCBP1), involved in translation [53, 54]. Phosphorylation of S6K1 by mTORC1 elicits a negative feedback loop as phospho-S6K1 disrupts signaling directly downstream of RTKs, ultimately reducing mTORC1 activity [55–57]. Recently, another negative feedback loop has been described whereby mTORC1 phosphorylates growth factor receptor bound protein 10 (GRB10) to block upstream RTK signaling [58, 59].

mTORC2 is stimulated by growth factors alone. In this case, growth factors stimulate RTK-PI3K signaling to promote mTORC2 association with the ribosome and thereby mTORC2 activation [60]. mTORC2-ribosome interaction requires both subunits of the ribosome but is independent of translation. Association of mTORC2 with a fully formed ribosome could link growth capacity of the cell with mTORC2 signaling. The best characterized targets of mTORC2 are members of the AGC kinase family, including AKT, protein kinase C (PKC), and serum glucocorticoid-regulated kinase (SGK), that regulate cell physiology in many ways [30].

mTOR signaling also has effects on organismal physiology. Mouse models displaying altered mTORC1 or mTORC2 signaling specifically in metabolic organs such as liver, adipose, and muscle have been generated [61]. Abrogation of mTORC1 signaling has more severe effects, as seen in liver and muscle, than abrogation of mTORC2 signaling [62, 63]. Along these lines, mTORC2 signaling is dispensable in maintenance of normal skin epidermis and prostate epithelium [64, 65]. This argues for a possible therapeutic window for mTORC2 inhibition, at least in treating skin and prostate disorders. Data from mouse models on effects of mTOR alteration argue for a central role of mTOR in metabolic disorders such as metabolic syndrome, diabetes, and obesity (reviewed in [66, 61]). Indeed, mTOR signaling alteration plays a role in many diseases (listed in Table 1). Below, we focus on the role of mTOR in neurological disorders and cancer.

IMPLICATIONS AND INDICATIONS
Diseases linked to aberrant mTOR signaling

Disease	Possible mechanism (preclinical studies)
Obesity	mTOR stimulates lipid biosynthesis and increases fat deposition in adipose tissue [66].
Diabetes	mTOR upregulation causes insulin resistance and hyperglycemia [61,66]
Cardiac disease	mTOR upregulation is linked to cardiomyopathy, hypertrophy, and heart failure. Treatment of patients with rapalogs reduces these cardiac events [165]
Neurodegenerative Diseases (Parkinson's, Huntington's, Alzheimer's)	mTOR stimulates protein synthesis and downregulates autophagy possibly leading to protein aggregation. Rapamycin treatment reduces levels of protein aggregates in mouse models [73,74].
Tuberous sclerosis complex (TSC)	Deletion of TSC1 or TSC2 causes mTOR-driven tuberous sclerosis. In clinical trials, rapalogs have shown efficacy toward TSC symptoms such as subependymal giant cell astrocytes (SEGA), angiomyolymphomas (AML) in kidneys, lymphangioleiomyomatosis (LAM) in lungs, and epilepsy [166–169]. Everolimus and sirolimus are used in the clinic for treatment of SEGA and LAM in TSC patients
Epilepsy	The majority of TSC patients develop epilepsy. Moreover, mutations in DEPDC5, a member of GATOR1 complex, can cause familial focal epilepsy due to focal cortical dysplasia [170–172]. Everolimus has been shown to reduce epileptic symptoms in TSC patients [168]
Autism spectrum disorders (ASD)	mTOR affects protein turnover and autophagy to influence neuronal pruning and synapse plasticity [82,83]
Hutchinson-Gilford Progeria	mTOR signaling downregulates autophagy, possibly causing progerin accumulation. Rapamycin treatment enhances progerin degradation [173]
Cancer	mTOR stimulates protein, lipid, and nucleotide metabolism and inhibits autophagy to promote tumorigenesis. Rapalogs are used in the clinic for treatment of specific tumors [120]
Macular degeneration	mTOR can affect VEGF expression and angiogenesis that drive capillary overgrowth in eye choroid. Rapamycin can reduce VEGF expression and inhibit angiogenesis [27,174]
Polycystic kidney disease (PKD)	Polycysteine upregulates mTOR activity promoting growth and proliferation exacerbating the disease. Rapalogs are used in the clinic to ameliorate PKD symptoms [175]
Restenosis	mTOR activity promotes angiogenesis and tissue growth that could lead to restenosis. Rapalogs are used in the clinic in drug-eluting stents to prevent restenosis [121]
Inflammation	Inflammation activates mTOR signaling and mTOR can affect inflammation via T lymphocyte proliferation and differentiation. INK-128, a pan-mTOR inhibitor, can reduce inflammation in vitro [155]
Muscular dystrophy	Downregulation of mTORC1 signaling in mice from birth affects muscle protein synthesis and overall muscle physiology causing muscle atrophy. On the other hand, upregulation of mTOR signaling in the muscle inhibits autophagy leading to muscle degeneration. Rapamycin can ameliorate the muscle degeneration phenotype caused by mTORC1 upregulation [62,176,177]

mTOR in pathophysiology

mTOR in neurological disorders

mTOR controls protein synthesis and turnover, including autophagy, and as such is thought to play a role in neuronal growth and plasticity. Indeed, inhibition of mTORC1 by rapamycin treatment reduces rates of translation at the synapse in vitro, affecting synaptic plasticity, memory, and learning [67]. Inappropriate high mTORC1 activity at the synapse is also detrimental, as mice with heterozygous mutations in *TSC1* or *TSC2* demonstrate learning and memory impairments

that are reversible by rapamycin treatment [68, 67]. Thus, the amount of mTOR signaling is critical for proper function of neuronal synapses.

Neurodegenerative disorders such as Alzheimer's disease (AD) and Huntington's disease (HD) are also associated with dysregulation of mTOR signaling. These diseases are characterized by loss of neuronal plasticity and function due to accumulation of unfolded protein aggregates, for example β-amyloid aggregates [69]. mTOR, as well as AKT signaling, has been shown to be deregulated in brains from AD patients, and accumulation of β-amyloid aggregates correlates with increased mTOR signaling [70, 71]. Moreover, DEPTOR, an inhibitor of mTOR signaling, is reduced upon induction of amyloid B plaque formation in neuronal cells [72]. In the same study, DEPTOR levels were found to be reduced in a patient with late onset AD as compared to early onset AD. Consistent with a role of mTOR in AD and HD, rapamycin treatment reduces protein aggregates and improves cognitive and motor skills in mouse models [73, 74]. Rapamycin treatment reduces the amount of misfolded proteins by downregulating protein synthesis and upregulating autophagy. Thus, aberrant mTOR signaling could be responsible for aggregate formation, and mTOR may be targeted in the clinic to treat neurodegenerative disorders.

mTOR signaling is also thought to be involved in autism spectrum disorders (ASD). ASD patients present brain regions with abnormal neuronal plasticity and synapse formation due to high numbers of synapses and excess neuronal firing, causing over-connectivity [75]. Of all ASD cases, 5–10 % are thought to arise from single gene defects where mTOR signaling is directly affected. For example, roughly 30 % of TSC patients, where *TSC1* or *TSC2* is mutated, exhibit ASD symptoms and 1–2 % of all ASD patients have upregulation of mTOR signaling due to a mutation in phosphatase and tensin homologue (*PTEN*), encoding a negative regulator of the PI3K-AKT-mTOR signaling axis [75, 76]. The most common familial cause of ASD is fragile X syndrome (FXS) where roughly 40 % of male and 20 % of female patients show ASD symptoms [75]. A mouse model of FXS exhibits increased S6K1, 4EBP-1, and AKT phosphorylation. Moreover, downregulation of S6K1 in a mouse model of FXS ameliorates neuronal morphology and autistic behavior of these mice [77]. Interestingly, S6K1 was reported to phosphorylate and regulate FXS-related FMR1 activity; however, a recent report showed that FMR1 phosphorylation was not altered in *TSC1* knockout or *S6K1* knockout mice [78, 79]. mTOR could be a target in treating FXS, although more work is needed to uncover the link between S6K1 and FMR1 in FXS.

mTOR could also play a role in non-familial cases of ASD. Cap-dependent translation upregulation at the synapse is associated with increased autism-like symptoms in mice [80, 81]. mTORC1-4EBP1 signaling can play a role in ASD via affecting cap-dependent translation at the synapse. Excess synapses are eliminated during brain development by so-called neuronal pruning. Recently, it has been shown that mTOR prevents neuronal pruning by inhibiting autophagy, thereby causing ASD-like synaptic pathology [82]. Another recent study showed that mTOR upregulation increases proteosomal protein degradation in mouse neuronal cells [83]. Thus, mTOR controls protein turnover that is critical in maintaining synaptic plasticity.

mTOR in cancer

The mTOR, RTK-PI3K-AKT, and RAS-MEK-ERK pathways are the main growth controlling pathways in the cell, and mutations in all three account for the majority of tumor mutations. Mutations in *mTOR* itself are not common but have been described in tumors [84, 85]. These mutations primarily affect the FAT and kinase domains of mTOR and may activate mTORC1 and/or mTORC2. Curiously, some of the mutations confer rapamycin hypersensitivity that could be exploited in the clinic [84]. It would be of interest to test if these mutations drive tumorigenesis.

Altering levels of the core components of the mTOR complexes have been linked to tumorigenesis. In line with an oncogenic role of mTOR signaling, RICTOR was shown to be essential for tumorigenesis in a PTEN-driven prostate mouse model as well as a DMBA/TPA-treated skin carcinogenesis mouse model [64, 65]. Furthermore, overexpression of RICTOR or mLST8 induces tumorigenic and invasive properties in tumor cell lines [86, 87]. Surprisingly, a mouse model where RAPTOR is specifically deleted in the liver showed accelerated DEN-induced liver tumorigenesis [63]. The authors attributed this to an increase in liver damage and liver fibrosis caused by a reduction in protein synthesis upon knocking out RAPTOR. Transient members of the mTOR complexes have also been shown to be deregulated in cancer. PRAS40 is deregulated in many pathologies including cancer [88–90]. PRAS40 expression and phosphorylation are enhanced in many cancers where the PI3K-AKT-mTOR pathway is altered [91, 92]. This argues for a connection between PRAS40, AKT, and mTOR in disease. However, more work is needed to explain the connection between PRAS40 overexpression and the AKT-mTOR axis in the context of disease. PROTOR1 expression is increased a cohort of colon cancers and decreased in a cohort of breast cancers, arguing for tissue specificity of its actions [93]. Furthermore, DEPTOR has been reported to be both overexpressed and downregulated in various types of tumors (reviewed in [94]). This suggests that DEPTOR has a tissue-specific mode of action in tumorigenesis. It is feasible that, in tumors where DEPTOR is downregulated, it acts in an mTOR-dependent manner, whereas in tumors where DEPTOR is overexpressed, it acts in an mTOR-independent manner. Further characterization of PROTOR-mTOR and DEPTOR-mTOR relationships

in tumors is needed to determine if these players can be modulated with the aim of altering TOR activity in tumors.

A role for mTOR in cancers is underscored by the presence of familial cancer syndromes that are caused by loss-of-function mutations in negative regulators of mTOR signaling. For example, loss of *TSC1* or *TSC2* causes TSC and loss of *LKB1* or *PTEN* causes Peutz-Jegher syndrome and Cowden syndrome, respectively [76, 95, 96]. All three diseases are characterized by benign tumor-like growth, hamartomas, in various organs. mTOR signaling is upregulated in these diseases and in mouse models of Cowden syndrome mTOR plays an essential role in hamartoma formation [97]. Moreover, in *PTEN*-deficient tumor mouse models, mTOR signaling plays an essential role. mTORC2 is essential for tumor progression in a prostate cancer mouse model where PTEN is specifically deleted in prostate epithelial cells [65]. Also, rapamycin reduces tumor burden in a PTEN mouse model of pancreatic ductal adenocarcinomas [98]. Thus, tumors that arise upon loss of tumor suppressors in the mTOR pathway, such as PTEN, are dependent on mTOR signaling.

The major role of mTOR in tumorigenesis is thought to be in increasing protein synthesis via phosphorylation of 4EBP1 and S6K1. Extensive literature points to 4EBP1 phosphorylation as the major effector of mTOR in tumorigenesis, as it promotes selective translation of a specific class of oncogenic proteins [99, 27, 100]. Lately, a role for S6K1 signaling in tumorigenesis is also gaining importance. A recent study revealed a central role for S6K1 in colorectal cancer [101]. The authors show that translational elongation controlled by S6K1-mediated phosphorylation is the rate-limiting step for tumor progression, despite an increase in translation initiation and overall protein synthesis [101]. S6K1 was also found recently to upregulate c-Myc, an oncogenic protein, potentially driving tumorigenesis [102].

mTOR signaling also appears to drive tumor growth independently of protein synthesis. Both mTORC1 and mTORC2 regulate lipid biosynthesis, partly via SREBP1, a regulator of fatty acid biosynthesis and a protein with oncogenic properties [103–105]. Moreover, a recent report showed that mTOR indirectly increases levels of PGC1-α and FGF21, proteins involved in fatty acid oxidation and gluconeogenesis [106]. Thus, mTOR signaling could be important for tumor maintenance when tumors depend on lipid biogenesis to maintain growth. mTOR also upregulates glycolytic flux and angiogenesis in tumors by activating the transcription factor HIF1α [103]. Another feature of tumors is an overall increase in both DNA and RNA syntheses. TOR signaling controls tRNA and possibly rRNA synthesis [107–110]. Recently it was shown that mTORC1 signaling regulates pyrimidine synthesis, via S6K1 phosphorylation of CAD, an enzyme involved in the first three steps of pyrimidine synthesis [111, 112]. Pyrimidine biosynthesis in general and CAD activity in particular have been shown to be upregulated in tumors and tumor cell lines [113, 114]. Furthermore, CAD gene amplification has been shown to correlate with an increase in genomic instability and growth rate of tumor cell lines [115, 116]. Thus, mTORC1 phosphorylation of CAD may contribute to tumor progression and aggressiveness.

Metastasis, characterized by increases in tumor cell motility and invasion, is a feature of malignant tumors and the main cause of death among patients. Both mTORC1 and mTORC2 can affect tumor metastasis by increasing translation of pro-invasion proteins and by modulating expression and activity of small GTPases (RHOA, RAC1, CDC42) that are involved in cell motility [117, 43]. Mechanistically, mTORC2 can affect the actin cytoskeleton and cell motility via phosphorylating PKCα and Paxillin that have roles in actin fiber reorganization [118]. However, more work is needed to delineate the function of mTORC2 in cell motility and tumor metastasis.

Targeting mTOR in the clinic

Rapamycin and rapalogs

Rapamycin was initially developed as an antifungal agent but was later used as an immunosuppressant and an anti-cancer drug [119, 120]. The FDA approved rapamycin in 1999 for use as an immunosuppressant for the prevention of graft rejection in patients with kidney transplants. Since then, a number of rapamycin analogs (rapalogs) have been developed and are prescribed for use against specific types of tumors [121]. However, rapalogs are not broadly effective as anti-cancer drugs. This could be due to their inability to completely block mTORC1 activity, as they have virtually no effect on the 4EBP1 branch of mTORC1 signaling [122]. Furthermore, in many settings, the mTORC2 pathway is resistant to rapalogs [121]. Weak performance of rapalogs could also be due to negative feedback loops associated with mTORC1 signaling. Rapalogs inhibit mTORC1-S6K1 signaling thereby inhibiting a negative feedback loop, ultimately upregulating PI3K-AKT signaling. S6K1 inhibition can also activate the ERK-MAPK cascade. Indeed, patients treated with the rapalog everolimus show a dose-dependent increase in mitogen-activated protein kinase (MAPK) activity [123]. Accordingly, the current trend is toward getting approval for use of rapalogs in combination with another drug.

Pan-mTOR inhibitors

Another class of mTOR inhibitors is the so-called pan-mTOR inhibitors. Unlike rapamycin and the rapalogs which are allosteric inhibitors, the pan-mTOR inhibitors are ATP competitive inhibitors that interact with the catalytic cleft of mTOR and block both mTORC1 and mTORC2 activity. They have higher affinity for mTOR

than for PI3K or other PIKK members. PP242, the first pan-mTOR inhibitor, has 50 times more affinity for mTOR than for DNA-dependent protein kinase (DNA-PK) and roughly 100 times more affinity for mTOR than PI3K [124]. Pan-mTOR inhibitors block 4EBP1 and S6K phosphorylation, showing improved antiproliferative and proapoptotic effects compared to rapalogs. As they block phosphorylation of the eIF4E inhibitor 4EBP1, activity of pan-mTOR inhibitors could depend on the 4EBP1/eIF4E ratio, being more efficacious in tumors where the ratio is high. Indeed, a panel of cancer cell lines showed that loss of 4EBP1 or overexpression of eIF4E made these cells resistant to PP242 [125]. In another study, downregulation of 4EBP1 enhanced tumor formation in nude mice and rendered cell proliferation resistant to PP242 [126]. In the clinic, levels of 4E-BP1 also vary in tumor patients. A study showed that over 50 % of pancreatic ductal adenocarcinoma (PDAC) patients have a reduction in 4E-BP1 levels compared to normal pancreas [126]. In these patients, pan-mTOR inhibitors could have less of an effect. Preclinical data have also shown that pan-mTOR inhibitors could be effective in combination therapy. For example, AZD8055, a pan-mTOR inhibitor, has a much more potent cytotoxic effect, in multiple myeloma cell lines and in xenograft models of human breast cancer, when coupled with RTK inhibitors [127]. Clinical trials determining the efficacy of pan-mTOR inhibitors alone or in combination are underway. Preclinical data underscore the need to characterize the genetic landscape of a disease before using mTOR inhibitors. Used in the right set of patients, these inhibitors might be particularly effective.

PI3K/mTOR inhibitors

Dual PI3K/mTOR inhibitors are also ATP competitive inhibitors that, unlike pan-mTOR inhibitors, bind and inhibit both PI3K and mTOR. They are available and undergoing clinical trials. Early members of this class, such as PI103, were developed as PI3K inhibitors and were later shown to have an affinity for mTOR [128]. In preclinical studies, dual inhibitors, like the pan-mTOR inhibitors, display more potent cytotoxic properties than rapalogs [120]. Moreover, they potentiate the antiproliferative activity of the chemotherapeutic agent cisplatin and ionizing radiation [129, 130]. Importantly, because these drugs inhibit PI3K upstream of mTOR, in addition to mTOR itself, they prevent some negative feedback loops, such as S6K1- and GRB10-driven negative feedback loops, but not all [131, 132].

Besides potentially relieving repression of some feedback loops that trigger PI3K signaling, preclinical studies have uncovered other potential mechanisms that could hamper usage of dual inhibitors in the clinic. Activating mutations in the *KRAS* gene result in resistance to treatment of colorectal cancer cell lines with BEZ235, a PI3K/mTOR inhibitor [133]. Furthermore, amplification of *eIF4E* or *c-MYC* confers resistance to treatment with the same inhibitor in tumor cell lines [134]. Another concern for current dual PI3K/mTOR inhibitors might be their activity toward other PI3K-related kinases that ultimately can increase the toxicity of these inhibitors. These observations underscore the complexity of mechanisms involved in resistance to targeted anti-cancer therapies suggesting that combinatorial therapy or careful patient stratification may increase effectiveness of dual inhibitors.

Novel uses of mTOR inhibitors

mTOR inhibitors are used primarily for immunosuppression and treatment of cancer. However, mTOR inhibitors may benefit also patients with other disorders. For example, clinical trials have generated encouraging results for Palomid 529, a PI3K/mTOR dual inhibitor, in the treatment of age-related macular degeneration [135]. mTOR inhibitors conceptually could be used to treat neurodegenerative diseases and ASD. Rapamycin has shown neuroprotective and plaque-reducing effects in mouse models of Huntington's and Alzheimer's diseases [73, 74]. Disruption of mTOR signaling has also ameliorated autistic behavior of adult *TSC1* or *TSC2* heterozygous mice and other ASD mouse models [136–138]. These preclinical data make the case for mTOR inhibitors to be used in the treatment of neurological diseases. Indeed, there are currently ongoing clinical trials assessing the efficacy of everolimus, a rapalog, toward autistic symptoms of TSC patients. mTOR signaling is also involved in the physiology of normal neuronal synapses. Thus, in order to use mTOR inhibitors in patients with ASD, we need to define and make use of a therapeutic window in mTOR signaling alteration.

Rapalogs are being widely used as immunosuppressive agents. However, new data suggest that rapamycin could also have immunoprotective effects in old age. Treating 2-year-old mice with rapamycin for 6 weeks restored the immunological capacity to fight infection [139]. This was attributed to rejuvenation of the hematopoietic stem cells in old mice. Furthermore, in a clinical study of over 65-year-old healthy individuals, treatment with everolimus for 6 weeks improved their immune response to vaccination [140]. Effects of rapamycin on the immune system of old subjects could play a significant role in the ability of rapamycin to increase lifespan and improve age-related conditions, as seen in mice [141, 142]. At the very least, these findings potentially uncover new uses for mTOR inhibitors as immunoprotective agents in old age. Improving drug formulation or the dispensing regime of mTOR inhibitors could reduce side effects and enhance efficacy in treating neurological and age-related diseases [143].

Conclusions

Since its discovery, TOR has emerged as a central player in the regulation of cellular growth and metabolism. It controls many cellular anabolic and catabolic processes. Other signaling pathways, such as AMPK and ERK-MAPK, converge on the mTOR pathway thereby underscoring the central role of mTOR signaling. Thus, it should not come as a surprise that mTOR signaling plays an important role in a number of diseases including metabolic and neurological disorders and cancer (Table 1). mTOR regulation has received much attention in the pharmaceutical community. The "original" allosteric mTOR inhibitor rapamycin was first approved in 1999 as an immunosuppressant. Nearly a decade later, rapamycin analogs, rapalogs, were approved for cancer treatment and are currently prescribed for specific cases of tumors. More recently, ATP competitive catalytic site inhibitors were developed and are currently undergoing clinical trials. It will be of great interest to see if this new class of mTOR inhibitors will be effective in the treatment of cancer or other morbidities. With the advent of "omics" techniques, personalized medicine is within reach. Better knowledge of the genetic landscape of patients will allow us to better predict drug effects and use mTOR inhibitors on patients carrying a specific set of mutations, regardless of the disease. This might enhance the efficacy of mTOR inhibitors in the clinic. From the knowledge of the mTOR structure, even newer allosteric or ATP site inhibitors or mTORC2-specific inhibitors could be designed. In animal models, mTOR signaling is also linked to aging and healthspan. It would be interesting to determine if manipulating mTOR could produce a dramatic change in lifespan as well as healthspan of individuals.

Acknowledgments The authors acknowledge support from the Swiss National Science Foundation and the NCCR RNA and Disease Grant.

References

1. Heitman J, Movva NR, Hiestand PC, Hall MN (1991) FK 506-binding protein proline rotamase is a target for the immunosuppressive agent FK 506 in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 88(5):1948–1952
2. Helliwell SB, Wagner P, Kunz J, Deuter-Reinhard M, Henriquez R, Hall MN (1994) TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. Mol Biol Cell 5(1):105–118
3. Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, Hall MN (1993) Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. Cell 73(3):585–596
4. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature 369(6483):756–758
5. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH (1994) RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 78(1):35–43
6. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol 6(11):1122–1128
7. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell 10(3):457–468
8. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 14(14):1296–1302
9. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22(2):159–168
10. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA, Sabatini DM (2007) PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell 25(6):903–915
11. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH (2007) Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol 9(3):316–323
12. Malla R, Wang Y, Chan WK, Tiwari AK, Faridi JS (2015) Genetic ablation of PRAS40 improves glucose homeostasis via linking the AKT and mTOR pathways. Biochem Pharmacol 96(1):65–75
13. Pearce LR, Huang X, Boudeau J, Pawlowski R, Wullschleger S, Deak M, Ibrahim AF, Gourlay R, Magnuson MA, Alessi DR (2007) Identification of Protor as a novel Rictor-binding component of mTOR complex-2. Biochem J 405(3):513–522
14. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS, Sabatini DM (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell 137(5):873–886
15. Lempiainen H, Halazonetis TD (2009) Emerging common themes in regulation of PIKKs and PI3Ks. EMBO J 28(20):3067–3073
16. Sauer E, Imseng S, Maier T, Hall MN (2013) Conserved sequence motifs and the structure of the mTOR kinase domain. Biochem Soc Trans 41(4):889–895
17. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP (2013) mTOR kinase structure, mechanism and regulation. Nature 497(7448):217–223
18. Choi J, Chen J, Schreiber SL, Clardy J (1996) Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science 273(5272):239–242
19. Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS et al (2012) Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 335(6076):1638–1643
20. Schreiber KH, Ortiz D, Academia EC, Anies AC, Liao CY, Kennedy BK (2015) Rapamycin-mediated mTORC2 inhibition is determined by the relative expression of FK506-binding proteins. Aging Cell 14(2):265–273
21. Gaubitz C, Oliveira TM, Prouteau M, Leitner A, Karuppasamy M, Konstantinidou G, Rispal D, Eltschinger S, Robinson GC, Thore S et al (2015) Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. Mol Cell 58(6):977–988
22. Yip CK, Murata K, Walz T, Sabatini DM, Kang SA (2010) Structure of the human mTOR complex I and its implications for rapamycin inhibition. Mol Cell 38(5):768–774

23. Jain A, Arauz E, Aggarwal V, Ikon N, Chen J, Ha T (2014) Stoichiometry and assembly of mTOR complexes revealed by single-molecule pulldown. Proc Natl Acad Sci U S A 111(50): 17833–17838
24. Kim SG, Hoffman GR, Poulogiannis G, Buel GR, Jang YJ, Lee KW, Kim BY, Erikson RL, Cantley LC, Choo AY et al (2013) Metabolic stress controls mTORC1 lysosomal localization and dimerization by regulating the TTT-RUVBL1/2 complex. Mol Cell 49(1):172–185
25. Dibble CC, Manning BD (2013) Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol 15(6):555–564
26. Jewell JL, Guan KL (2013) Nutrient signaling to mTOR and cell growth. Trends Biochem Sci 38(5):233–242
27. Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149(2):274–293
28. Loewith R, Hall MN (2011) Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics 189(4):1177–1201
29. Ma XM, Blenis J (2009) Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 10(5): 307–318
30. Shimobayashi M, Hall MN (2014) Making new contacts: the mTOR network in metabolism and signalling crosstalk. Nat Rev Mol Cell Biol 15(3):155–162
31. Dibble CC, Elis W, Menon S, Qin W, Klekota J, Asara JM, Finan PM, Kwiatkowski DJ, Murphy LO, Manning BD (2012) TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. Mol Cell 47(4):535–546
32. Inoki K, Li Y, Zhu T, Wu J, Guan KL (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 4(9):648–657
33. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC (2002) Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. Mol Cell 10(1):151–162
34. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J (2005) Rheb binds and regulates the mTOR kinase. Curr Biol 15(8):702–713
35. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 320(5882):1496–1501
36. Bar-Peled L, Sabatini DM (2014) Regulation of mTORC1 by amino acids. Trends Cell Biol 24(7):400–406
37. Efeyan A, Comb WC, Sabatini DM (2015) Nutrient-sensing mechanisms and pathways. Nature 517(7534):302–310
38. Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M, Sabatini DM (2013) A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science 340(6136):1100–1106
39. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM (2012) Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. Cell 150(6):1196–1208
40. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 141(2):290–303
41. Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, Sonenberg N (1999) Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev 13(11):1422–1437
42. Gingras AC, Kennedy SG, O'Leary MA, Sonenberg N, Hay N (1998) 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. Genes Dev 12(4):502–513
43. Hsieh AC, Liu Y, Edlind MP, Ingolia NT, Janes MR, Sher A, Shi EY, Stumpf CR, Christensen C, Bonham MJ et al (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. Nature 485(7396):55–61
44. Larsson O, Morita M, Topisirovic I, Alain T, Blouin MJ, Pollak M, Sonenberg N (2012) Distinct perturbation of the translatome by the antidiabetic drug metformin. Proc Natl Acad Sci U S A 109(23):8977–8982
45. Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. Nature 485(7396):109–113
46. Damgaard CK, Lykke-Andersen J (2011) Translational coregulation of 5'TOP mRNAs by TIA-1 and TIAR. Genes Dev 25(19):2057–2068
47. Miloslavski R, Cohen E, Avraham A, Iluz Y, Hayouka Z, Kasir J, Mudhasani R, Jones SN, Cybulski N, Ruegg MA et al (2014) Oxygen sufficiency controls TOP mRNA translation via the TSC-Rheb-mTOR pathway in a 4E-BP-independent manner. J Mol Cell Biol 6(3):255–266
48. Patursky-Polischuk I, Kasir J, Miloslavski R, Hayouka Z, Hausner-Hanochi M, Stolovich-Rain M, Tsukerman P, Biton M, Mudhasani R, Jones SN et al (2014) Reassessment of the role of TSC, mTORC1 and microRNAs in amino acids-meditated translational control of TOP mRNAs. PLoS One 9(10), e109410. doi: 10.1371/journal.pone.0109410
49. Holz MK, Ballif BA, Gygi SP, Blenis J (2005) mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell 123(4):569–580
50. Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, Polakiewicz RD, Sonenberg N, Hershey JW (2004) Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. EMBO J 23(8):1761–1769
51. Dorrello NV, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE, Pagano M (2006) S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. Science 314(5798):467–471
52. Wang X, Li W, Williams M, Terada N, Alessi DR, Proud CG (2001) Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. EMBO J 20(16):4370–4379
53. Richardson CJ, Broenstrup M, Fingar DC, Julich K, Ballif BA, Gygi S, Blenis J (2004) SKAR is a specific target of S6 kinase 1 in cell growth control. Curr Biol 14(17):1540–1549
54. Wilson KF, Wu WJ, Cerione RA (2000) Cdc42 stimulates RNA splicing via the S6 kinase and a novel S6 kinase target, the nuclear cap-binding complex. J Biol Chem 275(48):37307–37310
55. Shah OJ, Wang Z, Hunter T (2004) Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr Biol 14(18):1650–1656
56. Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie NR, Cheng S, Shepherd PR et al (2004) The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. J Cell Biol 166(2):213–223
57. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J et al (2004) Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 431(7005):200–205
58. Hsu PP, Kang SA, Rameseder J, Zhang Y, Ottina KA, Lim D, Peterson TR, Choi Y, Gray NS, Yaffe MB et al (2011) The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science 332(6035):1317–1322
59. Yu Y, Yoon SO, Poulogiannis G, Yang Q, Ma XM, Villen J, Kubica N, Hoffman GR, Cantley LC, Gygi SP et al (2011)

Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. Science 332(6035):1322–1326
60. Zinzalla V, Stracka D, Oppliger W, Hall MN (2011) Activation of mTORC2 by association with the ribosome. Cell 144(5):757–768
61. Albert V, Hall MN (2014) mTOR signaling in cellular and organismal energetics. Curr Opin Cell Biol 33C:55–66
62. Bentzinger CF, Romanino K, Cloetta D, Lin S, Mascarenhas JB, Oliveri F, Xia J, Casanova E, Costa CF, Brink M et al (2008) Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy. Cell Metab 8(5):411–424
63. Umemura A, Park EJ, Taniguchi K, Lee JH, Shalapour S, Valasek MA, Aghajan M, Nakagawa H, Seki E, Hall MN et al (2014) Liver damage, inflammation, and enhanced tumorigenesis after persistent mTORC1 inhibition. Cell Metab 20(1):133–144
64. Carr TD, Feehan RP, Hall MN, Ruegg MA, Shantz LM (2015) Conditional disruption of rictor demonstrates a direct requirement for mTORC2 in skin tumor development and continued growth of established tumors. Carcinogenesis 36(4):487–497
65. Guertin DA, Stevens DM, Saitoh M, Kinkel S, Crosby K, Sheen JH, Mullholland DJ, Magnuson MA, Wu H, Sabatini DM (2009) mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. Cancer Cell 15(2):148–159
66. Cornu M, Albert V, Hall MN (2013) mTOR in aging, metabolism, and cancer. Curr Opin Genet Dev 23(1):53–62
67. Goorden SM, van Woerden GM, van der Weerd L, Cheadle JP, Elgersma Y (2007) Cognitive deficits in Tsc1+/− mice in the absence of cerebral lesions and seizures. Ann Neurol 62(6):648–655
68. Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ (2008) Reversal of learning deficits in a Tsc2+/− mouse model of tuberous sclerosis. Nat Med 14(8):843–848
69. Lipton JO, Sahin M (2014) The neurology of mTOR. Neuron 84(2):275–291
70. An WL, Cowburn RF, Li L, Braak H, Alafuzoff I, Iqbal K, Iqbal IG, Winblad B, Pei JJ (2003) Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. Am J Pathol 163(2):591–607
71. Griffin RJ, Moloney A, Kelliher M, Johnston JA, Ravid R, Dockery P, O'Connor R, O'Neill C (2005) Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss and altered distribution of Akt and PTEN are features of Alzheimer's disease pathology. J Neurochem 93(1):105–117
72. Davies J, Zachariades E, Rogers-Broadway KR, Karteris E (2014) Elucidating the role of DEPTOR in Alzheimer's disease. Int J Mol Med 34(5):1195–1200
73. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ et al (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet 36(6):585–595
74. Spilman P, Podlutskaya N, Hart MJ, Debnath J, Gorostiza O, Bredesen D, Richardson A, Strong R, Galvan V (2010) Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. PLoS One 5(4), e9979. doi:10.1371/journal.pone.0009979
75. Carter MT, Scherer SW (2013) Autism spectrum disorder in the genetics clinic: a review. Clin Genet 83(5):399–407
76. Crino PB, Nathanson KL, Henske EP (2006) The tuberous sclerosis complex. N Engl J Med 355(13):1345–1356
77. Bhattacharya A, Kaphzan H, Alvarez-Dieppa AC, Murphy JP, Pierre P, Klann E (2012) Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. Neuron 76(2):325–337
78. Bartley CM, O'Keefe RA, Bordey A (2014) FMRP S499 is phosphorylated independent of mTORC1-S6K1 activity. PLoS One 9(5), e96956. doi:10.1371/journal.pone.0096956
79. Narayanan U, Nalavadi V, Nakamoto M, Thomas G, Ceman S, Bassell GJ, Warren ST (2008) S6K1 phosphorylates and regulates fragile X mental retardation protein (FMRP) with the neuronal protein synthesis-dependent mammalian target of rapamycin (mTOR) signaling cascade. J Biol Chem 283(27):18478–18482
80. Gkogkas CG, Khoutorsky A, Cao R, Jafarnejad SM, Prager-Khoutorsky M, Giannakas N, Kaminari A, Fragkouli A, Nader K, Price TJ et al (2014) Pharmacogenetic inhibition of eIF4E-dependent Mmp9 mRNA translation reverses fragile X syndrome-like phenotypes. Cell Rep 9(5):1742–1755
81. Santini E, Huynh TN, MacAskill AF, Carter AG, Pierre P, Ruggero D, Kaphzan H, Klann E (2013) Exaggerated translation causes synaptic and behavioural aberrations associated with autism. Nature 493(7432):411–415
82. Tang G, Gudsnuk K, Kuo SH, Cotrina ML, Rosoklija G, Sosunov A, Sonders MS, Kanter E, Castagna C, Yamamoto A et al (2014) Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. Neuron 83(5):1131–1143
83. Zhang Y, Nicholatos J, Dreier JR, Ricoult SJ, Widenmaier SB, Hotamisligil GS, Kwiatkowski DJ, Manning BD (2014) Coordinated regulation of protein synthesis and degradation by mTORC1. Nature 513(7518):440–443
84. Grabiner BC, Nardi V, Birsoy K, Possemato R, Shen K, Sinha S, Jordan A, Beck AH, Sabatini DM (2014) A diverse array of cancer-associated MTOR mutations are hyperactivating and can predict rapamycin sensitivity. Cancer Discov 4(5):554–563
85. Hardt M, Chantaravisoot N, Tamanoi F (2011) Activating mutations of TOR (target of rapamycin). Genes Cells 16(2):141–151
86. Kakumoto K, Ikeda J, Okada M, Morii E, Oneyama C (2015) mLST8 promotes mTOR-mediated tumor progression. PLoS One 10(4), e0119015. doi:10.1371/journal.pone.0119015
87. Oneyama C, Kito Y, Asai R, Ikeda J, Yoshida T, Okuzaki D, Kokuda R, Kakumoto K, Takayama K, Inoue S et al (2013) MiR-424/503-mediated Rictor upregulation promotes tumor progression. PLoS One 8(11), e80300. doi:10.1371/journal.pone.0080300
88. Das F, Dey N, Venkatesan B, Kasinath BS, Ghosh-Choudhury N, Choudhury GG (2011) High glucose upregulation of early-onset Parkinson's disease protein DJ-1 integrates the PRAS40/TORC1 axis to mesangial cell hypertrophy. Cell Signal 23(8):1311–1319
89. Hao J, Li F, Liu W, Liu Q, Liu S, Li H, Duan H (2014) Phosphorylation of PRAS40-Thr246 involved in renal lipid accumulation of diabetes. J Cell Physiol 229(8):1069–1077
90. Malla R, Ashby CR Jr, Narayanan NK, Narayanan B, Faridi JS, Tiwari AK (2015) Proline-rich AKT substrate of 40-kDa (PRAS40) in the pathophysiology of cancer. Biochem Biophys Res Commun 463(3):161–166
91. Vincent EE, Elder DJ, Thomas EC, Phillips L, Morgan C, Pawade J, Sohail M, May MT, Hetzel MR, Tavare JM (2011) Akt phosphorylation on Thr308 but not on Ser473 correlates with Akt protein kinase activity in human non-small cell lung cancer. Br J Cancer 104(11):1755–1761
92. Yuan K, Wu H, Wang Y, Chen H, Jiao M, Fu R (2015) Phospho-PRAS40 predicts trastuzumab response in patients with HER2-positive metastatic breast cancer. Oncol Lett 9(2):785–789
93. Johnstone CN, Castellvi-Bel S, Chang LM, Sung RK, Bowser MJ, Pique JM, Castells A, Rustgi AK (2005) PRR5 encodes a conserved proline-rich protein predominant in kidney: analysis of genomic organization, expression, and mutation status in breast and colorectal carcinomas. Genomics 85(3):338–351
94. Wang Z, Zhong J, Inuzuka H, Gao D, Shaik S, Sarkar FH, Wei W (2012) An evolving role for DEPTOR in tumor development and progression. Neoplasia 14(5):368–375

95. Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, Aminoff M, Hoglund P et al (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 391(6663):184–187
96. Salmena L, Carracedo A, Pandolfi PP (2008) Tenets of PTEN tumor suppression. Cell 133(3):403–414
97. Squarize CH, Castilho RM, Gutkind JS (2008) Chemoprevention and treatment of experimental Cowden's disease by mTOR inhibition with rapamycin. Cancer Res 68(17):7066–7072
98. Morran DC, Wu J, Jamieson NB, Mrowinska A, Kalna G, Karim SA, Au AY, Scarlett CJ, Chang DK, Pajak MZ et al (2014) Targeting mTOR dependency in pancreatic cancer. Gut 63(9): 1481–1489
99. Hsieh AC, Ruggero D (2010) Targeting eukaryotic translation initiation factor 4E (eIF4E) in cancer. Clin Cancer Res 16(20): 4914–4920
100. Mamane Y, Petroulakis E, LeBacquer O, Sonenberg N (2006) mTOR, translation initiation and cancer. Oncogene 25(48): 6416–6422
101. Faller WJ, Jackson TJ, Knight JR, Ridgway RA, Jamieson T, Karim SA, Jones C, Radulescu S, Huels DJ, Myant KB et al (2015) mTORC1-mediated translational elongation limits intestinal tumour initiation and growth. Nature 517(7535):497–500
102. Csibi A, Lee G, Yoon SO, Tong H, Ilter D, Elia I, Fendt SM, Roberts TM, Blenis J (2014) The mTORC1/S6K1 pathway regulates glutamine metabolism through the eIF4B-dependent control of c-Myc translation. Curr Biol 24(19):2274–2280
103. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S et al (2010) Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Mol Cell 39(2):171–183
104. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, Guertin DA, Madden KL, Carpenter AE, Finck BN et al (2011) mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell 146(3):408–420
105. Yuan M, Pino E, Wu L, Kacergis M, Soukas AA (2012) Identification of Akt-independent regulation of hepatic lipogenesis by mammalian target of rapamycin (mTOR) complex 2. J Biol Chem 287(35):29579–29588
106. Cornu M, Oppliger W, Albert V, Robitaille AM, Trapani F, Quagliata L, Fuhrer T, Sauer U, Terracciano L, Hall MN (2014) Hepatic mTORC1 controls locomotor activity, body temperature, and lipid metabolism through FGF21. Proc Natl Acad Sci U S A 111(32):11592–11599
107. Kantidakis T, Ramsbottom BA, Birch JL, Dowding SN, White RJ (2010) mTOR associates with TFIIIC, is found at tRNA and 5S rRNA genes, and targets their repressor Maf1. Proc Natl Acad Sci U S A 107(26):11823–11828
108. Mayer C, Zhao J, Yuan X, Grummt I (2004) mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. Genes Dev 18(4):423–434
109. Michels AA, Robitaille AM, Buczynski-Ruchonnet D, Hodroj W, Reina JH, Hall MN, Hernandez N (2010) mTORC1 directly phosphorylates and regulates human MAF1. Mol Cell Biol 30(15): 3749–3757
110. Shor B, Wu J, Shakey Q, Toral-Barza L, Shi C, Follettie M, Yu K (2010) Requirement of the mTOR kinase for the regulation of Maf1 phosphorylation and control of RNA polymerase III-dependent transcription in cancer cells. J Biol Chem 285(20): 15380–15392
111. Ben-Sahra I, Howell JJ, Asara JM, Manning BD (2013) Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. Science 339(6125):1323–1328
112. Robitaille AM, Christen S, Shimobayashi M, Cornu M, Fava LL, Moes S, Prescianotto-Baschong C, Sauer U, Jenoe P, Hall MN (2013) Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. Science 339(6125):1320–1323
113. Aoki T, Weber G (1981) Carbamoyl phosphate synthetase (glutamine-hydrolyzing): increased activity in cancer cells. Science 212(4493):463–465
114. Sigoillot FD, Sigoillot SM, Guy HI (2004) Breakdown of the regulatory control of pyrimidine biosynthesis in human breast cancer cells. Int J Cancer 109(4):491–498
115. Otto E, McCord S, Tlsty TD (1989) Increased incidence of CAD gene amplification in tumorigenic rat lines as an indicator of genomic instability of neoplastic cells. J Biol Chem 264(6):3390–3396
116. Vatteroni L, Piras A, Mariani T, Caligo MA, Rainaldi G (1993) Accumulation of anchorage independent cells showing amplified genes (CAD) during the in vitro propagation of CHEF18 Chinese hamster cells. Cell Prolif 26(2):161–170
117. Gulhati P, Bowen KA, Liu J, Stevens PD, Rychahou PG, Chen M, Lee EY, Weiss HL, O'Connor KL, Gao T et al (2011) mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. Cancer Res 71(9): 3246–3256
118. Zhou H, Huang S (2011) Role of mTOR signaling in tumor cell motility, invasion and metastasis. Curr Protein Pept Sci 12(1):30–42
119. Martel RR, Klicius J, Galet S (1977) Inhibition of the immune response by rapamycin, a new antifungal antibiotic. Can J Physiol Pharmacol 55(1):48–51
120. Benjamin D, Colombi M, Moroni C, Hall MN (2011) Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov 10(11):868–880
121. Lamming DW, Ye L, Sabatini DM, Baur JA (2013) Rapalogs and mTOR inhibitors as anti-aging therapeutics. J Clin Invest 123(3): 980–989
122. Choo AY, Yoon SO, Kim SG, Roux PP, Blenis J (2008) Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. Proc Natl Acad Sci U S A 105(45):17414–17419
123. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC et al (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 118(9):3065–3074
124. Apsel B, Blair JA, Gonzalez B, Nazif TM, Feldman ME, Aizenstein B, Hoffman R, Williams RL, Shokat KM, Knight ZA (2008) Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. Nat Chem Biol 4(11):691–699
125. Alain T, Morita M, Fonseca BD, Yanagiya A, Siddiqui N, Bhat M, Zammit D, Marcus V, Metrakos P, Voyer LA et al (2012) eIF4E/4E-BP ratio predicts the efficacy of mTOR targeted therapies. Cancer Res 72(24):6468–6476
126. Martineau Y, Azar R, Muller D, Lasfargues C, El Khawand S, Anesia R, Pelletier J, Bousquet C, Pyronnet S (2014) Pancreatic tumours escape from translational control through 4E-BP1 loss. Oncogene 33(11):1367–1374
127. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E, Baselga J, Guichard S, Rosen N (2011) mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. Cancer Discov 1(3):248–259
128. Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B et al (2006) A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell 125(4):733–747
129. Gil del Alcazar CR, Hardebeck MC, Mukherjee B, Tomimatsu N, Gao X, Yan J, Xie XJ, Bachoo R, Li L, Habib AA et al (2014)

Inhibition of DNA double-strand break repair by the dual PI3K/mTOR inhibitor NVP-BEZ235 as a strategy for radiosensitization of glioblastoma. Clin Cancer Res 20(5):1235–1248

130. Yang F, Qian XJ, Qin W, Deng R, Wu XQ, Qin J, Feng GK, Zhu XF (2013) Dual phosphoinositide 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 has a therapeutic potential and sensitizes cisplatin in nasopharyngeal carcinoma. PLoS One 8(3), e59879. doi:10.1371/journal.pone.0059879
131. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, Majumder PK, Baselga J, Rosen N (2011) AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell 19(1):58–71
132. Serra V, Scaltriti M, Prudkin L, Eichhorn PJ, Ibrahim YH, Chandarlapaty S, Markman B, Rodriguez O, Guzman M, Rodriguez S et al (2011) PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. Oncogene 30(22):2547–2557
133. Kim A, Lee JE, Lee SS, Kim C, Lee SJ, Jang WS, Park S (2013) Coexistent mutations of KRAS and PIK3CA affect the efficacy of NVP-BEZ235, a dual PI3K/MTOR inhibitor, in regulating the PI3K/MTOR pathway in colorectal cancer. Int J Cancer 133(4): 984–996
134. Ilic N, Utermark T, Widlund HR, Roberts TM (2011) PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. Proc Natl Acad Sci U S A 108(37):E699–708
135. Dalal M, Jacobs-El N, Nicholson B, Tuo J, Chew E, Chan CC, Nussenblatt R, Ferris F, Meyerle C (2013) Subconjunctival Palomid 529 in the treatment of neovascular age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 251(12):2705–2709
136. Burket JA, Benson AD, Tang AH, Deutsch SI (2014) Rapamycin improves sociability in the BTBR T(+)Itpr3(tf)/J mouse model of autism spectrum disorders. Brain Res Bull 100:70–75
137. Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, Weatherill DB, Vasuta C, Yee S, Truitt M, Dallaire P et al (2013) Autism-related deficits via dysregulated eIF4E-dependent translational control. Nature 493(7432):371–377
138. Sato A, Kasai S, Kobayashi T, Takamatsu Y, Hino O, Ikeda K, Mizuguchi M (2012) Rapamycin reverses impaired social interaction in mouse models of tuberous sclerosis complex. Nat Commun 3:1292
139. Chen C, Liu Y, Liu Y, Zheng P (2009) mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. Sci Signal 2(98):ra75
140. Mannick JB, Del Giudice G, Lattanzi M, Valiante NM, Praestgaard J, Huang B, Lonetto MA, Maecker HT, Kovarik J, Carson S et al (2014) mTOR inhibition improves immune function in the elderly. Sci Transl Med 6(268):268ra179
141. Flynn JM, O'Leary MN, Zambataro CA, Academia EC, Presley MP, Garrett BJ, Zykovich A, Mooney SD, Strong R, Rosen CJ et al (2013) Late-life rapamycin treatment reverses age-related heart dysfunction. Aging Cell 12(5):851–862
142. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS et al (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460(7253):392–395
143. Pallet N, Legendre C (2013) Adverse events associated with mTOR inhibitors. Expert Opin Drug Saf 12(2):177–186
144. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 15(23):6541–6551
145. Facchinetti V, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, Lowry C, Newton AC, Mao Y, Miao RQ et al (2008) The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. EMBO J 27(14):1932–1943
146. Liu P, Begley M, Michowski W, Inuzuka H, Ginzberg M, Gao D, Tsou P, Gan W, Papa A, Kim BM et al (2014) Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus. Nature 508(7497):541–545
147. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307(5712):1098–1101
148. Bohn G, Allroth A, Brandes G, Thiel J, Glocker E, Schaffer AA, Rathinam C, Taub N, Teis D, Zeidler C et al (2007) A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein p14. Nat Med 13(1):38–45
149. Burguete-Garcia AI, Cruz-Lopez M, Madrid-Marina V, Lopez-Ridaura R, Hernandez-Avila M, Cortina B, Gomez RE, Velasco-Mondragon E (2010) Association of Gly972Arg polymorphism of IRS1 gene with type 2 diabetes mellitus in lean participants of a national health survey in Mexico: a candidate gene study. Metabolism 59(1):38–45
150. Celi FS, Negri C, Tanner K, Raben N, De Pablo F, Rovira A, Pallardo LF, Martin-Vaquero P, Stern MP, Mitchell BD et al (2000) Molecular scanning for mutations in the insulin receptor substrate-1 (IRS-1) gene in Mexican Americans with type 2 diabetes mellitus. Diabetes Metab Res Rev 16(5):370–377
151. Jellema A, Zeegers MP, Feskens EJ, Dagnelie PC, Mensink RP (2003) Gly972Arg variant in the insulin receptor substrate-1 gene and association with type 2 diabetes: a meta-analysis of 27 studies. Diabetologia 46(7):990–995
152. Rampersaud E, Damcott CM, Fu M, Shen H, McArdle P, Shi X, Shelton J, Yin J, Chang YP, Ott SH et al (2007) Identification of novel candidate genes for type 2 diabetes from a genome-wide association scan in the Old Order Amish: evidence for replication from diabetes-related quantitative traits and from independent populations. Diabetes 56(12):3053–3062
153. Sanchez-Cespedes M (2007) A role for LKB1 gene in human cancer beyond the Peutz-Jeghers syndrome. Oncogene 26(57): 7825–7832
154. Yuan TL, Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. Oncogene 27(41):5497–5510
155. Dazert E, Hall MN (2011) mTOR signaling in disease. Curr Opin Cell Biol 23(6):744–755
156. Cancer Genome Atlas Research N (2011) Integrated genomic analyses of ovarian carcinoma. Nature 474(7353):609–615
157. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S et al (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448(7152):439–444
158. Liu P, Gan W, Inuzuka H, Lazorchak AS, Gao D, Arojo O, Liu D, Wan L, Zhai B, Yu Y et al (2013) Sin1 phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorigenesis. Nat Cell Biol 15(11):1340–1350
159. Lu ZH, Shvartsman MB, Lee AY, Shao JM, Murray MM, Kladney RD, Fan D, Krajewski S, Chiang GG, Mills GB et al (2010) Mammalian target of rapamycin activator RHEB is frequently overexpressed in human carcinomas and is critical and sufficient for skin epithelial carcinogenesis. Cancer Res 70(8):3287–3298
160. Nardella C, Chen Z, Salmena L, Carracedo A, Alimonti A, Egia A, Carver B, Gerald W, Cordon-Cardo C, Pandolfi PP (2008) Aberrant Rheb-mediated mTORC1 activation and Pten haploinsufficiency are cooperative oncogenic events. Genes Dev 22(16):2172–2177
161. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ et al (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science 304(5670):554

162. Nanjundan M, Zhang F, Schmandt R, Smith-McCune K, Mills GB (2007) Identification of a novel splice variant of AML1b in ovarian cancer patients conferring loss of wild-type tumor suppressive functions. Oncogene 26(18):2574–2584
163. Roffey J, Rosse C, Linch M, Hibbert A, McDonald NQ, Parker PJ (2009) Protein kinase C intervention: the state of play. Curr Opin Cell Biol 21(2):268–279
164. Sato T, Nakashima A, Guo L, Coffman K, Tamanoi F (2010) Single amino-acid changes that confer constitutive activation of mTOR are discovered in human cancer. Oncogene 29(18):2746–2752
165. Sciarretta S, Volpe M, Sadoshima J (2014) Mammalian target of rapamycin signaling in cardiac physiology and disease. Circ Res 114(3):549–564
166. Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, Sauter M, Nonomura N, Brakemeier S, de Vries PJ et al (2013) Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet 381(9869):817–824
167. Krueger DA, Care MM, Agricola K, Tudor C, Mays M, Franz DN (2013) Everolimus long-term safety and efficacy in subependymal giant cell astrocytoma. Neurology 80(6):574–580
168. Krueger DA, Wilfong AA, Holland-Bouley K, Anderson AE, Agricola K, Tudor C, Mays M, Lopez CM, Kim MO, Franz DN (2013) Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. Ann Neurol 74(5):679–687
169. Kwiatkowski DJ, Palmer MR, Jozwiak S, Bissler J, Franz D, Segal S, Chen D, Sampson JR (2015). Response to everolimus is seen in TSC-associated SEGAs and angiomyolipomas independent of mutation type and site in TSC1 and TSC2. Eur J Hum Genet. doi:10.1038/ejhg.2015.47.
170. Dibbens LM, de Vries B, Donatello S, Heron SE, Hodgson BL, Chintawar S, Crompton DE, Hughes JN, Bellows ST, Klein KM et al (2013) Mutations in DEPDC5 cause familial focal epilepsy with variable foci. Nat Genet 45(5):546–551
171. Lal D, Reinthaler EM, Schubert J, Muhle H, Riesch E, Kluger G, Jabbari K, Kawalia A, Baumel C, Holthausen H et al (2014) DEPDC5 mutations in genetic focal epilepsies of childhood. Ann Neurol 75(5):788–792
172. Scheffer IE, Heron SE, Regan BM, Mandelstam S, Crompton DE, Hodgson BL, Licchetta L, Provini F, Bisulli F, Vadlamudi L et al (2014) Mutations in mammalian target of rapamycin regulator DEPDC5 cause focal epilepsy with brain malformations. Ann Neurol 75(5):782–787
173. Graziotto JJ, Cao K, Collins FS, Krainc D (2012) Rapamycin activates autophagy in Hutchinson-Gilford progeria syndrome: implications for normal aging and age-dependent neurodegenerative disorders. Autophagy 8(1):147–151
174. Stahl A, Paschek L, Martin G, Gross NJ, Feltgen N, Hansen LL, Agostini HT (2008) Rapamycin reduces VEGF expression in retinal pigment epithelium (RPE) and inhibits RPE-induced sprouting angiogenesis in vitro. FEBS Lett 582(20):3097–3102
175. Ibraghimov-Beskrovnaya O, Natoli TA (2011) mTOR signaling in polycystic kidney disease. Trends Mol Med 17(11):625–633
176. Bibee KP, Cheng YJ, Ching JK, Marsh JN, Li AJ, Keeling RM, Connolly AM, Golumbek PT, Myerson JW, Hu G et al (2014) Rapamycin nanoparticles target defective autophagy in muscular dystrophy to enhance both strength and cardiac function. FASEB J 28(5):2047–2061
177. De Palma C, Morisi F, Cheli S, Pambianco S, Cappello V, Vezzoli M, Rovere-Querini P, Moggio M, Ripolone M, Francolini M et al (2012) Autophagy as a new therapeutic target in Duchenne muscular dystrophy. Cell Death Dis 3:e418