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Review

mTOR and autophagy: A dynamic relationship governed by nutrients and energy

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ABSTRACT

Mechanistic target of rapamycin (mTOR) functions as a key homeostatic regulator of cell growth and orchestrates whether anabolic or catabolic reactions are favoured. mTOR complex 1 (mTORC1) manages multiple biosynthetic pathways and promotes cell growth when nutrients are in plentiful supply. Many advances have been made over the last decade on nutrient sensing centred on mTORC1. Recent research reveals that mTORC1 maintains nutrient homeostasis through lysosomal biogenesis and autophagic processes. Cells utilise autophagy to recycle damaged or unwanted organelles and macromolecules and in so doing, generate energy and recover precursor building blocks necessary for normal growth. It is clear that mTOR and autophagy are closely integrated within cells, where defects in signalling through both pathways are known to drive the onset of a range of human diseases, such as cancer and neurodegenerative disease. This review focuses on the dynamic signalling interplay between mTOR and autophagy, which is governed by a core set of proteins that sense nutrients at lysosomal membranes.

Contents

Abbreviations: Akt1, v-akt murine thymoma viral oncogene homolog 1; AMBRA, Autophagy/Beclin-1 regulator 1; AMPK, AMP-dependent protein kinase; ATG, autophagy-related; BECN1, beclin 1; DEPTOR, DEP domain containing mTOR-interacting protein; EGFR, epidermal growth factor receptors; FIP200, focal adhesion kinase family interacting protein of 200 kDa; PIKFYVE, FYVE finger containing phosphatidylinositol 5-kinase; $[Ca^{2+}]i$, intracellular pool of Ca^{2+}; LAMTOR1-5, late endosomal/lysosomal adaptor, MAPK and mTOR activator 1-5; GATOR, Rag GTPases and GTRs; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; HEK, human embryonic kidney; IGF, insulin-like growth factor; RNAi, interfering RNA; IBP, interferon regulatory factor-4 binding protein; mTOR, mechanistic target of rapamycin; MAP1LC3A, microtubule-associated protein 1 light chain 3 alpha; mTORC1, mTOR complex 1; mTORC2, mTOR complex2; MEF, mouse embryonic fibroblast; PI, phosphatidylinositol; PIK3CA, PI-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PRAS40, proline-rich Akt substrate of 40 kDa; PX, Phox homology; PAT1, proton-assisted amino acid transporter; Raptor, rapamycin-associated protein of TOR; Rictor, rapamycin insensitive companion of TOR; ROS, reactive oxygen species; SQSTM1, Sequestome 1; TFE3, transcription factor binding to IGHM enhancer 3; TFEB, transcription factor EB; TBC1D7, Tre2-Bub2-Cdc16-1 domain family member 7; TRAF6, TNF receptor-associated factor 6, E3 ubiquitin protein ligase; TSC, Tuberous Sclerosis Complex; ULK1, unc-51 like autophagy activating kinase 1; v-ATPase, vacuolar H^+-ATPase; VPS34, vacuolar protein sorting-34.

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1. Introduction

Careful management of cell growth is coordinated through anabolic and catabolic processes and is in part, regulated through mechanistic target of rapamycin (mTOR). Anabolism consumes energy and simple precursors (i.e., ATP, amino acids, fatty acids and nucleotides) to generate more complex molecules essential for cell growth. To regenerate energy and precursor building blocks during cellular growth, unwanted complex molecules can also be broken down by catabolism. Consequently, anabolic and catabolic processes often work in unison and are tightly controlled through sensing cellular nutrient and energy levels, as well as growth factor and hormonal inputs. mTOR is a serine/threonine protein kinase that is integral to two distinct cellular complexes, termed mTOR complex 1 (mTORC1) and mTORC2, where these complexes sense and integrate a variety of inputs, including growth signals, nutrients and energy status. mTORC1 consists of the core components, mTOR, rapamycin-associated protein of TOR (Raptor) and mLST8 (also known as GbetaL) [1]. In addition, proline-rich Akt substrate of 40 kDa (PRAS40) [2-4] and DEP domain containing mTOR-interacting protein (DEPTOR) [5] can also associate with and negatively regulate mTORC1. mTOR and LST8 are also integral to mTORC2, while components distinctive to mTORC2 include rapamycin insensitive companion of TOR (rictor), SIN1 and Protor (reviewed in Ref. [6]). mTORC1 is the better studied of the two complexes and, by managing multiple biosynthetic pathways, plays a key anabolic role in promoting cell growth and proliferation. Through direct phosphorylation and activation of v-Akt murine thymoma viral oncogene homolog 1 (Akt1) [7], mTORC2 indirectly promotes mTORC1 activation further down the signalling pathway. As well as promoting cell growth and proliferation, mTORC2 influences cell morphology through regulation of cytoskeletal organisation.

A cell must first increase its biomass prior to cell division and mTORC1 does this through a number of coordinated mechanisms. mTORC1 builds up cellular protein content through ribosomal biogenesis and enhances initiation and elongation of protein translation (reviewed in Ref. [8]). To enhance protein build-up when nutrients and energy are sufficient, mTORC1 down-regulates macroautophagy (referred to as autophagy hereafter). Autophagy is a catabolic process where macromolecules are sequestered in double membrane bound autophagosomes that fuse with lysosomes to allow their enzymatic break down. In conditions of nutrient and energy sufficiency, mTORC1 is active, and through phosphorylation of early autophagy promoting complexes mTORC1 ensures that autophagy is inhibited. Although autophagy is down-regulated by mTORC1 when nutrients are plentiful, a low basal level of autophagy still occurs to prevent build-up of damaged organelles and aggregated or misfolded macromolecules. Revealing dynamic signalling interplay between autophagy and mTORC1, autophagic signalling switches mTORC1 off when nutrients and energy become limiting.

2. mTORC1 control of autophagy

2.1. Autophagy initiation is controlled through ULK1 and VPS34

Autophagy initiation is coordinated by two kinases, unc-51 like kinase 1 (ULK1, also known as autophagy-related (ATG)-1) and vacuolar protein sorting-34 (VPS34, also known as PIK3C3). ULK1 is a Ser/Thr protein kinase, while VPS34 is a class III phosphoinositol 3-kinase. As part of larger protein complex, ULK1 is activated following nutrient depletion and is considered upstream of VPS34 [9,10]. Activation of both ULK1 and VPS34 drives the recruitment of additional ATG proteins to phagophore membranes and promotes autophagosomal maturation. As well as regulating autophagy, ULK1 and VPS34 are known to influence signal transduction through mTORC1 (discussed in detail below).

2.2. Autophagy is governed by opposing kinase activities from mTORC1, ULK1 and AMPK

ULK1 functions in a complex with ATG13, and focal adhesion kinase family interacting protein of 200 kDa (FIP200, also known as RB1-inducible coiled-coil 1) [11,12]. Nutrient withdrawal stimulates this ULK1-ATG13-FIP200 complex and initiates autophagy via ULK1 autophosphorylation and phosphorylation of the binding partners, ATG13 and FIP200 [11,12]. mTORC1 plays a central role in the regulation of ULK1 and autophagy initiation, where inhibition of mTORC1 upon rapamycin treatment enhances the kinase activity of ULK1, while mTORC1 activation through Rheb overexpression potently represses ULK1 [11]. mTORC1 inhibits ULK1 through at least two mechanisms, with the first involving direct protein phosphorylation. Historically, it was known from yeast studies that TOR was an upstream kinase of Atg1 (the yeast homologue of ULK1) and that phosphorylation of Atg13 by TOR reduced the affinity of Atg13 for Atg1. The Atg1-Atg13 association and subsequent activation of Atg1 are required for autophagy induction in yeast, so TOR-mediated dissociation of the Atg1 complex prevents autophagy when nutrients are sufficient [13]. Several studies published in 2009 demonstrated that mTORC1 could negatively regulate the mammalian ULK1-ATG13-FIP200 complex in a comparable manner [11,12,14]. Although the integrity of the ULK1-ATG13-FIP200 complex is not regulated in response to nutrients (unlike the yeast Atg1-Atg13 complex), mTORC1 directly phosphorylates Atg13 and ULK1 in vitro [11,14]. This occurs through association of the mTORC1 component, Raptor, with ULK1 under nutrient-rich conditions [14]. Serine 758 was identified as the major mTORC1-mediated phosphorylation site on ULK1 and is considered to inhibit ULK1 [15,16].

In addition to ULK1 phosphorylation, mTORC1 indirectly destabilises ULK1 and impairs autophagy through phosphorylation of Autophagy/Beclin-1 regulator 1 (AMBRA1) [17]. Phosphorylation of AMBRA1 at Ser52 by mTORC1 prevents Lys-63-linked ubiquitination of ULK1 by TNF receptor-associated factor 6, E3 ubiquitin protein ligase (TRAF6). Lys-63-linked ubiquitination of ULK1 causes self-association, which enhances stability and through trans-autophosphorylation promotes ULK1 activation. During conditions that favour mTORC1 activation, AMBRA1 is kept in an inactive state by mTORC1 phosphorylation and is tethered to intracellular vesicles as part of a dynein motor complex. However, upon conditions when mTORC1 is inactivated, autophagy is promoted through rapid Lys-63-ubiquitination of ULK1 by the AMBRA1-TRAF6 complex, causing ULK1 self-association and enhancement of its kinase activity.

AMP-dependent protein kinase (AMPK, also known as PRKAA2) plays an important homeostatic role in the regulation of ULK1 and mTORC1 and is dependent on the energy status of the cell. In conditions of glucose starvation when cellular energy levels are low, AMPK binds to and activates ULK1 through phosphorylation [15,16,18–20]. Revealing a feedback mechanism, phosphorylation of ULK1 by mTORC1 was shown to impede the ability of AMPK to activate ULK1 [15,16]. ULK1 activation by AMPK is also further amplified through several signalling mechanisms that lead to mTORC1 inhibition. Firstly, AMPK phosphorylates Raptor, which consequently leads to association of 14-3-3 with the phosphorylated Raptor protein and disruption of mTORC1 signal transduction [21]. Secondly, AMPK activates the upstream negative mTORC1 regulator, Tuberous Sclerosis Complex 2 (TSC2) through direct phosphorylation [22]. As a third mechanism, AMPK also indirectly switches mTORC1 off through activation of ULK1, leading to ULK1

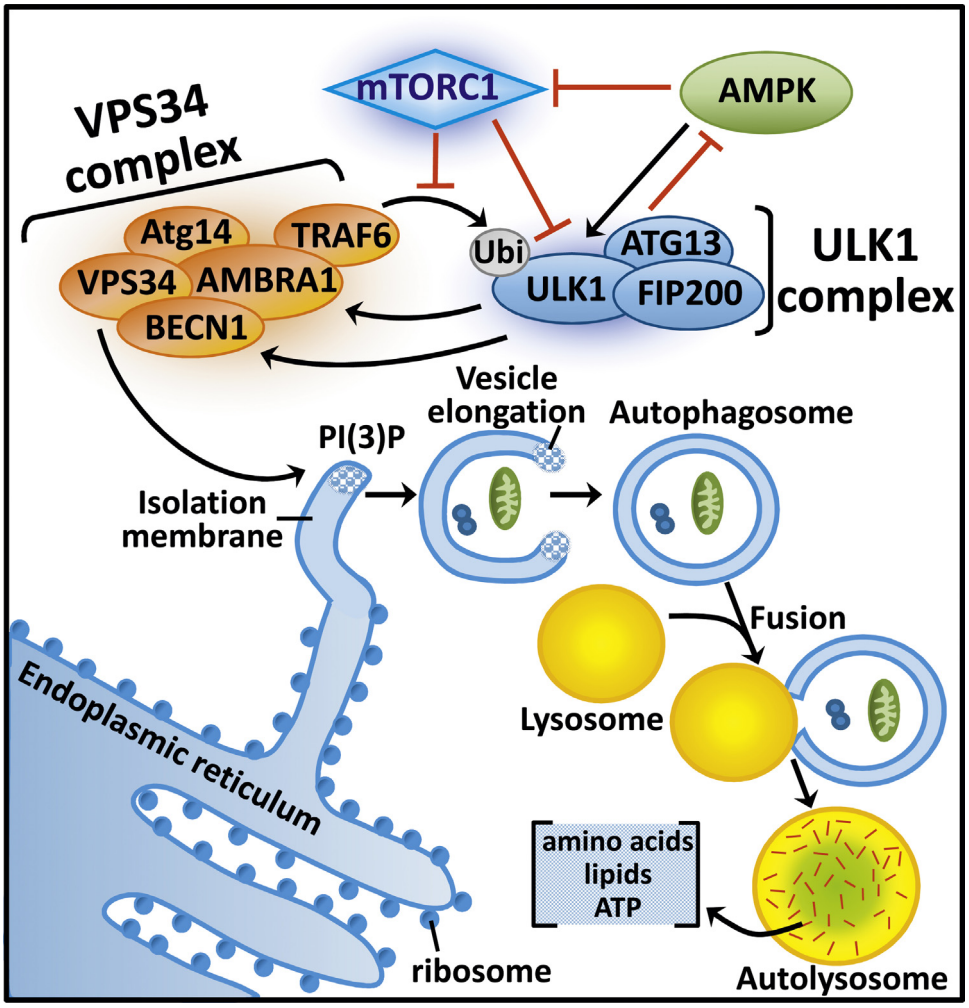

Fig. 1. Signalling cross-talk between mTORC1, AMPK, ULK1 and VPS34 and induction of autophagy. When nutrients are in supply, mTORC1 negatively regulates ULK1 through direct phosphorylation and destabilisation of ULK1 protein (via phosphorylation of AMBRA1 and impairment of TRAF6-mediated ubiquitination of ULK1). During conditions of energy starvation, AMPK activates ULK1 through protein phosphorylation. A negative feedback mechanism exists where ULK1 inhibits AMPK through phosphorylation. Upon nutrient withdrawal (when mTORC1 is inactive) ULK1 initiates autophagy in part through phosphorylation of AMBRA1 and BECN1, which activates VPS34 to cause maturation of the autophagosome from the ER. The autophagosome fuses with the lysosome to generate the autolysosome, resulting in degradation of internal contents that are recycled.

binding and inhibitory phosphorylation of Raptor [23,24]. A further level of complexity is presented by the observation that ULK1 also inhibits AMPK through phosphorylation [25]. Presumably this ULK1 feedback mechanism towards AMPK ensures that the autophagy pathway will not be constitutively on for long periods of time, which would be detrimental to the cell.

2.3. Phosphatidylinositol (PI) phospholipids in endomembrane processes, autophagy and mTORC1 activation

The endomembrane system is composed of multiple membrane structures in eukaryotic cells, such as the plasma membrane, nuclear envelope, endoplasmic reticulum (ER), Golgi apparatus, endosomes, cell membrane, peroxisomes, autophagosomes and lysosomes. Proteins can either be trafficked between membrane structures via vesicular transport or instead are actively recruited to specific signalling platforms on the membrane surface. Lysosomes are the end point of the degradation pathway within this endomembrane system. Autophagosomes are initially sculpted from several endomembrane structures, such as the ER and plasma membrane, and when fully formed are actively trafficked to lysosomes. ULK1 initiates this autophagy process through phosphorylation of AMBRA1 [26]. ULK1-dependent phosphorylation of AMBRA1 leads to translocation of the AMBRA1-ATG14-BECN1-VPS34 complex from the dynein motor complex to the endoplasmic reticulum (ER). ULK1 was also shown to phosphorylate beclin 1 (BECN1, also known as ATG6), which was required for VPS34 activation and induction of autophagy [27]. At the ER, VPS34 catalyses the conversion of phosphatidylinositol (PI) to phosphatidylinositol-3-phosphate (PI(3)P), a phospholipid central for membrane modelling and trafficking in autophagy. VPS34 enriches the ER membrane with PI(3)P to form the phagophore-assembly site, a signalling platform that initiates autophagosome nucleation. The enriched pool of PI(3)P binds effector protein complexes that possess either FYVE (named after, Fab1p, YOTB, Vac1p and EEA1) or PI3P-targeting PX (Phox homology) domains [28]. Through recruitment of membrane scaffold proteins such as Zinc finger, FYVE domain containing 1 (also known as DFCP1), PI(3)P enriched omegasome membrane structures grow out from the ER. During amino acid starvation, the AMBRA1-ATG14-BECN1-VPS34 complex promotes membrane expansion of the omegasome that then develops into an isolation membrane. ULK1-ATG13-FIP200, ATG12-ATG5-ATG16 and membrane vesicle bound ATG9 are actively recruited to expanding isolation membranes where the leading edges of the membrane will eventually close to engulf cytoplasmic components and form the autophagosome.

Autophagosomal maturation and the involvement of mTORC1, ULK1 and VPS34 is summarised in Fig. 1.

As well as having a positive role in autophagy regulation, VPS34 is also necessary for amino acid induced mTORC1 activation. For instance, ablation of VPS34 in mouse embryonic fibroblasts (MEFs) suppressed the activation of mTORC1 upon acute amino acid stimulation, while the basal level of mTORC1 signalling remained unaltered [29]. A positive role of VPS34 in mTORC1 signalling is also supported through several interfering RNA (RNAi) studies, where mTORC1 signalling after amino acid stimulation was blocked upon RNAi knockdown of VPS34 [30,31] and was also dependent on Ca^{2+}/calmodulin signalling [32]. Showing a positive connection of Ca^{2+} signalling to mTORC1, metal chelators that target the intracellular pool of Ca^{2+} ($[Ca^{2+}]i$) such as BAPTA-acetoxymethyl ester are known to ablate amino acid induced signalling through mTORC1. Gulati et al. revealed that $[Ca^{2+}]i$ increased after amino acid stimulation, which enhanced calmodulin-VPS34 binding and activated mTORC1 [32]. Over-expression of the FYVE domain, which sequesters PI(3)P away from PI(3)P-interacting proteins, potently impaired amino acid induced mTORC1 signalling [30,31]. Such evidence reveals that docking of proteins to PI(3)P via FYVE (and/or PX) domains are likely necessary for amino acids to elicit a robust level of mTORC1 activation.

Two different membrane PI(3)P pools might account for the differential effect of mTORC1 signalling by VPS34 depending on amino acid status [32]. On one hand, VPS34 induces autophagy upon nutrient withdrawal, which is dependent on a ER membrane-specific pool of PI(3)P (conditions that would typically repress mTORC1). While on the other hand, activation of mTORC1 after amino acid stimulation requires rising $[Ca^{2+}]i$ and a pool of PI(3)P. Of interest, through the WD40 repeats of Raptor, mTORC1 binds $PI(3,5)P_2$, which is necessary for its activation upon amino acid stimulation [33]. The study by Bridges et al. [33], revealed that in 3T3-L1 adipocytes phosphorylation of PI(3)P to $PI(3,5)P_2$ was through FYVE finger containing phosphatidylinositol 5-kinase (PIKfyve) and was necessary for mTORC1 activation. At endomembranes where mTORC1 is also considered to be localised, PIKfyve forms a complex with SAC3, associated regulator of PIKfyve, and ATG18 (also known as the WD40 repeat domain, phosphoinositide interacting 1). The involvement of PIKfyve implies that the activation of mTORC1 by VPS34 is not directly through PI(3)P, but is instead more likely through $PI(3,5)P_2$ enriched membranes. Indeed, lipid transfection of $PI(3,5)P_2$, but not PI(3)P, was able to elicit a level of S6K1 activation, albeit activation was increased by a modest level when compared to insulin stimulation [33]. Showing an upstream mitogenic input towards PIKfyve, PIKfyve was recently shown to be positively regulated by Akt1, where active PIKfyve targets epidermal growth factor receptors (EGFR) for lysosomal degradation and therefore blocks EGFR recycling through endocytosis [34]. PIKfyve is known to be involved in the regulation of autophagy through binding with ATG18 [35]. In a manner similar to Raptor, ATG18 binds to $PI(3,5)P_2$ via WD40 repeat domains [26]. Again indicating that PIKfyve is likely involved in endosomal processes, ectopically expressed green fluorescent protein-tagged WD40 protein was shown to co-localise with both mTOR and lysosomal-associated membrane protein 2 (a marker of lysosomes and late endosomes) in human embryonic kidney (HEK)293A cells [33]. Given this specific lysosomal location of mTORC1 in nutrient dependent activation, it is probable that enrichment of both PI(3)P and $PI(3,5)P_2$ occurs at lysosomal membranes and that activation of mTORC1 through VPS34 requires a lysosomal-specific Ca^{2+} channel. Although regulatory PI phospholipids are relatively low in abundance, they are clearly important for membrane remodelling and autophagy maturation. As well as PI(3)P and $PI(3,5)P_2$, several other PI phospholipid species are also essential for autophagy processes, such as $PI(4,5)P_2$ that becomes concentrated at the plasma membranes and instigates endocytosis (for a comprehensive review see Ref. [36]).

2.4. Sensing of intracellular amino acids at lysosomes

Cells have evolved an elaborate nutrient sensing node at the level of lysosomes that connects autophagy to mTORC1 signalling and is dependent on a class of small G proteins, called the Rag GTPases. The Rag proteins form functional heterodimers, where the active complex consists of GTP-bound RagA or RagB associated with either GDP-bound RagC or RagD [37]. During conditions when amino acids are plentiful, RagA and RagB switch to the active GTP-bound state which binds Raptor to translocate mTORC1 to both the 'Ragulator complex' [38] and the v-ATPase at lysosomes [39] (Fig. 2). Rag GTPases reside on the lysosomal surface, irrespective of their GTP-loaded states or amino acid availability.

Immunofluorescence studies show that mTORC1 localises to lysosomes upon stimulation with either amino acids or when active GTP-RagA/B and GDP-RagC/D mutant heterodimers are co-expressed [37]. Rheb is also found to be tethered to lysosomal membranes and is dependent on C-terminal farnesylation [40]. The TSC1, TSC2 and Tre2-Bub2-Cdc16-1 domain family member 7 (TBC1D7) protein complex functions to repress mTORC1 by acting as a Rheb GTPase activating protein (GAP) [41]. Growth factor signalling pathways converge on lysosomal localised TSC1/TSC2/TBC1D7 that is bound to inactive GDP-bound Rheb, where phosphorylation of TSC2 by Akt1 leads to cytosolic translocation of TSC1/TSC2/TBC1D7 away from Rheb [40]. In the absence of the TSC1/TSC2/TBC1D7 complex, Rheb is converted to a GTP-bound state that potently activates mTORC1, but only when mTORC1 is lysosomal bound. Therefore, Rag-mediated recruitment of mTORC1 to lysosomes by nutrients is a prerequisite before Rheb can relay growth signals via insulin or growth factors through proximally localised mTORC1 [37]. Showing another level of regulation at lysosomes, recently it was revealed that the Rag GTPases also actively recruited the TSC1/TSC2/TBC1D7 complex to lysosomes during nutrient deprivation to ensure inactivation of Rheb/mTORC1 [42].

The Rag proteins bind to the Ragulator complex, consisting of five proteins referred to as the late endosomal/lysosomal adaptor, MAPK and mTOR activator 1-5 (LAMTOR1-5), on the membrane surface of the lysosome. Anchorage of LAMTOR1 to lysosomal membranes through N-terminal myristoylation and palmitoylation creates a scaffold platform for association with the other 4 Ragulator components that function as two distinct heterodimers, LAMTOR2-3 and LAMTOR4-5 [38]. Knockdown experiments on LAMTOR1-5 revealed that the Ragulator complex is necessary for translocation of the Rag heterodimers to lysosomal membranes and activation of mTORC1 when nutrients are plentiful [38]. The Ragulator complex functions as a guanine nucleotide exchange factor (GEF) towards RagA and RagB that switches their conformation to an active GTP-bound state [43]. At lysosomes, the tumour suppressor protein Folliculin (FLCN) was shown to bind to and function as a GAP towards RagC and RagD [44,45]. The Ragulator complex and FLCN, therefore, work in concert to favour composition of the active Rag heterodimer, i.e., GTP-bound RagA/B with GDP-bound RagC/D.

The Rag heterodimers are further regulated by GATOR (Rag GTPases and GTRs)-1 and GATOR2, which are also multi-protein complexes that are lysosomal localised [46]. GATOR1 interacts directly with the Rag proteins and functions as a GAP towards RagA and RagB to negatively impact mTORC1 signalling. GATOR2 is epigenetically upstream of GATOR1 and functions as a negative regulator of GATOR1.

As lysosomes replenish the amino acid reserves through catabolism, these amino acids are actively sensed internally within the lysosomal lumen. The vacuolar H^+-ATPase (v-ATPase), composing of two multiprotein complexes (termed V1 and V0), is an

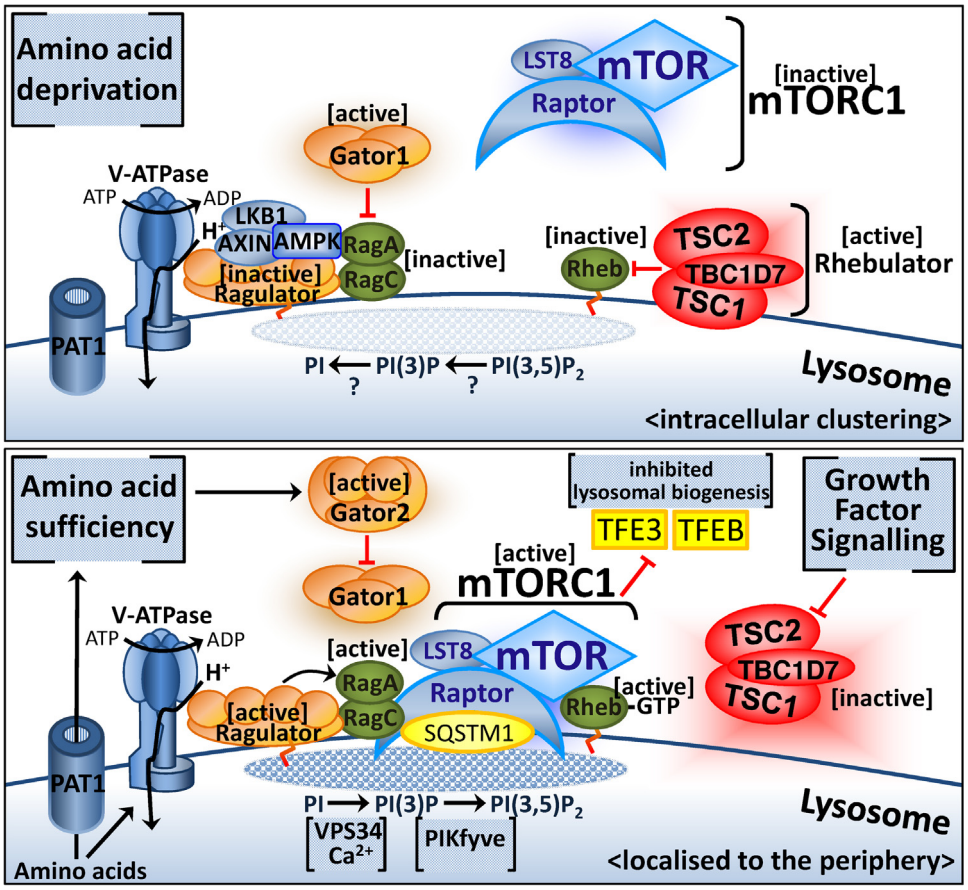

Fig. 2. Nutrient sensing of mTORC1 at lysosomes. During conditions of amino acid deprivation (upper panel), mTORC1 is cytoplasmic and is inactive. Inactive GDP-bound Rheb is favoured due to recruitment of an active TSC1/TSC2/TBC1D7 (loosely coined as the 'Rhebulator') to the lysosome (which is enhanced upon nutrient withdrawal) and functions as a RhebGAP. GATOR1 inhibits the Rag heterodimers as a RagA and RagB GAP. When amino acids are in supply (lower panel), lysosomes are located to the periphery of the cell. Amino acids within the lysosomal lumen signal through the Ragulator complex (termed the 'inside-out signal'), which activates the Rag GTPases. During energy starvation, AMPK binds to the Ragulator complex to switch it off (upper panel). Amino acid transporters (such as PAT1) transport the amino acids into the cytoplasm. Active Rag GTPases actively recruit mTORC1 through Raptor binding to the lysosomal surface. To further facilitate Raptor docking, Raptor associates with SQSTM1 and $PI(3,5)P_2$ enriched membrane pools (generated via VPS34 in a Ca^{2+}-dependent manner and PIKfyve). Upon growth factor signalling, TSC1/TSC2/TBC1D7 is inactivated that results in conversion of Rheb to an active GTP-bound state and mTORC1 activation (but only when mTORC1 is docked to lysosomes, i.e., when nutrients are present). mTORC1 negatively represses lysosomal biogenesis through phosphorylation and impairment of TFE3 and TFEB.

ATP-dependent proton pump that monitors the amino acid levels within the lysosome, where this signal is relayed to the Ragulator complex on the outside (and is coined the 'inside-out signal') [39]. Inhibition of v-ATPase as well as nutrient starvation was observed to block the interaction of the Ragulator complex with v-ATPase and prevented activation of the Rag heterodimers, via inhibition of the Ragulator GEF activity. Lysosomal amino acids are then transported out of the lysosome through PAT1 (proton-assisted amino acid transporter) and other amino acid transporters. PAT1 also interacts with the Rag GTPases [47], and is important for mTORC1 localisation to late endosomes (where knockdown of PAT1 prevented co-localisation of mTORC1 with the late endosomal marker LAMP2).

The v-ATPase also serves as an energy sensor, where the v-ATPase responds to glucose starvation through recruitment of axis inhibition protein (AXIN) [48]. Of interest, AXIN was recently shown to act as a scaffold to recruit LKB1-AMPK to late endosomes during glucose starvation where the AXIN-LKB1-AMPK complex binds to LAMTOR1 [48]. Zhang et al. showed that low concentrations of AMP (5 μM) activated endosomal AMPK when LAMTOR1 was present, while a higher concentration of AMP (200 μM) was required in the absence of LAMTOR1. Such evidence reveals that the Ragulator complex enhances allosteric activation of AMPK by AMP (presumably at lysosomes). In this study, dissociation of mTORC1 from endosomal membranes upon glucose starvation was observed to be dependent on AXIN. In summary, recruitment of AMPK by AXIN to the Ragulator complex functions as a negative input to prevent Rag/mTORC1 activation, which is regulated during energy starvation involving v-ATPase [48].

Sequestome 1 (SQSTM1) is an active constituent in the autophagic process through binding to microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A, also known as LC3) and GABA(A) receptor-associated protein family members (reviewed in Ref. [49]). Interestingly, SQSTM1 functions as a positive regulator of mTORC1 through interaction with RagC and RagD as part of an active Rag conformation and also to Raptor. Through these protein associations, SQSTM1 acts as a scaffold on lysosomal membranes for mTORC1 [50]. During autophagy, SQSTM1 is incorporated into the autophagosome and degraded, thus limiting mTORC1 activity. It has been proposed that autophagic degradation of SQSTM1 in response to starvation might contribute to mTORC1 inactivation and would help sustain autophagy [51].

Showing another facet of regulation, mTORC1 inhibits a set of transcription factors that are responsible for lysosomal biogenesis, transcription factor EB (TFEB) [52] and transcription factor binding to IGHM enhancer 3 (TFE3) [53]. Under amino acid sufficiency, TFEB and TFE3 are tethered to lysosomes via the active Rag heterodimers where mTORC1 phosphorylates TFEB at SerS142 [52] and TFE3 at Ser311 [53], retaining both transcription factors in the cytosol and thus blocking their transcriptional activity. Upon amino

acid starvation, TFE3 and TFEB are rapidly dephosphorylated, leading to nuclear translocation and transcription of genes involved in lysosomal biogenesis. Cellular positioning of lysosomes within the cell is also important in the regulation of mTORC1. Korolchuk et al. determined that intracellular clusters of lysosomes scatter to the periphery of the cell in a nutrient dependent manner that was necessary for mTORC1 activation (via proximal signalling inputs from the plasma membrane), inhibition of autophagosome synthesis and reduced autophagosome-lysosome fusion [54].

2.5. mTORC2 and autophagy regulation

While mTORC1 is clearly imbedded in lysosomal signalling and autophagy, there is much less experimental evidence showing that mTORC2 is involved. However, through Akt1, mTORC2 can indirectly repress autophagy. As well as repressing autophagy through Akt1/mTORC1 activation, mTORC2 also represses autophagy through the Akt1/forkhead box O3 (FOXO)3A arm of the signalling pathway [55]. In skeletal muscle, it was found that Akt1 activation prevented the increase in transcript levels of autophagy genes, such as MAP1LC3A, GABARAPL1 and BCL2/adenovirus E1B 19 kDa interacting protein 3, normally induced by fasting [56]. This effect was independent of rapamycin, suggesting no mTORC1 involvement. However, inhibition of mTORC2 through Rictor knockdown allowed FOXO3A nuclear translocation and a build-up of autophagic vesicles, indicating that mTORC2 activation was necessary for autophagy suppression in this system [56]. A similar mTORC2/Akt1/FOXO3A pathway was implicated in suppressing autophagy in breast cancer cells [57]. Knockdown of interferon regulatory factor-4 binding protein (IBP) expression in these breast cancer cell lines reduced signalling through mTORC2/Akt1/FOXO3A, enhanced autophagy, and inhibited xenograft tumour growth and metastasis in vivo. A dual inhibitor of mTORC1/2 was also effective in suppressing tumour growth, while rapamycin treatment had little effect [57]. This study revealed that IBP has anti-autophagic properties and drives tumourigenesis through mTORC2 signalling and suppression of autophagy.

Given the involvement of Akt1 and mTORC1 as negative regulators of autophagy, it would be expected that stimulation with insulin-like growth factor (IGF)-1 would repress autophagy. However quite unexpectedly, knockdown of the IGF-1 receptor and impaired IGF-1 signalling, actually decreased autophagy [58]. This effect was found to be due to the influence of mTORC2 on the actin cytoskeleton and endocytic pathways. Rictor knockdown strategies to prevent mTORC2 signalling reduced Protein kinase C-α/β phosphorylation and severely disrupted the actin cytoskeleton. Consequently, this led to reduced endocytosis and impairment of early autophagosome precursor formation [58]. This work indicates that a controlled level of mTORC2 signalling is required for formation and trafficking of autophagosomes. Given that mTORC2 appears to have a dual role as a negative and positive regulator of autophagy, caution is required when considering the use of dual mTORC1/2 kinase inhibitors, as long-term treatment will likely compromise normal autophagic processes.

3. Autophagy, mTOR and disease

3.1. Autophagy defects upon loss of TSC2 and hyperactivation of mTORC1

In the genetic condition, Tuberous Sclerosis Complex (TSC), loss of function of *TSC1* or *TSC2* causes hyperactive mTORC1 activity and downregulates basal autophagy in dividing cells [59,60]. However, autophagy impairment is not observed in all *Tsc2*-deficient cells. For instance, neurons lacking *Tsc2* exhibit autophagic organelle accumulation and demonstrate autophagic activity, albeit with slightly reduced autophagic flux compared to controls [60]. This indicates autophagy can be differently regulated in *Tsc2*-deficient neurons compared to proliferative cell lines, such as fibroblasts. Although surprising at first, the higher level of autophagy observed in *Tsc2*-deficient neurons was due to compensatory activation of autophagy through AMPK as a consequence of rising AMP levels and energy stress. This leads to activation of ULK1 through phosphorylation of Ser555 by AMPK, which is sufficient to dominantly drive autophagic processes in conditions when mTORC1 signalling has been heightened [60].

TSC1/2 also functions as a sensor of reactive oxygen species (ROS) that is tightly linked to regulation of mTORC1 and autophagy. Recently, it was discovered that a pool of TSC1/2 resides at peroxisomes and responds to ROS to induce autophagy [61]. In this study, over-expression of TSC1/TSC2 in cells which are treated with exogenous ROS (H_2O_2) results in rapid suppression of mTORC1 and enhancement of autophagy. This indicates that at the peroxisome, TSC functions to repress mTORC1 and regulate autophagy in response to oxidative stress [61]. Given that mitophagy (autophagy of mitochondria) specifically targets defective mitochondria through their higher levels of ROS generation (reviewed in Ref. [62]), it would be of interest to examine whether TSC1/2 functions in mitophagy regulation at the level of peroxisomes.

3.2. Autophagy induction with mTOR inhibitors as a therapeutic strategy

Autophagy dysfunction is increasingly emerging as a modulator of disease onset and progression. BECN1 is mono-allelically deleted in 40–75% of sporadic human breast cancers and ovarian cancers [63], indicating the likely importance of a functional autophagy pathway in cancer prevention. In a number of neurodegenerative conditions, impaired autophagy is commonly associated with an accumulation of protein aggregates and neuronal cell death (reviewed in Ref. [64]). Autophagy induction through use of mTORC1 inhibitors has some therapeutic potential for neurodegenerative conditions and cancer. However, it is likely that autophagy induction through mTORC1 inhibition would not be fully effective when mutations to key autophagy genes have occurred, i.e., in the context of cancer, an intact signalling pathway from mTORC1 through to the autophagic pathway would be essential for autophagy induction. However, autophagy has been observed to play both pro- and anti-oncogenic roles in cancer development, for example by suppressing cancer initiation but promoting the growth of established cancers (reviewed in Ref. [65]). Clearly, more research will be required to better define which cancer cells would respond best to autophagy drugs.

Typically, neurodegenerative diseases involve the accumulation of pathogenic proteins that lead to neuronal cell death. It was originally hypothesised that mTORC1 inhibition would protect neurons via autophagic clearance of these pathogenic proteins. However, in cell line models of Huntington's disease it was found that the allosteric inhibitor, everolimus (a rapalogue), impaired mTORC1 activity but was not sufficient to induce aggregate clearance through enhanced autophagy [66]. Similar observations have been made in a transgenic mouse model of Huntington's disease, where everolimus could slowly penetrate the mouse brain and inhibit mTORC1, but had no effect on protein levels of the Huntingtin mutant [67]. Unlike the treatment with rapalogues, it was found that either ATP-competitive mTOR inhibitors or shRNA depletion of mTOR, was sufficient to induce aggregate clearance. Such data implies that a more robust level of mTORC1 inhibition (or dual inhibition of both mTORC1 and mTORC2) is likely required

for therapeutic benefit for Huntington's disease [66]. However, as long-term mTORC2 inhibition compromises early autophagosome precursor formation [58], it is unlikely that dual inhibition of mTORC1 and mTORC2 would be a feasible strategy to induce autophagy for sustained periods of time.

While therapeutic targeting of autophagy to treat disease is clearly in its infancy, some initial cell line studies have indicated that autophagy induction through treatment with rapamycin might hold some promise. In some situations, autophagy activation after rapamycin treatment is sufficient for aggregate clearance. The Parkinson's disease aggregate, α-synuclein, was found to be degraded by autophagy in PC12 cells following rapamycin treatment, along with Ala30Pro and Ala53Thr α-synuclein mutants [68]. Similarly, in a mouse model of Alzheimer's disease, rapamycin treatment lowered the levels of amyloid β and slowed disease progression, potentially as a consequence of stimulating autophagy [69]. Furthermore, activation of autophagy by rapamycin showed beneficial effect on TDP-43 proteinopathies [70] and prion disease [71]. The explanation behind why certain cell types are more sensitive to autophagy induction by rapamycin may lie in the stability of the mTORC1 complex [72]. It is proposed that cells with a more unstable mTORC1 conformation may undergo more complete mTORC1 inhibition by allosteric inhibitors, such as rapamycin, thereby allowing activation of autophagy. Of interest, for those cells where rapamycin alone was ineffective at inducing autophagy, nonefficacious low concentrations of ATP-competitive inhibitors of mTOR combined with saturating concentrations of rapamycin could robustly activate autophagy [72]. Therefore, combination treatment of ATP-competitive mTOR inhibitors with rapalogues may be more successful at activating autophagy in rapamycin insensitive cells.

Chronic myeloid leukaemia cells expressing the BCR-ABL oncoprotein have also been treated with mTOR inhibitors. The dual mTORC1/2 catalytic inhibitor, OSI-027, was found to induce autophagy in BCR-ABL expressing cells, which acted as a protective mechanism for the leukaemic cells [73]. However, simultaneous treatment with OSI-027 and the autophagy inhibitor chloroquine overcame the use of autophagy as a defensive mechanism in these cells, inducing an apoptotic response instead [73]. In contrast, in an alternative BCR-ABL cell model (p190 BCR-ABL transformed progenitor B cell lines) the dual mTORC1/2 inhibitor, PP242, induced autophagy while exerting potent antileukaemic effects [74]. This finding of autophagy induction through mTOR inhibition having both pro-survival and pro-death effects is reflected in acute myeloid leukaemia cell models. Treatment of acute myeloid leukaemia cell lines and primary circulating peripheral leukaemic blasts from patients with OSI-027 induced autophagy and reduced colony formation, while simultaneous blockade of both mTOR and autophagy signalling resulted in enhanced antileukaemic responses, as evidenced by reduced colony formation and increased apoptosis [75]. Additionally, a dose-dependent effect of mTORC1/2 inhibition has been observed in AML cell lines, with autophagy induction by high doses of the dual mTORC1/2 inhibitor, AZD8055, resulting in a cytoprotective effect, but autophagy was implicated in AML cell death when low doses of AZD8055 were used [76]. The same inhibitor, AZD8055, appears to induce autophagy and reduce cell proliferation in some non-small cell lung carcinoma cell lines [77].

This snapshot of the differential efficacy of mTOR inhibitors to reduce disease burden in conditions where autophagy dysfunction is implicated highlights the challenges of translating such a treatment to a clinical setting. In models of neurodegenerative diseases and leukaemia described above, the impact of mTOR inhibitors that are either allosteric (such as rapalogues) or catalytic are highly variable, although beneficial effects of treatment were observed in some model systems. Expanding our knowledge of how autophagy induction can prevent pathogenesis, together with further understanding of the underlying signalling pathways that connect mTOR and autophagy may shed further light on which treatments will have most impact depending on the disease.

4. Future research

Much progress has been made on 'nutrient sensing' through mTORC1 at the level of lysosomes. No doubt, these nutrient sensing signalling pathways will be further delineated with future research. We still do not understand how amino acids are sensed within the lumen of the lysosome, and how this internal signal is then relayed through to the Ragulator and Gator complexes on the outside of the lysosome. Another area of research that requires additional insight is how the membrane pools of PI(3)P and PI(3,5)P_2 are managed within the endomembrane system to regulate both mTORC1 signalling and autophagy. Current research clearly reveals that signal transduction through mTORC1 and mTORC2 are fundamental for autophagy regulation, with close signalling interplay between mTORC1 and ULK1, and mTORC2 being critically involved in autophagosomal trafficking. How mTORC1 and mTORC2 both regulate vesicular trafficking is an area of research that certainly requires more attention. Future basic research that helps determine novel signalling events that govern mTOR and autophagy will no doubt help steer future therapeutic end points. How we can exploit drugs that target mTORC1, mTORC2 and autophagy to effectively treat a range of human disease still remains an area of clinical importance.

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References

[1] Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, 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.
[2] Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, et al. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell 2007;25:903–15.
[3] Fonseca BD, Smith EM, Lee VH, MacKintosh C, Proud CG. PRAS40 is a target for mammalian target of rapamycin complex 1 and is required for signaling downstream of this complex. J Biol Chem 2007;282:24514–24.
[4] Oshiro N, Takahashi R, Yoshino K, Tanimura K, Nakashima A, Eguchi S, et al. The proline-rich Akt substrate of 40 kDa (PRAS40) is a physiological substrate of mammalian target of rapamycin complex 1. J Biol Chem 2007;282:20329–39.
[5] Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, et al. DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell 2009;137:873–86.
[6] Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 2011;12:21–35.
[7] Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005;307:1098–101.
[8] Proud CG. Signalling to translation: how signal transduction pathways control the protein synthetic machinery. Biochem J 2007;403:217–34.
[9] Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. Autophagy 2010;6:764–76.
[10] Russell RC, Tian Y, Yuan H, Park HW, Chang YY, Kim J, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol 2013;15:741–50.
[11] Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 2009;20:1992–2003.
[12] Ganley IG, Lam du H, Wang J, Ding X, Chen S, Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 2009;284:12297–305.
[13] Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, Ohsumi Y. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. J Cell Biol 2000;150:1507–13.

[14] Hosokawa N, Sasaki T, Iemura S, Natsume T, Hara T, Mizushima N. Atg101, a novel mammalian autophagy protein interacting with Atg13. Autophagy 2009;5:1–7.
[15] Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011;13:132–41.
[16] Shang L, Chen S, Du F, Li S, Zhao L, Wang X. Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. Proc Natl Acad Sci U S A 2011;108:4788–93.
[17] Nazio F, Strappazzon F, Antonioli M, Bielli P, Cianfanelli V, Bordi M, et al. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. Nat Cell Biol 2013;15:406–16.
[18] Lee JW, Park S, Takahashi Y, Wang HG. The association of AMPK with ULK1 regulates autophagy. PLoS ONE 2010;3:e15394.
[19] Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 2011;331:456–61.
[20] Bach M, Larance M, James DE, Ramm G. The serine/threonine kinase ULK1 is a target of multiple phosphorylation events. Biochem J 2011;440:283–91.
[21] Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 2008;30:214–26.
[22] Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell 2003;115:577–90.
[23] Dunlop EA, Hunt DK, Acosta-Jaquez HA, Fingar DC, Tee AR. ULK1 inhibits mTORC1 signaling, promotes multisite Raptor phosphorylation and hinders substrate binding. Autophagy 2011;7:737–47.
[24] Jung CH, Seo M, Otto NM, Kim DH. ULK1 inhibits the kinase activity of mTORC1 and cell proliferation. Autophagy 2011;7:1212–21.
[25] Löffler AS, Alers S, Dieterle AM, Keppeler H, Franz-Wachtel M, Kundu M, et al. Ulk1-mediated phosphorylation of AMPK constitutes a negative regulatory feedback loop. Autophagy 2011;7:696–706.
[26] Di Bartolomeo S, Corazzari M, Nazio F, Oliverio S, Lisi G, Antonioli M, et al. The dynamic interaction of AMBRA1 with the dynein motor complex regulates mammalian autophagy. J Cell Biol 2010;191:155–68.
[27] Russell RC1, Tian Y, Yuan H, Park HW, Chang YY, Kim J, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol 2013;15:741–50.
[28] Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol 2008;182:685–701.
[29] Jaber N1, Dou Z, Chen JS, Catanzaro J, Jiang YP, Ballou LM, et al. Class III PI3K Vps34 plays an essential role in autophagy and in heart and liver function. Proc Natl Acad Sci U S A 2012;109:2003–8.
[30] Byfield MP, Murray JT, Backer JM. hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. J Biol Chem 2005;280:33076–82.
[31] Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, Gulati P, et al. Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. Proc Natl Acad Sci U S A 2005;102:14238–43.
[32] Gulati P, Gaspers LD, Dann SG, Joaquin M, Nobukuni T, Natt F, et al. Amino acids activate mTOR complex 1 via Ca^{2+}/CaM signaling to hVps34. Cell Metab 2008;7:456–65.
[33] Bridges D, Ma JT, Park S, Inoki K, Weisman LS, Saltiel AR. Phosphatidylinositol 3,5-bisphosphate plays a role in the activation and subcellular localization of mechanistic target of rapamycin 1. Mol Biol Cell 2012;23:2955–62.
[34] Er EE, Mendoza MC, Mackey AM, Rameh LE, Blenis J. AKT facilitates EGFR trafficking and degradation by phosphorylating and activating PIKfyve. Sci Signal 2013;6:ra45.
[35] Jin N, Chow CY, Liu L, Zolov SN, Bronson R, Davisson M, et al. VAC14 nucleates a protein complex essential for the acute interconversion of PI3P and PI(3,5)P(2) in yeast and mouse. EMBO J 2008;27:3221–34.
[36] Dall'Armi C, Devereaux KA, Paolo GD. The role of lipids in the control of autophagy. Curr Biol 2013;23:R33–45.
[37] Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008;320:1496–501.
[38] Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 2010;141:290–303.
[39] Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. Science 2011;334:678–83.
[40] Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, et al. Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. Cell 2014;156:771–85.
[41] Dibble CC, Elis W, Menon S, Qin W, Klekota J, Asara JM, et al. TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. Mol Cell 2012;47:535–46.
[42] Demetriades C, Doumpas N, Teleman AA. Regulation of TORC1 in response to amino acid starvation via lysosomal recruitment of TSC2. Cell 2014;156:786–99.
[43] Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the Rag GTPases that signal amino acid to mTORC1. Cell 2012;150:1196–208.
[44] Petit CS, Roczniak-Ferguson A, Ferguson SM. Recruitment of folliculin to lysosomes supports the amino acid-dependent activation of Rag GTPases. J Cell Biol 2013;202:1107–22.
[45] Tsun ZY, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, Kim C, et al. The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. Mol Cell 2013;52:495–505.
[46] Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, 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.
[47] Ögmundsdóttir MH, Heublein S, Kazi S, Reynolds B, Visvalingam SM, Shaw MK, et al. Proton-assisted amino acid transporter PAT1 complexes with Rag GTPases and activates TORC1 on late endosomal and lysosomal membranes. PLoS ONE 2012;7:e36616.
[48] Zhang CS, Jiang B, Li M, Zhu M, Peng Y, Zhang YL, et al. The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism. Cell Metab 2014, http://dx.doi.org/10.1016/j.cmet.2014.06.014.
[49] Bitto A, Lerner CA, Nacarelli T, Crowe E, Torres C, Sell C. p62/SQSTM1 at the interface of aging, autophagy, and disease. Age 2014;36:9626.
[50] Duran A, Amanchy R, Linares JF, Joshi J, Abu-Baker S, Porollo A, et al. p62 is a key regulator of nutrient sensing in the mTORC1 pathway. Mol Cell 2011;44:134–46.
[51] Komatsu M, Kageyama S, Ichimura Y. p62/SQSTM1/A170: physiology and pathology. Pharmacol Res 2012;66:457–62.
[52] Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Erdin S, et al. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. EMBO J 2012;31:1095–108.
[53] Martina JA, Diab HI, Lishu L, Jeong-A L, Patange S, Raben N, et al. The nutrient-responsive transcription factor TFE3 promotes autophagy, lysosomal biogenesis, and clearance of cellular debris. Sci Signal 2014;7:ra9.
[54] Korolchuk VI, Saiki S, Lichtenberg M, Siddiqi FH, Roberts EA, Imarisio S, et al. Lysosomal positioning coordinates cellular nutrient responses. Nat Cell Biol 2011;13:453–60.
[55] Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, 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.
[56] Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, et al. FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab 2007;6:458–71.
[57] Chen S, Han Q, Wang X, Yang M, Zhang Z, Li P, et al. IBP-mediated suppression of autophagy promotes growth and metastasis of breast cancer cells via activating mTORC2/Akt/FOXO3a signaling pathway. Cell Death Dis 2013;4:e842.
[58] Renna M, Bento CF, Fleming A, Menzies FM, Siddiqi FH, Ravikumar B, et al. IGF-1 receptor antagonism inhibits autophagy. Hum Mol Genet 2013;22:4528–44.
[59] Parkhitko A, Myachina F, Morrison TA, Hindi KM, Auricchio N, Karbowniczek M, et al. Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent. Proc Natl Acad Sci U S A 2011;108:12455–60.
[60] Di Nardo A, Wertz MH, Kwiatkowski E, Tsai PT, Leech JD, Greene-Colozzi E, et al. Neuronal Tsc1/2 complex controls autophagy through AMPK dependent regulation of ULK1. Hum Mol Genet 2014;23:3865–74.
[61] Zhang J, Kim J, Alexander A, Cai S, Tripathi DN, Dere R, et al. A tuberous sclerosis complex signalling node at the peroxisome regulates mTORC1 and autophagy in response to ROS. Nat Cell Biol 2013;15:1186–96.
[62] Campello S, Strappazzon F, Cecconi F. Mitochondrial dismissal in mammals, from protein degradation to mitophagy. Biochim Biophys Acta 2014;1837:451–60.
[63] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999;402:672–6.
[64] Sarkar S. Regulation of autophagy by mTOR-dependent and mTOR-independent pathways: autophagy dysfunction in neurodegenerative diseases and therapeutic application of autophagy enhancers. Biochem Soc Trans 2013;41:1103–30.
[65] White E. Deconvoluting the context-dependent role for autophagy in cancer. Nat Rev Cancer 2012;12:401–10.
[66] Roscic A, Baldo B, Crochemore C, Marcellin D, Paganetti P. Induction of autophagy with catalytic mTOR inhibitors reduces huntingtin aggregates in a neuronal cell model. J Neurochem 2011;119:398–407.
[67] Fox JH, Connor T, Chopra V, Dorsey K, Kama JA, Bleckmann D, et al. The mTOR kinase inhibitor Everolimus decreases S6 kinase phosphorylation but fails to reduce mutant huntingtin levels in brain and is not neuroprotective in the R6/2 mouse model of Huntington's disease. Mol Neurodegener 2010;5:26.
[68] Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. α-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem 2003;278:25009–13.
[69] Spilman P, Podlutskaya N, Hart MJ, Debnath J, Gorostiza O, Bredesen D, et al. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. PLoS ONE 2010;5:e9979.

[70] Wang IF, Guo BS, Liu YC, Wu CC, Yang CH, Tsai KJ, et al. Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43. Proc Natl Acad Sci USA 2012;109:15024–9.
[71] Cortes CJ, Qin K, Cook J, Solanki A, Mastrianni JA. Rapamycin delays disease onset and prevents PrP plaque deposition in a mouse model of Gerstmann–Sträussler–Scheinker disease. J Neurosci 2012;32:12396–405.
[72] Nyfeler B, Bergman P, Triantafellow E, Wilson CJ, Zhu Y, Radetich B, et al. Relieving autophagy and 4EBP1 from rapamycin resistance. Mol Cell Biol 2011;31:2867–76.
[73] Carayol N, Vakana E, Sassano A, Kaur S, Goussetis DJ, Glaser H, et al. Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells. Proc Natl Acad Sci U S A 2010;107:12469–74.
[74] Janes MR, Limon JJ, So L, Chen J, Lim RJ, Chavez MA, et al. Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. Nat Med 2010;16:205–13.
[75] Altman JK, Szilard A, Goussetis DJ, Sassano A, Colamonici M, Gounaris E, et al. Autophagy is a survival mechanism of acute myeloid leukemia precursors during dual mTORC2/mTORC1 targeting. Clin Cancer Res 2014;20:2400–9.
[76] Willems L, Chapuis N, Puissant A, Maciel TT, Green AS, Jacque N, et al. The dual mTORC1 and mTORC2 inhibitor AZD8055 has anti-tumor activity in acute myeloid leukemia. Leukemia 2012;26:1195–202.
[77] Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE, et al. AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. Cancer Res 2010;70:288–98.