REVIEW

National Science Review
6: 1149–1162, 2019
doi: 10.1093/nsr/nwz171
Advance access publication 19 November 2019

CHEMISTRY

Special Topic: Chemistry and Molecular Medicine

Sin1-mediated mTOR signaling in cell growth, metabolism and immune response

Chun Ruan[1,†], Xinxing Ouyang[1,†], Hongzhi Liu[1,†], Song Li[1], Jingsi Jin[1], Weiyi Tang[2], Yu Xia[2] and Bing Su[1,2,*]

ABSTRACT

The mammalian target of rapamycin (mTOR) is an evolutionarily conserved Ser/Thr protein kinase with essential cellular function via processing various extracellular and intracellular inputs. Two distinct multi-protein mTOR complexes (mTORC), mTORC1 and mTORC2, have been identified and well characterized in eukaryotic cells from yeast to human. Sin1, which stands for Sty1/Spc1-interacting protein1, also known as mitogen-activated protein kinase (MAPK) associated protein (MAPKAP)1, is an evolutionarily conserved adaptor protein. Mammalian Sin1 interacts with many cellular proteins, but it has been widely studied as an essential component of mTORC2, and it is crucial not only for the assembly of mTORC2 but also for the regulation of its substrate specificity. In this review, we summarize our current knowledge of the structure and functions of Sin1, focusing specifically on its protein interaction network and its roles in the mTOR pathway that could account for various cellular functions of mTOR in growth, metabolism, immunity and cancer.

Keywords: Sin1, AGC kinases, mTOR complex, Akt, metabolism and immune response

[1]Shanghai Institute of Immunology, Department of Immunology and Microbiology, and the Minister of Education Key Laboratory of Cell Death and Differentiation, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China and [2]Zhiyuan College, Shanghai Jiao Tong University, Shanghai 200240, China

*Corresponding author. E-mail: bingsu@sjtu.edu.cn
†Equally contributed to this work.

Received 12 September 2019; **Revised** 22 October 2019; **Accepted** 22 October 2019

INTRODUCTION

The mammalian target of rapamycin (mTOR) regulates diverse cellular and molecular functions that ultimately control cell and body growth [1–4]. Our understanding of mTOR function and regulation begins with the finding of a bacterially produced compound called rapamycin. Rapamycin was originally identified from *Streptomyces hygroscopicus* isolated from soil samples from Easter Island, known to locals as *Rapa nui* [5–7]. Rapamycin can bind to a conserved cellular protein called FKBP12 and allosterically inhibit mTOR activity [3,8,9]. The molecular function of mTOR involves the regulation of translation initiation, ribosome biogenesis, protein maturation, autophagy, actin cytoskeleton reorganization and transcription etc. [3,10–12]. Studies, using gene-deficient mice, that impair functions of mTOR and its regulators, have greatly expanded our knowledge about mTORC's function in the development and homeostasis of different cells in different organisms [2,13–16]. On the other hand, abnormality in the mTOR-regulated pathways is associated with numerous pathological conditions including metabolic diseases, cancer, immune disorders, and cardiovascular and neurological diseases [2,17–20]. Since the mTOR pathway is extensively reviewed elsewhere [1,3,21], this review will briefly discuss the overall mTOR signaling and mainly focus on Sin1, one of the key adaptor molecules in mTORC2, for its regulation and function in the mTOR signaling pathway.

Mammalian TOR belongs to a family of phosphoinositide 3-kinase (PI3K)-related kinases (PIKKs) [22,23]. Interestingly, members of this family share homology with lipid kinases but instead of lipid, they phosphorylate Ser/Thr residues in proteins [23,24]. Many PIKKs form multi-protein complexes and the binding partners often dictate their substrate specificity [25,26]. Components of the TOR pathway are generally highly conserved [3,10,27] and mTOR forms at least two distinct protein complexes termed mTOR complex

© The Author(s) 2019. Published by Oxford University Press on behalf of China Science Publishing & Media Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

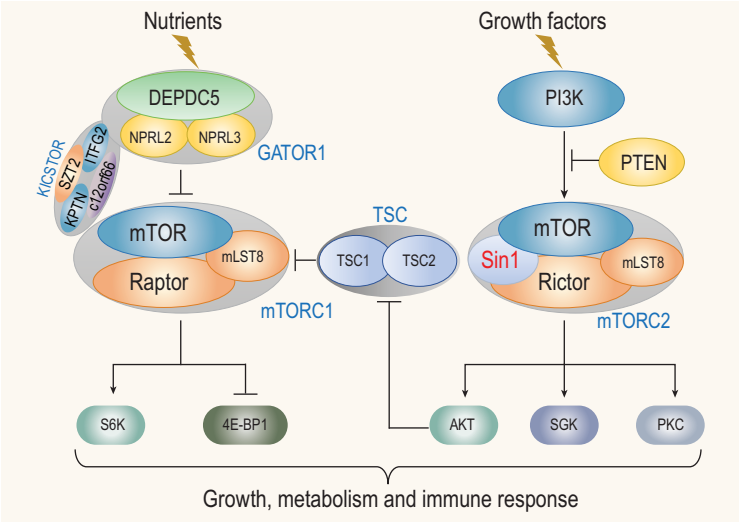

Figure 1. Illustration of the key components of two mTOR complexes and their responses to nutrients and growth factors. In response to nutrients and growth factors, mTORC1 and mTORC2 phosphorylate their respective substrates as indicated to control cell growth, metabolism and immunity.

(mTORC) 1 and mTORC2 (Fig. 1) that perform different cellular functions [2,10]. mTORC1 is sensitive to rapamycin and nutrient status [12,28]. In contrast, mTORC2 only responds partially to rapamycin treatment and is stimulated by growth factors [10,29].

mTORC1 core components consist of mTOR, regulatory-associated protein of mTOR (Raptor) and mLST8 (also called GβL) [28,30,31]. Other subunits include PRAS40 and Deptor [32,33] (Fig. 1). mTORC1 responds to nutrients, for example amino acids and glucose. These signals can regulate mTORC1 temporally and spatially via phosphorylation of Raptor [34]. mLST8, a protein consisting of seven WD40 repeats, is an essential subunit not only for mTORC1 but also mTORC2. Consistently, mLST8 ablation in mice completely abolishes mTORC2 activity [35]. mLST8 binds to the kinase domain of mTOR to stimulate mTOR catalytic activity [36]. Recent structural study of the mTOR complex has revealed parts of the mTORC1 structure at the atomic level [37], confirming such an mLST8–mTOR interaction. mTORC1 additionally interacts with PRAS40, FKBP38 and Deptor to control the activity of mTORC1 under different growth conditions [27,38].

The activity of mTORC1 is controlled by diverse cues ranging from the levels of amino acids, glucose, oxygen, energy and redox status, to mitogens such as growth factors and cytokines, and is acutely inhibited by rapamycin [39–41]. When amino acids are sufficient, small GTPases Rag (heterodimers of either RagA or RagB with either RagC or RagD) becomes active, which in turn anchors mTORC1 to lysosomes [40,42]. Cellular redox status also regulates mTORC1 activity via cysteine oxidants in the absence of amino acids [39,43]. In contrast, growth factors induce mTORC1 activation via the PI3K–Akt signaling pathway [44]. Activated Akt is able to inactivate the tuberous sclerosis complex (TSC1/TSC2) proteins by phosphorylation of TSC2, which is a GTPase-activating protein (GAP) for Rheb, a positive regulator of mTORC1, thus leading to augmented mTORC1 activity [45]. Multiple pathways in mammals convey the growth and nutritional signals to mTORC1 via regulation of TSC1/TSC2 [46] (Fig. 1).

mTORC2 contains the conserved core components rapamycin-insensitive component of TOR (Rictor), Sin1 and mLST8, and other proteins including PRR5/Protor and PRR5L [47–49]. The stability and integrity of mTORC2 depend on both Rictor and Sin1. Rictor has a N-armadillo domain and a conserved C-terminus that contains multiple potential phosphorylation sites [50]. Among these sites, Thr1135 is the target of AGC family kinases such as S6K and Akt, and thus regulated by various nutrients and growth factors [51,52]. Interestingly, Thr1135 phosphorylation seems not to affect the phosphorylation of the well-known mTORC2 substrates Akt and SGK1. Instead, it may disrupt the association of Rictor and Cullin-1, affecting the ubiquitination of SGK1 [52]. Sin1 deficiency causes a decreased protein level of Rictor and abolishes Rictor interaction with mTOR [10,53], while Rictor-deficiency also affects Sin1 protein level [35,53,54], suggesting that they may require each other for stability.

Besides the well-studied mTORC1 and mTORC2 complexes, recent proteomic studies have identified various proteins associated with key subunits of mTOR such as Sin1 and mLST8 [55]. They form mTOR-like complexes and regulate important cellular processes under specific physiological conditions. For example, in lymphoma cells, CDK9 interacts with mLST8 in nuclei and promotes transcription of leukemogenesis-related genes [55]. CDK9 may also interact with Sin1/Rictor in mTORC2 for LARP1 phosphorylation to potentially promote tumor growth [55]. Deletion of Rictor in mouse brown adipocytes led to increased lipid intake and catabolism, which appeared to be independent of Akt, suggesting a non-canonical mTOR signaling pathway [55]. There was an increased mTOR/SIRT6 interaction and FoxO1 deacetylation in Rictor-deficient brown adipocytes, which may be the cause of increased lipid intake and catabolism [56]. However, in the following sections, we will review in detail the structure and function of Sin1, and discuss recent development of Sin1-mediated mTOR signaling in cell growth, metabolism and immune function.

SIN1 IS A CONSERVED ADAPTOR MOLECULE ESSENTIAL FOR MTORC2 FUNCTION

Discovery of Sin1

Sty1/Spc1-*in*teracting protein 1 (Sin1), also known as mitogen-activated protein kinase (*MAPK*) *a*ssociated *p*rotein (MAPKAP)1, was first identified in 1999 in fission yeast *Schizosaccharomyces pombe* as an adaptor protein interacting with Sty1 (also known as Spc1), a member of the eukaryotic stress-activated protein kinase (SAPK) or mitogen-activated protein kinase (MAPK) family [57]. Under cellular stresses, Sin1 is phosphorylated in a Sty1/Spc1-dependent manner. Interestingly, Sin1 is not required for Sty1/Spc1 activation but is required for stress-dependent transcription via its substrate, Atf1. Cells lacking Sin1 display multiple stress sensitivity including heat shock, osmotic stress, oxidative stress and so on [58]. In mammalian cells, Sin1 was also found to negatively regulate the MAPK pathway via direct interaction with MAP3K2, MEKK2 [59]. Later through both biochemistry and genetics studies, Sin1 was shown to be essential for the assembly and substrate specification of mTORC2 [10,53]. Intensive studies of mammalian Sin1 in mice and cell lines have revealed the essential roles of Sin1 in embryonic development, cancer, immune function and other organ development that will be discussed in more detail in the following sections.

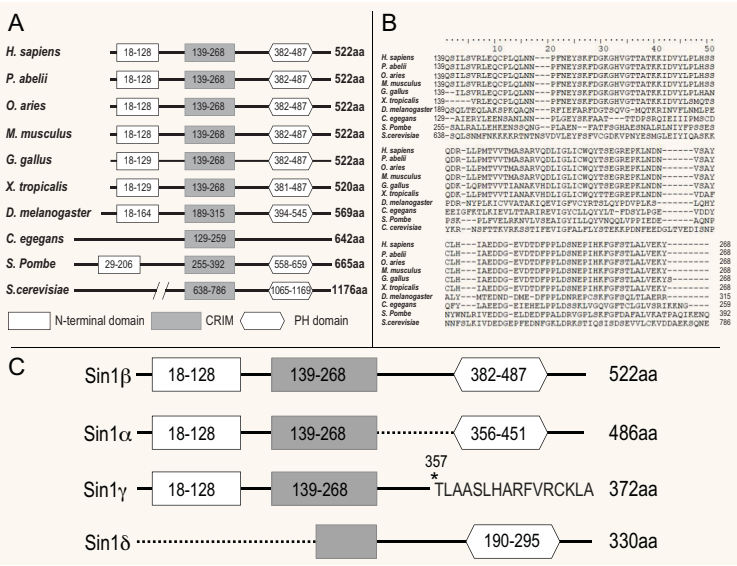

Figure 2. Sequence alignment of evolutionarily conserved Sin1 proteins. (A) A diagram showing the evolutionarily conserved domains in Sin1 proteins among different species. Empty rectangle, N-terminal region; filled rectangle, conserved region in the middle (CRIM); hexagon, PH domain. (B) Sequence alignment of the CRIM domain of Sin1 from different species. (C) Alternative splicing isoforms of human Sin1.

Conservation of the Sin1 molecule

The Sin1 protein is conserved from yeast to mammals and its orthologues are highly conserved in vertebrates from fish to mammals [59–62] (Fig. 2A). This high conservation of Sin1 suggests that Sin1 may play an indispensable role in eukaryotic organisms. However, Sin1 orthologue is not found in Plantae genomes [63]. Comparison of the conserved sequences in various Sin1 orthologues has identified a stretch of sequence called conserved region in the middle (CRIM) that is conserved among all species including *Saccharomyces cerevisiae* [64] (Fig. 2B). In addition, a putative PH domain at the C-terminus is also conserved in most species except the worm *Caenorhabditis elegans*. Interestingly, the N-terminal region with about 100 amino acids of Sin1, which is sufficient for the assembly of mammalian TORC2 [36] (unpublished data), is missing in the worm and *S. cerevisiae* (Fig. 2A) [63]. Together, these data demonstrate that Sin1 structure and function are well conserved in various species (data based on Uniprot sequence).

An adaptor protein called Avo1 in the budding yeast *S. cerevisiae* was first studied as the key component of *S. cerevisiae* TORC2, which also contains TOR2, AVO2 (Rictor orthologue) and AVO3 (mLST8 orthologue) [31,65]. However, it was not considered as a Sin1 orthologue due to its low sequence homology (about 20%) with other Sin1 orthologues, and because of this, mammalian Sin1 was first thought not to be involved in mTOR function [1,31]. The Avo1-containing [65] TORC2 in *S. cerevisiae* was shown to regulate actin organization and maintain cell wall integrity via Ypk2 (yeast protein kinase 2) [66]. Interestingly, the conserved CRIM domain in Avo1 was found to recruit Ypk to TORC2 [31,67]. In addition to the CRIM domain, Avo1 also contains a putative PH domain (840–933 amino acids) and a sequence with weak homology to Ras-binding domain (RBD) found in many Ras target proteins [31] (Fig. 2A). This RBD domain is also found in the *Dictyostelium discoideum* Sin1 orthologue RIP3, indicating that Sin1 in these two species may bind and inhibit Ras protein signaling [68]. Such RBD sequence is also identified in mammalian Sin1, strongly suggesting that Sin1 plays an important role in regulating Ras signaling [10,58,59,69,70].

Mammalian Sin1 structure

Like many other members of the Sin1 family, mammalian Sin1 protein contains an N-terminal region, a CRIM domain (conserved region in the middle), a RBD [68] (Ras-binding domain) and a PH

(pleckstrin homology) domain in its C-terminal region (Fig. 2A). The N-terminal region of mammalian Sin1 is important for interaction with either Rictor or MEKK2 [36,59]. The RBD motif in mammalian Sin1 is suggested to bind to Ras protein and inhibit the Ras signaling [68]. The CRIM domain of Sin1 is highly conserved among all Sin1 orthologues [64]. It likely serves as a specific binding site for many of the mTORC2 substrates and thus contributes to the specificity of mTORC2 [71,72].

The PH domain in the C-terminal region of Sin1 contains a lipid-binding motif that could selectively bind phosphor-inositides in cellular membrane [71]. In PI3K-mediated mTORC2 activation, the PH domain of Sin1 specifically interacts with phosphatidylinositol (3,4,5)-trisphosphate [73] on the plasma membrane and is involved in mTORC2 activation via a 'release-of-inhibition' mechanism [72]. The PH domain is not required for the enzymatic activity of mTORC2 *in vitro* since the kinase activity of mTORC2 is comparable between full-length and PH domain-truncated Sin1 [36]. The Sin1 PH domain was also shown to interact with PKC [74,75], suggesting that Sin1 may regulate the activity of other kinases using similar protein–lipid interaction [72,76].

Detailed Sin1 structure studies using NMR, crystallization and cryo-EM have shed light on its function as well as evolution. The crystal structures of yeast and human Sin1 PH domains have revealed that though they adopted similar PH folding, their binding pockets for phospholipids are not the same, indicating that during evolution, Sin1 may evolve to recognize different substrates [77]. An NMR study of Sin1 CRIM domain in fission yeast *S. pombe* showed that it formed a ubiquitin-like structure with a characteristic acidic protrusion which contributed to the recruitment of TORC2 substrate Akt. Notably, this yeast Sin1 CRIM domain can be fused to any other subunits of TORC2 and retains the complex's kinase activity, even in Sin1-null cells [71]. In contrast, the Sin1 CRIM domain in budding yeast and human is not sufficient for the integrity and function of mTORC2 [36,78]. Recent cryo-EM studies have shed light on Sin1 structure in the context of the TORC2 protein complex. The yeast TORC2 contains two copies of Sin1 (Avo1) and it functions as a scaffold for the complex by interacting with almost all other subunits, including LST8, Avo3 and TOR [78]. In human mTORC2, mammalian Sin1 interacts with Rictor to form a steric hindrance near the rapamycin–FKBP12 complex binding region in mTOR. This may explain why mTORC2 is insensitive to rapamycin inhibition. However, due to the flexibility of Sin1 protein, its accurate conformation in the mTORC2 complex remains elusive in all structural studies so far [36].

Sin1 isoforms and intracellular localization

Mammalian Sin1 exists in multiple alternatively spliced isoforms (Fig. 2C) [60,64,79]. At least five different transcript variants and four distinct protein isoforms have been identified [54,64,80]. These isoforms vary in expression level, and cellular and tissue location [54,80]. Sin1α and Sin1β are the two main isoforms that form mTORC2. Sin1γ, which lacks the PH domain, cannot be localized to the plasma membrane. Instead, it was found to be co-localized with the centrosome and forms a distinct cylinder structure, and may regulate cell division [80]. Notably, Sin1γ-containing mTORC2 is resistant to insulin stimulation and its enzymatic activity is dramatically reduced [54,80]. Sin1δ, which lacks an N-terminal domain, does not co-immune precipitate with other components of mTORC2 and is not responsible for most of the mTORC2 functions, such as phosphorylation of Akt at Ser473 and the formation of a Sin1–Rictor–mTOR complex [80]. Its precise function remains unclear. These observations confirm the importance of the N-terminal region of Sin1 for mTORC2 complex integrity.

Subcellularly, active mTORC1 mainly localizes on the surface of lysosomes [40] while mTORC2 was originally reported to be present on mitochondria-associated endoplasmic reticulum membranes [81]. Using a cell-compartment-specific reporter system, Ebner *et al.* broadened the localization of mammalian Sin1 to many organelles such as plasma membrane, mitochondria outer membrane and endosomes. Notably, the subcellular localization of mTORC2 affects its response to PI3K signaling [82]. Extracellular signals such as hormones also regulate mTORC2 function by dictating its subcellular localization [83]. Another example is upon activation of iNKT cells, augmented glycolysis activates mTORC2 by recruiting it to mitochondria via hexokinase-II. This step is crucial to regulate the production of IFN-γ in matured iNKT cells [82,84]. However, how, and the precise cellular location by which mTORC2 is regulated in catalyzing the turn motif phosphorylation of Akt and cPKC remain unclear [85,86].

Sin1 interacts with many protein kinases

Early studies of mouse and human Sin1 show that it is an interacting protein for MAP3K2 MEKK2 and

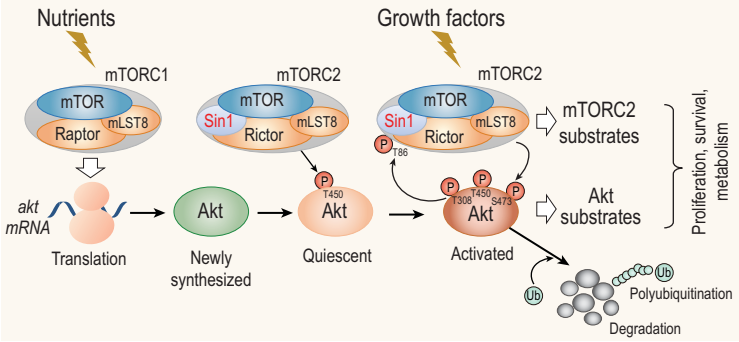

Figure 3. mTORC1 and mTORC2 regulate Akt protein synthesis, stability and activation. When nutrients are sufficient, mTORC1 is activated to direct Akt protein synthesis. During Akt translation, mTORC2 is recruited to ribosomes and to phosphorylate the newly synthesized Akt at Thr450. Thr450 phosphorylation stabilizes the Akt protein by preventing K48-ubiquitination-mediated degradation. When cells receive signals from growth factors, a small fraction of resting Akt is further phosphorylated at Ser473 by mTORC2 and at Thr308 by PDK1, thus becoming fully activated. Active Akt not only controls its own substrates but also phosphorylates Sin1 at Thr86 to augment the activity of mTORC2, resulting in a positive feedback loop for more mTORC2 substrate phosphorylation. Alternatively, Sin1 could be phosphorylated at both Thr86 and Thr398, leading to dissociation of mTORC2 to negatively regulate the mTORC2–Akt axis. Finally, Akt Ser473 phosphorylation also primes Akt for the Lys48-linked polyubiquitination, leading to its degradation, thus preventing over-activation of the PI3K–Akt pathway.

involved in negatively regulating the MAPK signaling [59]. Sin1 could form a stable complex with the inactive and non-phosphorylated MEKK2, thereby preventing its dimerization and activation, resulting in inhibition of MEKK2/c-Jun N-terminal kinase (JNK) signaling [59,79]. Another study showed that human Sin1 and its isoform Sin1α bound to JNK *in vitro* and *in vivo*, but not to p38- or ERK1/2-family MAPKs [61]. Overexpression of full-length Sin1 suppressed basal JNK activity and UV-C-induced activation of JNK in certain cell types, suggesting that Sin1 may serve as a scaffold protein in the regulation of JNK signaling [61]. In another study, it was shown that Sin1 was capable of binding to both ATF-2 and p38, and enhanced ATF-2-dependent transcription in an SAPK signaling pathway [87]. It was also reported that ovine Sin1 (ovSin1) could bind with ovIFNAR2 constitutively and the two proteins were co-localized to the plasma membrane and perinuclear structures [88]. Furthermore, it was reported that Sin1 may have specific and conserved sequences for IFNAR2 interaction [60]. These studies may provide a possible link between type I IFN function and Sin1-mediated signaling pathways. In this regard, it was reported that Sin1/mTORC2 played a role in type I IFN-induced expression of ISG and type I IFN biological responses via engagement of the Akt/mTORC1 axis, including IFN-induced phosphorylation of S6 kinase and phosphorylation of 4EBP1 [89]. In addition, targeted disruption of Sin1 led to decreased activation of the STAT1 signaling pathway and type I IFN-induced gene transcription in antiproliferative responses [90]. More recently, it was found that Sin1 could regulate IFNγ-induced genes and type II IFN-mediated biological responses via both Akt/mTORC1 activation and tyrosine phosphorylation of STAT1 [91].

Sin1 is essential for Akt HM and TM site phosphorylation

The most characterized mTORC2 substrates include Akt, conventional PKC, and other AGC (protein kinase A, G, C families) protein family members [4]. Although studies using Sin1-deficient cells first confirmed the essential role of Sin1 in mTORC2-mediated phosphorylation of Akt Ser473 at its hydrophobic motif [10,53], it did reveal another important function of Sin1 and mTORC2 until later. It was known for quite a long time that Akt is constitutively phosphorylated at the Thr450 (murine, human Thr451) site within its turn motif [92,93]. Due to its constitutive nature, this phosphorylation was first thought to have no significant function [92]. It was first speculated to be an autophosphorylation. Later studies suggest that it may contribute to the induction of Akt activity [94]. However, following the identification of Sin1 as the most important component of mTORC2 and required for the assembly of the kinase complex for Akt Ser473 phosphorylation, it was soon shown that Sin1-mTORC2 also served as the kinase for Akt Thr450 and PCK at the turn motif (TM) independent of Ser473 phosphorylation [85,86].

Remarkably, the Akt Thr450 residue is highly conserved not only across species but also exists in many AGC kinases including PKC and PKA [85,86]. Although phosphorylation of this site was identified in the middle 90's of the last century [92] as discussed above long before the function of mTORC2 was studied, the importance and implication of this site phosphorylation was not appreciated until the discovery of Sin1-mediated mTOR function [85,86]. Its major function is to regulate the stability of conventional (c)PKC and Akt [72,85,86,95]. When cellular nutrients are sufficient, mTORC1 stimulates Akt protein synthesis. During the translation process, mTORC2 is recruited to active ribosome and directly binds ribosomal protein L23 at the exit tunnel of the ribosomal complex [95] where mTORC2 phosphorylates newly synthesized Akt Thr450 at the turn motif (TM) but not Ser473 in the hydrophobic motif (HM) [95] (Fig. 3). This phosphorylation is critical for correct protein folding and stabilization of Akt by preventing co-translational ubiquitination and

degradation [85,86] (Fig. 3). Interestingly, in Sin1- or mTORC2-deficient cells, the partially folded Akt or cPKC was protected by the molecular chaperone HSP90, thus maintaining a similar half-life in cells as that in wild type cells [85,86]. Consistently, dual inhibition of Sin1–mTORC2 and the chaperone HSP90 pathway synergistically suppressed the growth of leukemia tumor cells [96]. In response to growth factors or other extracellular cues, a small fraction of quiescent Akt is further phosphorylated at Ser473 and Thr308 to become fully activated. Active Akt not only controls its well-characterized substrates but also phosphorylates Sin1 at Thr86. Some believe that this phosphorylation leads to the augmentation of mTORC2 activity, resulting in a positive feedback loop [72,97,98] (Fig. 3), while others have shown evidence that phosphorylated Thr86 causes disruption of the mTORC2 complex thus suppressing tumor cell growth [17] (Fig. 3). We speculate that under different cellular environments, the same modification of Sin1 may lead to different outcomes. Furthermore, the Akt Ser473 phosphorylation primes Akt for Lys-48-linked polyubiquitination, leading to its degradation [99] (Fig. 3). This Sin1–mTORC2-mediated down-regulation of active Akt is likely a crucial step to prevent overt activation of the PI3K–Akt pathway, which has been linked to numerous human diseases [2,17].

The TORC2–AGC kinase signaling axis is evolutionarily conserved. Both *S. cerevisiae* and *S. pombe* have Akt orthologues (YPK and Gad8, respectively) that can be phosphorylated by TORC2. However, their protein sequences are very different from mammalian Akt. In addition, Thr450 in the TM is also conserved in other AGC kinases like cPKC (Fig. 4). Therefore we speculate that besides Akt, this Sin1–mTOR-regulated kinase activation mechanism may also affect other kinases downstream of mTOR by regulating their stability and activity through the TM and HM sites.

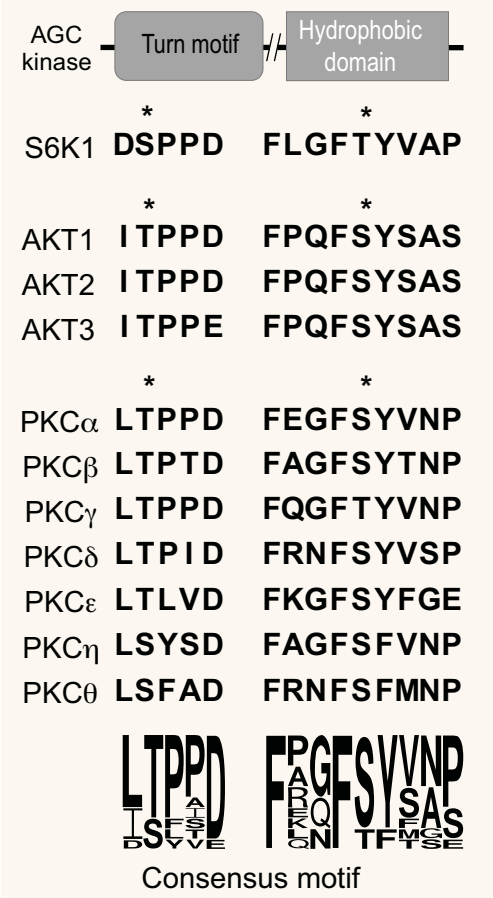

Figure 4. Conservation of the turn motif (TM) and hydrophobic motif (HM) of members of the AGC kinase family, and sequence alignment of the TM and HM domains of Akt and PKC. All the evolutionarily conserved sites that can be phosphorylated by mTORC2 are indicated by asterisks. The degree of the conservation of the consensus residues in the TM and HM domains is illustrated by the size of the specified residues at the bottom. All sequences are based on human sequences downloaded from Uniprot.

PHYSIOLOGICAL FUNCTION OF SIN1

Sin1 function in immune responses

Sin1 plays a major role in the immune system, ranging from the development of immune cells, production of cytokines and generation of specific microenvironment [69,70,90,100]. Sin1 was first shown to regulate the number of Foxp3$^+$ natural T regulatory cells [101]. A recent study also demonstrates that Sin1 is involved in the metabolism and proliferation of DN T cells in the thymus [69]. Sin1/mTORC2 controls the proliferation and development of DN stage T cells by regulating glycolytic metabolism through an Akt–PPARγ–PKM2 axis [69]. Moreover, Sin1 controls the homing of naïve T cells. Under normal state, Sin1 suppresses CXCR4 expression, and Sin1 deletion leads to an increased accumulation of naïve T cells in bone marrow [102].

Likewise, Sin1 is required for B cell development, antibody generation and humoral immunity response upon viral infection [70,100,103]. Sin1 plays a vital role in transducing BCR-mediated PI3K signals to Akt and regulates the stability of c-Myc and the activity of mTORC1 via GSK3 and TSC1/2, respectively [100]. This partly explains why in Sin1-null B cells there is a limited expansion of the B cell pool [70]. HSCs with compromised Sin1 also generate fewer immature B cells than wild type, due to higher expression levels of *il7r* [104]. Sin1 deficiency

promotes *il7r* and RAG gene expression, pro-B cell survival and V(D)J recombination when cultured with IL-7. Although increased pro-B cell survival is observed, it is accompanied by decreased levels of IgM^+ immature cells, indicating stagnation of B cell development [100].

Although no apparent defects in neutrophil development is observed in Sin1-deficient mice [105], Sin1 may regulate the activity of platelets [106]. Sin1 was found highly phosphorylated at Thr86 in platelets from patients with ST-elevation myocardial infarction (STEMI), accompanied by elevated mTORC2 activation and Akt Ser473 phosphorylation [106]. Sin1 deficiency in platelets attenuates the microthrombosis after ischemic conditions. The capability of Sin1 to reduce ROS levels under hypoxic conditions may prevent platelet activation and embolization in ischemic cardiovascular diseases [106]. Mechanistically, Sin1 may mediate the αIIbβ3-initiated outside-in signaling.

Apart from mediating the development of immune cells, Sin1 also contributes to the formation of a specific microenvironment in bone marrow as knockdown of Sin1 at mRNA level reduces low-dose irradiation-induced Akt Ser473 phosphorylation and subsequent responses in mouse osteoblasts, which inhibit osteoblast differentiation [107]. Sin1 may also regulate multiple cytokine-induced pathways since dysregulation of Sin1 led to decreased STAT1 and suppressed the transcription of several IFN-γ-induced genes [91]. As discussed above, Sin1 could also regulate type I IFN production and its antitumor responses [89].

It has been shown that abnormal PKCδ, which forms an overly stable interaction with Sin1, may contribute to SHORT syndrome [75]. CCDC28B, a protein associated with the Bardet–Biedl syndrome, is shown to interact with Sin1 and disrupt cilia formation, which is linked with proper signal transduction and immune responses [108]. Nijmegen breakage syndrome (NBS) protein NBS1 interacts with Sin1/mTORC2 [109]. Reduced Sin1 activity may prevent chronic rejection in allograft models [96,110].

Other functions of mTORC2 may include the regulation of the generation of surface receptors [111], the lineage differentiation of T cell subsets [112–116], the development of memory T cells [117], the development and activation of other immune cells like macrophages, dendritic cells and NK cells [118–120], the production of cytokines [121], the long-term survival of immune cells [122] and so on. However, the precise role of Sin1 in these processes requires more thorough studies with specific cellular and mouse models.

Sin1/mTORC2 function in metabolism

mTOR is a well-studied key regulator of metabolism via integrating upstream signals from nutrients and growth factors upstream of many AGC kinases that subsequently act on a wide range of transcription factors, which controls the level of rate-limiting enzymes in the metabolic pathways [2,21,123,124]. AGC kinases can directly activate rate-limiting enzymes of metabolic pathways [125–127]. The deficiency of Sin1 is therefore expected to cause great changes in metabolism.

Changes in cell metabolism are tightly associated with the growth, activation and differentiation of many immune cells including $CD4^+$ T cells [118]. Studies in cell lines have uncovered the function of Sin1 in metabolism regulation. Sin1/mTORC2 could regulate mSREBP1 levels, and knockout of Sin1 decreased mSREBP1 levels and its target genes including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN), resulting in suppressed lipogenesis in cells [128]. It is also found that Sin1/mTORC2 was required for the hexosamine biosynthesis pathway (HBP), via glutamine: fructose-6-phosphate amidotransferase 1 (GFAT1) [129]. Sin1 also controls cell metabolism in response to extrinsic and intrinsic immune signals. Recent studies show that Sin1 is required for upregulation of PKM2 for glycolysis [69] and is crucial for proper B cell glycolysis and mitochondrial respiration under both resting and anti-IgM-stimulated conditions [70,130].

Sin1 function in cancer

Dysregulation in the mTORC2 pathway caused by overexpression or upregulation of Sin1 is associated with many types of cancer [21,131]. For example, Sin1 is upregulated in primary breast cancer tissues. Its overexpression is associated with higher proliferation and metastasis of tumor cells [132]. Sin1 is also overexpressed in clinically aggressive thyroid cancer types, including medullary thyroid carcinomas and aggressive variants of papillary thyroid carcinoma, accompanied by strong activation of Akt [133]. In the above cases, dysregulation of Sin1 was usually associated with over-activated Akt and high c-Myc levels, which is likely the reason for augmented tumor growth. Moreover, Sin1 may promote the metastasis and epithelial mesenchymal transition (EMT) of tumor cells. In NSCLC tissues, MLL expression is upregulated, which may activate Sin1 epigenetically to promote EMT and proliferation of NSCLC [134]. Elevated Sin1 level is also associated with higher invasion and metastasis of HCC cells and may facilitate the development of HCC [135].

Though the specific mechanism still remains to be discovered, it is reasonable to predict that drugs targeting the overly activated mTOR pathway, especially mTORC2, would have a clinical significance in cancer treatment. Indeed, suppression of mTORC2 by expressing the truncated form of Sin1 significantly impairs tumor growth in the xenograft mouse model [136]. Similarly, a tumor suppressor Pdcd4 that could attenuate Sin1 translation was shown to prevent invasion of colon carcinoma [137] and a synthesized Rictor inhibitor, also targeting mTORC2 activity, could inhibit glioblastoma growth in a xenograft model [138]. Better understanding of the Sin1/mTORC2 signaling pathway will provide more clues to develop efficient cancer diagnosis and treatment methods.

Sin1 function in neurons

TPKC, an important regulator of cytoskeletons, is directly regulated by Sin1/mTORC2, and its substrates include many important proteins in actin cytoskeletal rearrangement *in vivo*, including, but not limited to, GAP43, MARCKS and adducin [139]. In *Drosophila*, long-term memory was restored in aged flies and enhanced in young flies by direct activation of dTORC2. At the same time, actin polymerization in neurons was observed [140]. Similarly, deficiency of Rictor in mice reduced the level of actin polymerization, which is associated with impaired long-term memory and hippocampal potential [141]. Interestingly, short-term memory is not affected [142]. In humans, mTORC2 dysfunction is associated with irregular insulin/PI3K/Akt function, which is an important feature of Alzheimer's disease pathogenesis [143].

PERSPECTIVE AND FUTURE STUDIES OF SIN1 REGULATION AND FUNCTION

Targeting Sin1 as an mTORC2-specific inhibitor

Sin1, since its first discovery as an essential mTORC2 subunit in 2006, has been shown to have more and more indispensable functions in cell growth, metabolism, cancer and immunity. Given its potent roles in mTORC2 assembly, regulation and substrate specificity in immune function and cancer development, it is natural to believe that approaches targeting the Sin1–mTORC2 interaction may yield fruitful outcomes with a clinical impact. Considering the lack of mTORC2-specific inhibitors at the moment, it is likely that Sin1 could be an ideal target for mTORC2 inhibitors.

Although much has been learned about the structure and function of Sin1, more questions remain to be answered. For example, why does Sin1 have multiple isoforms and what are their physiological functions? What controls mTORC2 subcellular localization? How would mTORC2 signaling crosstalk with that of mTORC1, and how would it impact cellular metabolic re-programing? In addition, it is already known that Sin1 and mTOR subcellular localizations are not entirely overlapping, indicating that Sin1 has mTOR-independent functions. Consistently, although both Sin1 and Rictor are required for mTORC2 integrity and functions [10], deletion of Sin1 and Rictor individually shows different phenotypes in neutrophils [105], in T cells [101,111] and in other cell types [103]. These data strongly suggest that Sin1 may have distinct functions besides mTORC2.

Sin1 mediates the crosstalk between mTORC1 and mTORC2

It is well appreciated that the main function of mTORC1 is sensing the intracellular nutrient levels, and when adequate, directs the biosynthesis of macromolecules such as proteins and nucleic acids as building blocks for cell growth and proliferation. On the other hand, mTORC2 may not be directly involved in the biosynthesis of such macromolecules but instead, it senses the extracellular or intracellular conditions for cells to determine if it is suitable for starting a biosynthesis process. For instance, if there are abundant levels of growth factors, mTORC2 may send a signal to cells for rapid biosynthesis of macromolecules. In contrast, if cellular stresses are sensed by mTORC2, it may signal to the cells to stop biosynthesis of macromolecules, thus saving energy that would be needed to respond to the adverse situation. Therefore, one outstanding question in the field is how the two distinct mTOR complexes, mTORC1 and mTORC2, coordinate the nutrient- and growth factor- or cellular stresses-mediated signals in controlling cell growth and metabolism. Since the two mTOR complexes share key components, which have evolved to regulate similar cellular growth and metabolism processes, we believe that they should be involved in sensing nutrients and cellular signals synergistically to regulate those key cellular functions (Fig. 1).

Previous studies have provided strong evidence showing that mTORC2 could send positive signals via the AGC kinase Akt to inhibit the TSC complex, which is a negative regulator of mTORC1, thus augmenting the mTORC1 activity and promoting optimal cell growth [4,144,145]. Since this

cross-regulation of mTORC1 by mTORC2 is mediated directly through a small GTPase RheB, it is interesting to know if mTORC2 could also regulate mTORC1 by targeting its upstream nutrient-sensing components [12,146]. Multi-protein complexes such as Gator1, Gator2 and Kicstor etc. have been identified in the past several years acting upstream of mTORC1 with important roles in sensing nutrients such as glucose, amino acids and cellular energy levels [12,147–150]. Given that most of those studies focus only on the roles of those complexes in response to nutrients, little is known at the moment how signals activating through mTORC2 may be linked to these nutrient-sensing components of mTORC1. In our study, we found that mTORC2 deficiency could lead to increased mTORC1 activity under certain conditions [10,85] (data not shown), while over-activation of mTORC1 also restricts mTORC2 activity, either through the mTORC1-mediated phosphorylation and activation of Grb10, a negative regulator of insulin/IGF-1 receptor [12,151], or by S6K1 phosphorylation and degradation of insulin receptor substrate 1 (IRS1) to suppress mTORC2 activity [152]. Mathematical modeling of phospho-proteomic data in insulin-treated adipocytes revealed a network consisting of both a negative signal from S6K to Rictor/mTORC2 and a positive signal from Akt to Sin1/mTORC2 [97,98]. This model also predicts that under insulin-resistant diabetic conditions, mTORC1 and mTORC2 crosstalk to each other to enhance the positive feedback between mTORC2 and Akt, whereas the negative signal from S6K is reduced, resulting in increased overall Akt-Ser473 phosphorylation and likely Akt activity in diabetic patients [152]. Consistently, the computational simulation model that used constitutively active Akt with Ser473 phosphorylation in type 2 diabetes patients showed decreased mTORC1 activity [153]. It was also revealed that S6K could phosphorylate Rictor at Thr1135 to suppress mTORC2 formation [98]. Furthermore, it was found that Foxo1/3, a well-characterized downstream target of mTORC2, may activate Sestrin3 to decrease mTORC1 activity [154].

S6K may also phosphorylate Sin1 at Thr86 and Thr398, which was proposed to dissociate Sin1 from mTORC2, leading to a decreased mTORC2 activity [17] (Fig. 3). This negative feedback model seems to be supported by the fact that a Sin1-R81T mutation was associated with ovarian cancer [17]. This mutation results in a deficiency of Sin1 phosphorylation at Thr86 and sustained activation of mTORC2 and Akt [17]. Interestingly, it was also shown in this study by Liu *et al.* [17] and from an independent study [97] that Akt is also a kinase for Sin1 Thr86 phosphorylation. In this case, it was Akt that mediated Sin1 Thr86 phosphorylation to positively regulate mTORC2 activity [98], and a computational simulation also supports the positive regulation model [153]. Recent data from our group with Sin1 Thr86 phosphorylation-deficient knockin mice support Sin1 Thr86 phosphorylation as a positive regulator of mTORC2 (data not shown).

While mTORC1 and mTORC2 are thought to be activated by different upstream cues, increasing evidence suggests that a wide spectrum of environmental and intrinsic cues can activate both complexes at the same time or even synergistically. For instance, Ras homolog enriched in striatum [155], a small G protein, has been shown to interact with both complexes and activate mTOR activity [155]. DEPTOR, capable of binding both mTOR complexes, could suppress the formation of both complexes [156]. On the other hand, small GTPase Rac1 could positively regulate both mTOR complexes [157]. Activation of both mTORC1 and mTORC2 could lead to upregulation of protein translation via the mTORC1-mediated S6K activation [158] and the mTORC2-mediated Akt activation [159]. These data strongly indicate a common shared function of mTOR at the early stage of evolution. In this regard, phylogenetic studies of most components of the TOR pathway found that it originated before the Last Eukaryotic Common Ancestor [160]. mTORC1 and mTORC2 may result from an ancient genome duplication event [160]. Interestingly, in budding yeast, TOR2 is a subunit for both TORC1 and TORC2, suggesting that at this evolutionary stage the two complexes may have redundant function. Afterward, the two complexes diverged and evolved as separated modules to have different functions in different cellular processes [160].

Although most studies on mTORC2-mediated Akt phosphorylation and regulation focus on the HM site phosphorylation, it is still puzzling about the role of mTORC2 in the phosphorylation of the TM sites of AGC kinases such as Akt and cPKC that we identified a few years ago [10,85,86]. We showed that this highly conserved function of Sin1-mTORC2 is required for stabilizing the newly synthesized AGC kinases Akt and cPKC primarily through facilitating the proper folding of the nascent peptides. Since the phosphorylation of the Akt HM site is dependent on growth factor while that of the TM site seems not to be, we speculate that the phosphorylation of TM appeared earlier during evolution.

Whether it is a general form of regulation of the stability of many other nascent cellular proteins remains elusive. Considering that mTOR is a well-conserved protein kinase for protein synthesis, it is

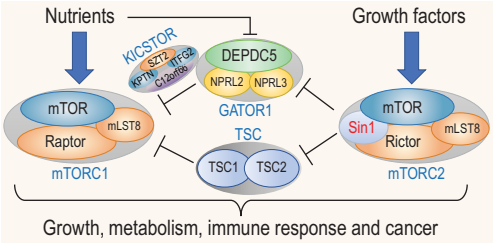

Figure 5. The newly proposed model for the crosstalk between mTORC1 and mTORC2 in regulation of cell growth, metabolism, immunity and cancer. In response to nutrients and growth factors, mTORC1 and mTORC2 phosphorylate their respective substrates as indicated to control major cellular functions as indicated. As illustrated, mTORC2 sends positive signals via Akt to mTORC1 by inhibiting its negative regulator TSC complex. Similarly, mTORC2 may also regulate mTORC1 activity by controlling the activity of its upstream nutrient-sensing components such as Gator1.

reasonable to believe that these two protein complexes should coordinately control the overall protein synthesis, which includes not only the polypeptide chain elongation, but also the quality of the nascent polypeptides. In this regard, to fully ensure that a newly synthesized protein functions properly, it is required to be properly folded, modified and transported to its proper location for function. In case any of those steps fail, the polypeptide should be degraded and recycled to prevent the accumulation of waste that is known to be the cause of many diseases [161,162]. We believe that mTORC2, which may not be directly involved in polypeptide enlongation, may serve this quality check and recycle function of the newly synthesized polypeptides. Consistently, this function of mTORC2 does not require growth factors or other extracellular stimuli, and could function constitutively [10,85].

Finally, the precise molecular mechanism by which the mTORC1 and mTORC2 coordinately control protein synthesis and metabolism remains largely unknown. In search for such a crosstalk between these two complexes, we have recently identified components of Gator1 that could be regulated by Sin1, most likely via the mTORC2 function (data not shown and Fig. 5). Combined with the well-established mTORC2–Akt–TSC axis in mTORC1 regulation, we propose a new model showing that Sin1–mTORC2 may also directly regulate the Gator1–Kicstor complex to impact the mTORC1 activity, and together to control cell growth, metabolism, immune responses, and perhaps even cancer development. Future investigation of this area is likely to yield fruitful results and may lead to the identification of new therapeutic targets for the treatment of various diseases due to abnormal metabolic regulation.

ACKNOWLEDGEMENTS

We thank all members of the Su Lab for helpful discussions, critical reading of the manuscript and careful improvement of the figures.

FUNDING

This work was supported by the National Natural Science Foundation of China (81430033) and the Shanghai Science and Technology Committee (16410723300).

Conflict of interest statement. None declared.

REFERENCES

1. Wullschleger S, Loewith R and Hall MN. TOR signaling in growth and metabolism. *Cell* 2006; **124**: 471–84.
2. Saxton RA and Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell* 2017; **168**: 960–76.
3. Sabatini DM. Twenty-five years of mTOR: uncovering the link from nutrients to growth. *Proc Natl Acad Sci USA* 2017; **114**: 11818–25.
4. Su B and Jacinto E. Mammalian TOR signaling to the AGC kinases. *Crit Rev Biochem Mol Biol* 2011; **46**: 527–47.
5. Vezina C, Kudelski A and Sehgal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot* 1975; **28**: 721–6.
6. Sehgal SN, Baker H and Vezina C. Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *J Antibiot* 1975; **28**: 727–32.
7. Martel RR, Klicius J and Galet S. Inhibition of the immune response by rapamycin, a new antifungal antibiotic. *Can J Physiol Pharmacol* 1977; **55**: 48–51.
8. Vilella-Bach M, Nuzzi P and Fang Y *et al.* The FKBP12-rapamycin-binding domain is required for FKBP12-rapamycin-associated protein kinase activity and G1 progression. *J Biol Chem* 1999; **274**: 4266–72.
9. Jacinto E and Hall MN. TOR signalling in bugs, brain and brawn. *Nat Rev Mol Cell Biol* 2003; **4**: 117–26.
10. Jacinto E, Facchinetti V and Liu D *et al.* SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* 2006; **127**: 125–37.
11. Gulhati P, Bowen KA and Liu J *et al.* mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Res* 2011; **71**: 3246–56.
12. Hsu PP, Kang SA and Rameseder J *et al.* The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. *Science* 2011; **332**: 1317–22.
13. Murakami M, Ichisaka T and Maeda M *et al.* mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. *Mol Cell Biol* 2004; **24**: 6710–8.

14. Polak P and Hall MN. mTOR and the control of whole body metabolism. *Curr Opin Cell Biol* 2009; **21**: 209–18.
15. Alessi DR, Pearce LR and Garcia-Martinez JM. New insights into mTOR signaling: mTORC2 and beyond. *Sci Signal* 2009; **2**: pe27.
16. Yang Q and Guan KL. Expanding mTOR signaling. *Cell Res* 2007; **17**: 666–81.
17. Liu P, Gan W and Inuzuka H *et al.* Sin1 phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorigenesis. *Nat Cell Biol* 2013; **15**: 1340–50.
18. Altman JK, Sassano A and Kaur S *et al.* Dual mTORC2/mTORC1 targeting results in potent suppressive effects on acute myeloid leukemia (AML) progenitors. *Clin Cancer Res* 2011; **17**: 4378–88.
19. Chong ZZ, Shang YC and Maiese K. Cardiovascular disease and mTOR signaling. *Trends Cardiovasc Med* 2011; **21**: 151–5.
20. Wong M. Mammalian target of rapamycin (mTOR) pathways in neurological diseases. *Biomed J* 2013; **36**: 40–50.
21. Laplante M and Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**: 274–93.
22. Helliwell SB, Wagner P and Kunz J *et al.* TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. *Mol Biol Cell* 1994; **5**: 105–18.
23. Manning G, Plowman GD and Hunter T *et al.* Evolution of protein kinase signaling from yeast to man. *Trends Biochem Sci* 2002; **27**: 514–20.
24. Bosotti R, Isacchi A and Sonnhammer EL. FAT: a novel domain in PIK-related kinases. *Trends Biochem Sci* 2000; **25**: 225–7.
25. Lovejoy CA and Cortez D. Common mechanisms of PIKK regulation. *DNA Repair (Amst)* 2009; **8**: 1004–8.
26. Baretic D and Williams RL. PIKKs–the solenoid nest where partners and kinases meet. *Curr Opin Struct Biol* 2014; **29**: 134–42.
27. Laplante M and Sabatini DM. mTOR signaling at a glance. *J Cell Sci* 2009; **122**: 3589–94.
28. Kim DH, Sarbassov DD and Ali SM *et al.* mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 2002; **110**: 163–75.
29. Hagiwara A, Cornu M and Cybulski N *et al.* Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. *Cell Metab* 2012; **15**: 725–38.
30. Hara K, Maruki Y and Long X *et al.* Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* 2002; **110**: 177–89.
31. Loewith R, Jacinto E and Wullschleger S *et al.* Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell* 2002; **10**: 457–68.
32. Kim DH, Sarbassov DD and Ali SM *et al.* GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell* 2003; **11**: 895–904.
33. Sancak Y, Thoreen CC and Peterson TR *et al.* PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* 2007; **25**: 903–15.
34. Foster KG, Acosta-Jaquez HA and Romeo Y *et al.* Regulation of mTOR complex 1 (mTORC1) by raptor Ser863 and multisite phosphorylation. *J Biol Chem* 2010; **285**: 80–94.
35. Guertin DA, Stevens DM and Thoreen CC *et al.* Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Dev Cell* 2006; **11**: 859–71.
36. Chen X, Liu M and Tian Y *et al.* Cryo-EM structure of human mTOR complex 2. *Cell Res* 2018; **28**: 518–28.
37. Yang D, Li R and Wang H *et al.* Clinical implications of progranulin in gastric cancer and its regulation via a positive feedback loop involving AKT and ERK signaling pathways. *Mol Med Rep* 2017; **16**: 9685–91.
38. Dunlop EA and Tee AR. Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms. *Cell Signal* 2009; **21**: 827–35.
39. Sarbassov DD and Sabatini DM. Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex. *J Biol Chem* 2005; **280**: 39505–9.
40. Sancak Y, Bar-Peled L and Zoncu R *et al.* Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* 2010; **141**: 290–303.
41. Bar-Peled L and Sabatini DM. Regulation of mTORC1 by amino acids. *Trends Cell Biol* 2014; **24**: 400–6.
42. Sancak Y, Peterson TR and Shaul YD *et al.* The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 2008; **320**: 1496–501.
43. Yoshida S, Hong S and Suzuki T *et al.* Redox regulates mammalian target of rapamycin complex 1 (mTORC1) activity by modulating the TSC1/TSC2-Rheb GTPase pathway. *J Biol Chem* 2011; **286**: 32651–60.
44. Inoki K, Li Y and Zhu T *et al.* TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 2002; **4**: 648–57.
45. Manning BD and Cantley LC. Rheb fills a GAP between TSC and TOR. *Trends Biochem Sci* 2003; **28**: 573–6.
46. Efeyan A and Sabatini DM. Nutrients and growth factors in mTORC1 activation. *Biochem Soc Trans* 2013; **41**: 902–5.
47. Pearce LR, Huang X and Boudeau J *et al.* Identification of Protor as a novel Rictor-binding component of mTOR complex-2. *Biochem J* 2007; **405**: 513–22.
48. Woo SY, Kim DH and Jun CB *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**: 25604–12.
49. Thedieck K, Polak P and Kim ML *et al.* PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. *PLoS One* 2007; **2**: e1217.
50. Dibble CC, Asara JM and Manning BD. Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1. *Mol Cell Biol* 2009; **29**: 5657–70.
51. Julien LA, Carriere A and Moreau J *et al.* mTORC1-activated S6K1 phosphorylates Rictor on threonine 1135 and regulates mTORC2 signaling. *Mol Cell Biol* 2010; **30**: 908–21.
52. Gao D, Wan L and Wei W. Phosphorylation of Rictor at Thr1135 impairs the Rictor/Cullin-1 complex to ubiquitinate SGK1. *Protein Cell* 2010; **1**: 881–5.
53. Yang Q, Inoki K and Ikenoue T *et al.* Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. *Genes Dev* 2006; **20**: 2820–32.
54. Frias MA, Thoreen CC and Jaffe JD *et al.* mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol* 2006; **16**: 1865–70.
55. Beauchamp EM, Abedin SM and Radecki SG *et al.* Identification and targeting of novel CDK9 complexes in acute myeloid leukemia. *Blood* 2019; **133**: 1171–85.
56. Jung SM, Hung CM and Hildebrand SR *et al.* Non-canonical mTORC2 signaling regulates brown adipocyte lipid catabolism through SIRT6-FoxO1. *Mol Cell* 2019; **75**: 807–822.e8.
57. Reiter W, Watt S and Dawson K *et al.* Fission yeast MAP kinase Sty1 is recruited to stress-induced genes. *J Biol Chem* 2008; **283**: 9945–56.
58. Wilkinson MG, Pino TS and Tournier S *et al.* Sin1: an evolutionarily conserved component of the eukaryotic SAPK pathway. *EMBO J* 1999; **18**: 4210–21.
59. Cheng J, Zhang D and Kim K *et al.* Mip1, an MEKK2-interacting protein, controls MEKK2 dimerization and activation. *Mol Cell Biol* 2005; **25**: 5955–64.

60. Wang SZ and Roberts RM. The evolution of the Sin1 gene product, a little known protein implicated in stress responses and type I interferon signaling in vertebrates. *BMC Evol Biol* 2005; **5**: 13.
61. Schroder W, Bushell G and Sculley T. The human stress-activated protein kinase-interacting 1 gene encodes JNK-binding proteins. *Cell Signal* 2005; **17**: 761–7.
62. Tatebe H and Shiozaki K. Evolutionary conservation of the components in the TOR signaling pathways. *Biomolecules* 2017; **7**: 77.
63. Xiong Y and Sheen J. The role of target of rapamycin signaling networks in plant growth and metabolism. *Plant Physiol* 2014; **164**: 499–512.
64. Schroder W, Cloonan N and Bushell G *et al.* Alternative polyadenylation and splicing of mRNAs transcribed from the human Sin1 gene. *Gene* 2004; **339**: 17–23.
65. Cybulski N and Hall MN. TOR complex 2: a signaling pathway of its own. *Trends Biochem Sci* 2009; **34**: 620–7.
66. Liao HC and Chen MY. Target of rapamycin complex 2 signals to downstream effector yeast protein kinase 2 (Ypk2) through adheres-voraciously-to-target-of-rapamycin-2 protein 1 (Avo1) in Saccharomyces cerevisiae. *J Biol Chem* 2012; **287**: 6089–99.
67. Kamada Y, Fujioka Y and Suzuki NN *et al.* Tor2 directly phosphorylates the AGC kinase Ypk2 to regulate actin polarization. *Mol Cell Biol* 2005; **25**: 7239–48.
68. Schroder WA, Buck M and Cloonan N *et al.* Human Sin1 contains Ras-binding and pleckstrin homology domains and suppresses Ras signalling. *Cell Signal* 2007; **19**: 1279–89.
69. Ouyang X, Han Y and Qu G *et al.* Metabolic regulation of T cell development by Sin1-mTORC2 is mediated by pyruvate kinase M2. *J Mol Cell Biol* 2019; **11**: 93–106.
70. Li M, Lazorchak AS and Ouyang X *et al.* Sin1/mTORC2 regulate B cell growth and metabolism by activating mTORC1 and Myc. *Cell Mol Immunol* 2019; **16**: 757–69.
71. Tatebe H, Murayama S and Yonekura T *et al.* Substrate specificity of TOR complex 2 is determined by a ubiquitin-fold domain of the Sin1 subunit. *eLife* 2017; **6**: e19594.
72. Liu P, Gan W and Chin YR *et al.* PtdIns(3,4,5)P3-dependent activation of the mTORC2 kinase complex. *Cancer Discov* 2015; **5**: 1194–209.
73. Kim SJ, DeStefano MA and Oh WJ *et al.* mTOR complex 2 regulates proper turnover of insulin receptor substrate-1 via the ubiquitin ligase subunit Fbw8. *Mol Cell* 2012; **48**: 875–87.
74. Cameron AJ, Linch MD and Saurin AT *et al.* mTORC2 targets AGC kinases through Sin1-dependent recruitment. *Biochem J* 2011; **439**: 287–97.
75. Alcantara D, Elmslie F and Tetreault M *et al.* SHORT syndrome due to a novel de novo mutation in *PRKCE* (protein kinase Cε) impairing TORC2-dependent AKT activation. *Hum Mol Genet* 2017; **26**: 3713–21.
76. Rebecchi MJ and Scarlata S. Pleckstrin homology domains: a common fold with diverse functions. *Annu Rev Biophys Biomol Struct* 1998; **27**: 503–28.
77. Pan D and Matsuura Y. Structures of the pleckstrin homology domain of Saccharomyces cerevisiae Avo1 and its human orthologue Sin1, an essential subunit of TOR complex 2. *Acta Crystallogr F Struct Biol Cryst Commun* 2012; **68**: 386–92.
78. Gaubitz C, Oliveira TM and Prouteau M *et al.* Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. *Mol Cell* 2015; **58**: 977–88.
79. Cheng J, Yu L and Zhang D *et al.* Dimerization through the catalytic domain is essential for MEKK2 activation. *J Biol Chem* 2005; **280**: 13477–82.
80. Yuan Y, Pan B and Sun H *et al.* Characterization of Sin1 isoforms reveals an mTOR-dependent and independent function of Sin1gamma. *PLoS One* 2015; **10**: e0135017.
81. Betz C, Stracka D and Prescianotto-Baschong C *et al.* Feature article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. *Proc Natl Acad Sci USA* 2013; **110**: 12526–34.
82. Ebner M, Sinkovics B and Szczygiel M *et al.* Localization of mTORC2 activity inside cells. *J Cell Biol* 2017; **216**: 343–53.
83. Gleason CE, Oses-Prieto JA and Li KH *et al.* Phosphorylation at distinct subcellular locations underlies specificity in mTORC2-mediated activation of SGK1 and Akt. *J Cell Sci* 2019; doi: 10.1242/jcs.224931.
84. Fu S, Zhu S and Tian C *et al.* Immunometabolism regulates TCR recycling and iNKT cell functions. *Sci Signal* 2019; **12**: eaau1788.
85. Facchinetti V, Ouyang W and Wei H *et al.* The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *EMBO J* 2008; **27**: 1932–43.
86. Ikenoue T, Inoki K and Yang Q *et al.* Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J* 2008; **27**: 1919–31.
87. Makino C, Sano Y and Shinagawa T *et al.* Sin1 binds to both ATF-2 and p38 and enhances ATF-2-dependent transcription in an SAPK signaling pathway. *Genes Cells* 2006; **11**: 1239–51.
88. Wang SZ and Roberts RM. Interaction of stress-activated protein kinase-interacting protein-1 with the interferon receptor subunit IFNAR2 in uterine endometrium. *Endocrinology* 2004; **145**: 5820–31.
89. Kaur S, Sassano A and Majchrzak-Kita B *et al.* Regulatory effects of mTORC2 complexes in type I IFN signaling and in the generation of IFN responses. *Proc Natl Acad Sci USA* 2012; **109**: 7723–8.
90. Kaur S, Kroczynska B and Sharma B *et al.* Critical roles for Rictor/Sin1 complexes in interferon-dependent gene transcription and generation of antiproliferative responses. *J Biol Chem* 2014; **289**: 6581–91.
91. Kroczynska B, Blyth GT and Rafidi RL *et al.* Central regulatory role for SIN1 in interferon gamma (IFNgamma) signaling and generation of biological responses. *J Biol Chem* 2017; **292**: 4743–52.
92. Alessi DR, Andjelkovic M and Caudwell B *et al.* Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J* 1996; **15**: 6541–51.
93. Bellacosa A, Chan TO and Ahmed NN *et al.* Akt activation by growth factors is a multiple-step process: the role of the PH domain. *Oncogene* 1998; **17**: 313–25.
94. Hauge C, Antal TL and Hirschberg D *et al.* Mechanism for activation of the growth factor-activated AGC kinases by turn motif phosphorylation. *EMBO J* 2007; **26**: 2251–61.
95. Oh WJ, Wu CC and Kim SJ *et al.* mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. *EMBO J* 2010; **29**: 3939–51.
96. Zhang F, Lazorchak AS and Liu D *et al.* Inhibition of the mTORC2 and chaperone pathways to treat leukemia. *Blood* 2012; **119**: 6080–8.
97. Humphrey SJ, Yang G and Yang P *et al.* Dynamic adipocyte phosphoproteome reveals that Akt directly regulates mTORC2. *Cell Metab* 2013; **17**: 1009–20.
98. Yang G, Murashige DS and Humphrey SJ *et al.* A positive feedback loop between Akt and mTORC2 via SIN1 phosphorylation. *Cell Rep* 2015; **12**: 937–43.
99. Wu YT, Ouyang W and Lazorchak AS *et al.* mTOR complex 2 targets Akt for proteasomal degradation via phosphorylation at the hydrophobic motif. *J Biol Chem* 2011; **286**: 14190–8.
100. Lazorchak AS, Liu D and Facchinetti V *et al.* Sin1-mTORC2 suppresses rag and il7r gene expression through Akt2 in B cells. *Mol Cell* 2010; **39**: 433–43.

101. Chang X, Lazorchak AS and Liu D *et al.* Sin1 regulates Treg-cell development but is not required for T-cell growth and proliferation. *Eur J Immunol* 2012; **42**: 1639–47.
102. Arojo OA, Ouyang X and Liu D *et al.* Active mTORC2 signaling in naive T cells suppresses bone marrow homing by inhibiting CXCR4 expression. *J Immunol* 2018; **201**: 908–15.
103. Huang L, Zhang Y and Xu C *et al.* Rictor positively regulates B cell receptor signaling by modulating actin reorganization via ezrin. *PLoS Biol* 2017; **15**: e2001750.
104. Lazorchak AS and Su B. Perspectives on the role of mTORC2 in B lymphocyte development, immunity and tumorigenesis. *Protein Cell* 2011; **2**: 523–30.
105. He Y, Li D and Cook SL *et al.* Mammalian target of rapamycin and Rictor control neutrophil chemotaxis by regulating Rac/Cdc42 activity and the actin cytoskeleton. *Mol Biol Cell* 2013; **24**: 3369–80.
106. Xu Y, Ouyang X and Yan L *et al.* Sin1 (stress-activated protein kinase-interacting protein) regulates ischemia-induced microthrombosis through integrin alphaIIbbeta3-mediated outside-in signaling and hypoxia responses in platelets. *Arterioscler Thromb Vasc Biol* 2018; **38**: 2793–805.
107. Xu Y, Fang SJ and Zhu LJ *et al.* DNA-PKcs-SIN1 complexation mediates low-dose X-ray irradiation (LDI)-induced Akt activation and osteoblast differentiation. *Biochem Biophys Res Commun* 2014; **453**: 362–7.
108. Cardenas-Rodriguez M, Irigoin F and Osborn DP *et al.* The Bardet-Biedl syndrome-related protein CCDC28B modulates mTORC2 function and interacts with SIN1 to control cilia length independently of the mTOR complex. *Hum Mol Genet* 2013; **22**: 4031–42.
109. Wang JQ, Chen JH and Chen YC *et al.* Interaction between NBS1 and the mTOR/Rictor/SIN1 complex through specific domains. *PLoS One* 2013; **8**: e65586.
110. Zhang L, You J and Sidhu J *et al.* Abrogation of chronic rejection in rat model system involves modulation of the mTORC1 and mTORC2 pathways. *Transplantation* 2013; **96**: 782–90.
111. Chou PC, Oh WJ and Wu CC *et al.* Mammalian target of rapamycin complex 2 modulates alphabetaTCR processing and surface expression during thymocyte development. *J Immunol* 2014; **193**: 1162–70.
112. Linke M, Fritsch SD and Sukhbaatar N *et al.* mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett* 2017; **591**: 3089–103.
113. Zeng H, Cohen S and Guy C *et al.* mTORC1 and mTORC2 kinase signaling and glucose metabolism drive follicular helper T cell differentiation. *Immunity* 2016; **45**: 540–54.
114. Xu L, Huang Q and Wang H *et al.* The kinase mTORC1 promotes the generation and suppressive function of follicular regulatory T cells. *Immunity* 2017; **47**: 538–551.e5.
115. Lee K, Gudapati P and Dragovic S *et al.* Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity* 2010; **32**: 743–53.
116. Delgoffe GM, Pollizzi KN and Waickman AT *et al.* The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol* 2011; **12**: 295–303.
117. Pollizzi KN, Patel CH and Sun IH *et al.* mTORC1 and mTORC2 selectively regulate $CD8^+$ T cell differentiation. *J Clin Invest* 2015; **125**: 2090–108.
118. Michalek RD, Gerriets VA and Jacobs SR *et al.* Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory $CD4^+$ T cell subsets. *J Immunol* 2011; **186**: 3299–303.
119. Weichhart T, Hengstschlager M and Linke M. Regulation of innate immune cell function by mTOR. *Nat Rev Immunol* 2015; **15**: 599–614.
120. Wang F, Meng M and Mo B *et al.* Crosstalks between mTORC1 and mTORC2 variagate cytokine signaling to control NK maturation and effector function. *Nat Commun* 2018; **9**: 4874.
121. Saleiro D and Platanias LC. Intersection of mTOR and STAT signaling in immunity. *Trends Immunol* 2015; **36**: 21–9.
122. Perkey E, Fingar D and Miller RA *et al.* Increased mammalian target of rapamycin complex 2 signaling promotes age-related decline in CD4 T cell signaling and function. *J Immunol* 2013; **191**: 4648–55.
123. Laplante M and Sabatini DM. Regulation of mTORC1 and its impact on gene expression at a glance. *J Cell Sci* 2013; **126**: 1713–9.
124. Duvel K, Yecies JL and Menon S *et al.* Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* 2010; **39**: 171–83.
125. Mossmann D, Park S and Hall MN. mTOR signalling and cellular metabolism are mutual determinants in cancer. *Nat Rev Cancer* 2018; **18**: 744–57.
126. Buller CL, Loberg RD and Fan MH *et al.* A GSK-3/TSC2/mTOR pathway regulates glucose uptake and GLUT1 glucose transporter expression. *Am J Physiol Cell Physiol* 2008; **295**: C836–43.
127. Masui K, Tanaka K and Akhavan D *et al.* mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO acetylation and upregulation of c-Myc. *Cell Metab* 2013; **18**: 726–39.
128. Li S, Oh YT and Yue P *et al.* Inhibition of mTOR complex 2 induces GSK3/FBXW7-dependent degradation of sterol regulatory element-binding protein 1 (SREBP1) and suppresses lipogenesis in cancer cells. *Oncogene* 2016; **35**: 642–50.
129. Moloughney JG, Kim PK and Vega-Cotto NM *et al.* mTORC2 responds to glutamine catabolite levels to modulate the hexosamine biosynthesis enzyme GFAT1. *Mol Cell* 2016; **63**: 811–26.
130. Pearce EL and Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* 2013; **38**: 633–43.
131. Liu P, Gan W and Inuzuka H *et al.* Author correction: Sin1 phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorigenesis. *Nat Cell Biol* 2019; **21**: 662–3.
132. Wang D, Wu P and Wang H *et al.* SIN1 promotes the proliferation and migration of breast cancer cells by Akt activation. *Biosci Rep* 2016; **36**: e00424.
133. Moraitis D, Karanikou M and Liakou C *et al.* SIN1, a critical component of the mTOR-Rictor complex, is overexpressed and associated with AKT activation in medullary and aggressive papillary thyroid carcinomas. *Surgery* 2014; **156**: 1542–9, discussion 48–9.
134. Hu Z, Wang Y and Wang Y *et al.* SIN1, a critical component of the mTOR-Rictor complex, is overexpressed and associated with AKT activation in medullary and aggressive papillary thyroid carcinomas. *Biochem Biophys Res Commun* 2017; **483**: 645–51.
135. Xu J, Li X and Yang H *et al.* SIN1 promotes invasion and metastasis of hepatocellular carcinoma by facilitating epithelial-mesenchymal transition. *Cancer* 2013; **119**: 2247–57.
136. Cameron AJM, Veeriah S and Marshall JJT *et al.* Uncoupling TORC2 from AGC kinases inhibits tumour growth. *Oncotarget* 2017; **8**: 84685–96.
137. Wang Q, Zhu J and Wang YW *et al.* Tumor suppressor Pdcd4 attenuates Sin1 translation to inhibit invasion in colon carcinoma. *Oncogene* 2017; **36**: 6225–34.
138. Benavides-Serrato A, Lee J and Holmes B *et al.* Specific blockade of Rictor-mTOR association inhibits mTORC2 activity and is cytotoxic in glioblastoma. *PLoS One* 2017; **12**: e0176599.
139. Angliker N and Ruegg MA. In vivo evidence for mTORC2-mediated actin cytoskeleton rearrangement in neurons. *Bioarchitecture* 2013; **3**: 113–8.

140. Johnson JL, Huang W and Roman G *et al.* TORC2: a novel target for treating age-associated memory impairment. *Sci Rep* 2015; **5**: 15193.
141. Huang W, Zhu PJ and Zhang S *et al.* mTORC2 controls actin polymerization required for consolidation of long-term memory. *Nat Neurosci* 2013; **16**: 441–8.
142. Josselyn SA and Frankland PW. mTORC2: actin on your memory. *Nat Neurosci* 2013; **16**: 379–80.
143. Lee HK, Kwon B and Lemere CA *et al.* mTORC2 (Rictor) in Alzheimer's disease and reversal of amyloid-beta expression-induced insulin resistance and toxicity in rat primary cortical neurons. *J Alzheimers Dis* 2017; **56**: 1015–36.
144. Cai SL, Tee AR and Short JD *et al.* Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. *J Cell Biol* 2006; **173**: 279–89.
145. Manning BD, Tee AR and Logsdon MN *et al.* Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 2002; **10**: 151–62.
146. Duran RV and Hall MN. Regulation of TOR by small GTPases. *EMBO Rep* 2012; **13**: 121–8.
147. Bar-Peled L, Chantranupong L and Cherniack AD *et al.* A tumor suppressor complex with GAP activity for the rag GTPases that signal amino acid sufficiency to mTORC1. *Science* 2013; **340**: 1100–6.
148. Peng M, Yin N and Li MO. SZT2 dictates GATOR control of mTORC1 signalling. *Nature* 2017; **543**: 433–7.
149. Gu X, Orozco JM and Saxton RA *et al.* SAMTOR is an S-adenosylmethionine sensor for the mTORC1 pathway. *Science* 2017; **358**: 813–8.
150. Wolfson RL, Chantranupong L and Wyant GA *et al.* KICSTOR recruits GATOR1 to the lysosome and is necessary for nutrients to regulate mTORC1. *Nature* 2017; **543**: 438–42.
151. Yu Y, Yoon SO and Poulogiannis G *et al.* Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. *Science* 2011; **332**: 1322–6.
152. Harrington LS, Findlay GM and Gray A *et al.* The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J Cell Biol* 2004; **166**: 213–23.
153. Magnusson R, Gustafsson M and Cedersund G *et al.* Cross-talks via mTORC2 can explain enhanced activation in response to insulin in diabetic patients. *Biosci Rep* 2017; **37**: BSR20160514.
154. Chen XG, Liu F and Song XF *et al.* Rapamycin regulates Akt and ERK phosphorylation through mTORC1 and mTORC2 signaling pathways. *Mol Carcinog* 2010; **49**: 603–10.
155. Subramaniam S, Napolitano F and Mealer RG *et al.* Rhes, a striatal-enriched small G protein, mediates mTOR signaling and L-DOPA-induced dyskinesia. *Nat Neurosci* 2012; **15**: 191–3.
156. Catena V and Fanciulli M. Deptor: not only a mTOR inhibitor. *J Exp Clin Cancer Res* 2017; **36**: 12.
157. Saci A, Cantley LC and Carpenter CL. Rac1 regulates the activity of mTORC1 and mTORC2 and controls cellular size. *Mol Cell* 2011; **42**: 50–61.
158. Harris TE, Chi A and Shabanowitz J *et al.* mTOR-dependent stimulation of the association of eIF4G and eIF3 by insulin. *EMBO J* 2006; **25**: 1659–68.
159. Wang L, Liu F and Adamo ML. Cyclic AMP inhibits extracellular signal-regulated kinase and phosphatidylinositol 3-kinase/Akt pathways by inhibiting Rap1. *J Biol Chem* 2001; **276**: 37242–9.
160. van Dam TJ, Zwartkruis FJ and Bos JL *et al.* Evolution of the TOR pathway. *J Mol Evol* 2011; **73**: 209–20.
161. Lu HC, Chung SS and Fornili A *et al.* Anatomy of protein disorder, flexibility and disease-related mutations. *Front Mol Biosci* 2015; **2**: 47.
162. Hung MC and Link W. Protein localization in disease and therapy. *J Cell Sci* 2011; **124**: 3381–92.