Exploiting cancer vulnerabilities: mTOR, autophagy and homeostatic imbalance

Charlotte E. Johnson and Andrew R. Tee*

Division of Cancer and Genetics, Cardiff University, Heath Park, Cardiff, CF14 4XN, UK.

***Corresponding author:** Dr. Andrew R. Tee, Institute of Cancer and Genetics, Cardiff University, Cancer Genetics Building, 1st Floor, Heath Park, Cardiff, CF14 4XN, UK.
Telephone number: +44 (0)2920 687856, e-mail address: teea@cardiff.ac.uk

Keywords: mTOR, cancer, autophagy, ER stress, AMPK, ULK1,
Running title: mTOR, autophagy and cancer

Abbreviations list
AMBRA, Autophagy/Beclin-1 regulator 1; AMPK, AMP-dependent protein kinase; ATG, autophagy-related; AXIN, axis inhibition protein; CASTOR, Cytosolic Arginine Sensor For MTORC1; BECN1, beclin 1; DEPTOR, DEP domain containing mTOR-interacting protein; FIP200, focal adhesion kinase family interacting protein of 200 kDa; ER, endoplasmic reticulum; LAMTOR1-5, late endosomal/lysosomal adaptor, MAPK and mTOR activator 1-5; FLCN, Folliculin; GATOR, Rag GTPases and GTRs; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex2; PRAS40, proline-rich Akt substrate of 40 kDa; PAT1, proton-assisted amino acid transporter; Raptor, rapamycin-associated protein of TOR; Rheb, Ras homologue enriched in brain;Rictor, rapamycin insensitive companion of TOR; SESN, sestrin; TFE3, transcription factor binding to IGHM enhancer 3; TFEB, transcription factor EB; 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;

Summary points
- nutrient sensing and mitogenic signaling through mTOR
- nutrient and energy interplay between mTORC1, AMPK and ULK1
- regulation of autophagy by mTORC1
- mTORC1 and autophagy in cancer
- loss of energy and nutrient homeostasis and cancer

Abstract
Mechanistic target of rapamycin complex 1 (mTORC1) at lysosomes plays a pivotal role in cell growth control where an array of large multi-protein complexes relay nutrient, energy and growth signal inputs through mTORC1. In cancer cells, such regulation often becomes disconnected, leading to uncontrolled cell growth and an elevation of cellular stress. Consequently, cancer cells often loose homeostatic balance as they grow in unfavorable conditions, i.e., when nutrients and energy are limiting yet mTORC1 is still aberrantly activated. Cancer cells loose signaling flexibility because of hyperactive mTORC1 that leads to heightened cellular stress and loss of nutrient and energy homeostasis, all of which are

potential avenues for cancer therapy. Cancer cells can take advantage of mTORC1 to drive cell growth and proliferation while also maintaining cancer cell survival. Autophagy regulation by mTORC1 is critically involved in nutrient and energy homeostasis, cell growth control and survival. Targeting mTORC1 and autophagy as a potential strategy to treat cancer has grown in interest over the last few decades. This review will explore the signaling pathways central to mTORC1 and autophagy regulation, and cancer vulnerabilities when considering anti-cancer therapies.

Introduction

Mechanistic target of rapamycin complex 1 (mTORC1) senses and integrates a variety of inputs, including growth signals, amino acid levels and energy status and then orchestrates cell growth control in a multifaceted manner. For a cell to rapidly proliferate it needs to build-up its biomass before the cell can effectively divide. Cellular components are doubled and include not only protein signaling complexes, receptors, transporters and enzymes but also cellular scaffold proteins that permit the cytoskeleton to expand as the cell steadily grows. To rapidly increase cellular biomass, mTORC1 promotes ribosomal biogenesis to enhance the cell's protein translation machinery (reviewed in [1]). Consequently, the capacity of the cell to manufacture *de novo* protein is greatly augmented by mTORC1 and is essential for rapidly proliferating cells. Generation of *de novo* protein can be considered a rate limiting factor for cells that are either in the G_1 or G_2 growth phase of the cell cycle. Given the large quantity of amino acids and ATP that is consumed as a cell produces protein, tight regulation ensures that mTORC1 is only active when there is a sufficient supply of nutrients and energy. It is not surprising that mTORC1 is often aberrantly activated in cancer. As well as enhancing tumor growth and proliferative rate, mTORC1 is involved in metabolic transformation, neovascularisation and metastasis. mTORC1 contributes to tumor growth through the regulation of protein translation, hypoxia signaling, autophagy and synthetic anabolic pathways in the pentose phosphate pathway that builds tumor mass while maintaining energy and nutrient homeostasis (reviewed in [2]). mTORC1 activation correlates with metastasis, poor patient survival and resistance to anticancer agents [3].

mTORC1 hyper-activation through genetic mutations of upstream components occurs in sporadic cancer but also underlies several tumor predisposition syndromes, such as tuberous sclerosis complex (TSC) [4] and Cowden disease/PTEN hamartoma syndrome [5]. A lot of our current understanding of mTORC1 signal transduction and cell growth control has been gained through research on such rare genetic disorders. TSC is an autosomal dominant condition caused through mutations in either *TSC1* or *TSC2* and is characterized by tumor growth in multiple organs, neurocognitive problems and epilepsy [4]. The TSC1 and TSC2 proteins form a functional tumor suppressor complex which has GTPase-activating protein (GAP) activity towards Ras homologue enriched in brain (Rheb). Rheb loaded with GTP potently activates mTORC1, while hydrolysis of GTP through interaction with TSC1/TSC2 converts Rheb to an inactive GDP-bound state and switches mTORC1 off [6,7]. Consequently, loss-of-function mutations in either *TSC1* or *TSC2* cause aberrant signal transduction through mTORC1. *TSC1* and *TSC2* are mutated in approximately 15% of bladder cancer and 3% of clear cell renal carcinomas. *TSC2* is mutated in 3% of bladder cancers and in 8% of well-differentiated pancreatic neuroendocrine tumors [8-10]. Whereas, activating mutations within the mTOR kinase domain have been identified in multiple cancer subtypes,

with the highest number in kidney, colorectal, endometrial, and lung cancers [11]. Mutations within *TSC1*, *TSC2* and *mTOR* are uncommon in sporadic cancer, while mutations within genes that function upstream of mTOR are much more common, i.e., within *PTEN* or *RAS*. Additionally, gene amplification of growth factor receptors that activate these pathways, such as epidermal growth factor receptors and platelet-derived growth factor receptors are examples that result in increased mTORC1 signaling. Frequent mutations that affect the wider PI3K/PTEN/Akt-TSC1/TSC2-Rheb/mTORC1 signaling network have also been reported in renal cancers and head and neck cancer [12,13]. The signaling pathway is illustrated in Figure 1.

mTOR signal transduction

mTOR is a serine/threonine protein kinase that is integral to mTORC1. mTORC1 consists of the core components; mTOR, rapamycin-associated protein of TOR (Raptor) and mLST8. In addition, proline-rich Akt substrate of 40 kDa (PRAS40) [14] and DEP domain containing mTOR-interacting protein (DEPTOR) can also associate with and negatively regulate mTORC1 [15]. mTOR and mLST8 are also integral to a second complex called mTORC2. Components distinctive to mTORC2 include rapamycin insensitive companion of TOR (Rictor), mSIN1 and Protor [16]. mTORC1 is the better studied of the two complexes involved in managing multiple biosynthetic pathways and plays a key anabolic role in promoting cell growth and proliferation. As well as promoting cell growth and proliferation, mTORC2 influences cell morphology through cytoskeletal organization. mTORC2 can also indirectly promote mTORC1 activation via activation of Akt. mTORC2 directly phosphorylates Akt on Ser473, which enhances Akt activity when additionally phosphorylated at Ser308 by PDK1 within the PI3K signaling pathway [17]. Activation of Akt then promotes signal transduction through mTORC1 via switching off the TSC1/TSC2 tumor suppressor complex. Consequently, mTORC2 could be considered to function upstream of mTORC1.

When complexed with TSC1, TSC2 functions as a GAP towards a small G-protein called Rheb, reverting Rheb to an inactive GDP-bound form. Reversion of Rheb to an inactive GDP-bound form turns mTORC1 off [6,7]. Rheb becomes activated when the TSC1/TSC2 complex is repressed through an array of upstream kinases within mitogenic and hormone induced pathways (depicted in Figure 1). PI3K/AKT and Ras/MAPK/ERK/RSK cell signaling pathways regulate mTORC1 signaling via TSC1/TSC2. Both AKT (also referred to as PKB) [18] and RSK [19] can phosphorylate TSC2 on over-lapping and distinct residues that lead to inactivation of TSC1/TSC2. As well as growth signaling inputs, mTORC1 is also regulated by energy and this occurs through the energy sensing AMP-dependent protein kinase (AMPK). AMPK becomes active in response to energy stress, when cellular ATP levels decline and AMP levels sequentially increase. AMPK phosphorylates TSC2 on Thr1227 and Ser1345, which enhances the function of TSC1/TSC2 to act as a RhebGAP [20]. AMPK is itself activated by the serine/threonine kinase LKB1/STK11 [19] or the Ca^{2+}/CaM-dependent protein kinase kinase β (CaMKKβ) [21] that phosphorylates the α-subunit of AMPK on Thr172. AMPK-dependent phosphorylation of TSC2 on Thr1227 and Ser1345 [22] is lost when inactivating mutations within LKB1 occur, giving rise to Peutz-Jeghers Syndrome that predisposes patients to hamartomatous polyps in the gastrointestinal tract and development of cancer [23].

mTORC1 can only be activated when nutrients are present. There are multiple mechanisms of nutrient sensing involving mTORC1 and lysosomes. Initially, Sancak *et al*. [24] and Kim *et al*. [25] identified the Rag small G-proteins as important mediators of amino acid signaling. Four Rag GTPases were identified as Raptor interacting proteins. The active complex consists of either RagA-GTP or RagB-GTP associated with either RagC-GDP or RagD-GDP as a heterodimer [24]. During conditions when amino acids are sufficient, RagA and RagB switch to an active GTP-bound state causing Raptor association. The Rag heterodimer then translocates mTORC1 to the 'Ragulator complex' [26] that is closely associated with v-ATPase on lysosomal membranes [27] (Figure 2). Immunofluorescence studies revealed that mTORC1 localized to lysosomes either in the presence of amino acids or when active Rag heterodimers were expressed [24]. It should be noted that Rheb is also lysosomally localized via C-terminal prenylation and the association of mTORC1 with Rheb on lysosomal membranes is required for its activity [28]. Therefore, under conditions of nutrient withdrawal, mTORC1 is cytoplasmically localized in an inactive state as it cannot interact with lysosomal-tethered Rheb. When nutrients become plentiful, the Rag proteins and mTORC1 are actively translocated to the Ragulator complex on the membrane surface of lysosomes, where Rheb-GTP is then required for mTORC1 activation.

The Ragulator complex consists of five proteins (MP1, p14, p18, HBXIP and C7ORF59), which are referred to as the late endosomal/lysosomal adaptor, MAPK and mTOR activator 1-5 (LAMTOR1-5) [26]. The Ragulator complex functions as a guanine nucleotide exchange factor (GEF) towards the RagA and RagB small G proteins to convert them to an active GTP-bound state [29]. At lysosomes, the tumor suppressor protein Folliculin (FLCN) also positively regulates these Rag heterodimers by acting as a GAP towards RagC and RagD [30,31]. For instance, these two positive signaling inputs switch these Rag heterodimers to an active state, where RagA/B becomes GTP-bound and RagC/D becomes GDP-bound. These Rag heterodimers are also regulated by RagGTPases and GTRs-1 (GATOR) and GATOR2, and like the Ragulator complex are lysosomal localized and function as a multi-protein complex [32]. As a negative regulator of mTORC1, GATOR1 acts as a GAP towards RagA and RagB to switch them to an inactive state, preventing Rag-mediated translocation of mTORC1 to the lysosome. A recently identified protein complex called KICSTOR was found to be necessary for tethering GATOR1 to the lysosome [33,34]. GATOR2 lies upstream of GATOR1 and functions as a negative regulator of GATOR1. GATOR2 is positively regulated by nutrients. The branched chained amino acid, leucine, is sensed via three Sestrins (SESN1– SESN3), leading to activation of GATOR2. Sestrins are thought to be the main leucine sensor that activates mTORC1. Activation of GATOR2 then sequentially inactivates GATOR1, switching the Rag heterodimers to an active conformation to translocate mTORC1 to lysosomes to promote mTORC1 [35-36].

Lysosomal recruitment of mTORC1 by the Rag GTPase also allows mTORC1 to directly interface with AMPK and ULK1 (unc-51 like autophagy activating kinase 1). Energy and nutrients are replenished within the lysosome through catabolism, where these amino acids are thought to be actively sensed within the lumen of the lysosome and are dependent on the vacuolar H^+-ATPase (v-ATPase). The v-ATPase monitors the amino acid levels within the lysosome and relays this signal to the Ragulator complex on the outside, termed the 'inside-out signal' [27]. The amino acid signal to the v-ATPase is likely relayed via amino acid

transporters that reside on the lysosomal membrane. Amino acids are transported out of the lysosome by amino acid transporters that include PAT1 (proton-assisted amino acid transporter) and SLC38A9. SLC38A9 was shown to interact with the Rag-Ragulator-v-ATPase complex on lysosomes and is required for arginine-mediated activation of mTORC1 [37-39]. The v-ATPase is also critically involved in sensing the energy status of the cell. Under conditions of glucose starvation, axis inhibition protein (AXIN) is recruited to the v-ATPase on lysosomal membranes [40]. When cells are energy starved, AXIN functions as a scaffold to recruit LKB1-AMPK to lysosomes, where this AXIN-LKB1-AMPK complex binds to LAMTOR1 as part of the Ragulator complex. Importantly, LAMTOR1 dramatically reduces the concentration of AMP that is required to promote association of AXIN-LKB1 with AMPK, which is necessary for AMPK activation. The presence of LAMTOR1 lowers the AMP concentration to 5 μM that is required for AMPK activation at lysosomes, while much higher AMP concentrations (>150 μM) are required when LAMTOR1 is absent. Therefore, recruitment of AMPK by AXIN to the Ragulator complex is regulated during energy starvation involving the v-ATPase and switches mTORC1 off [40].

The lysosome not only acts as a signaling hub to relay the nutrient and energy status of the cell to mTORC1, lysosomes also relay the signaling inputs from growth factor receptors on the plasma membrane. To efficiently interface with the outside signals via growth factor receptors, the localization of lysosomes is dynamically regulated. Lysosomes are translocated in the direction of the plasma membrane when nutrients and energy are in sufficient supply [41]. Proximal localization of lysosomal-tethered mTORC1 and Rheb to the plasma membrane ensures for rapid signal transduction via growth factor receptors to TSC1/TSC2 and sequential Rheb/mTORC1 activation. Conversely, lysosomal activity is down-regulated when proximal to the plasma membrane to minimize catabolism while cells actively grow. When nutrients become limiting, lysosomes are translocated inwards towards the nuclear periphery where autophagosomal flux and lysosomal activity is enhanced. Such a mechanism to partition mTORC1 away from plasma membrane localized growth factor receptors leads to rapid mTORC1 inhibition. To further deactivate mTORC1 under nutrient deprivation, mTORC1 is displaced from the lysosomal surface, away from Rheb.

Nutrients are sensed by many signaling inputs. A recent finding revealed that when arginine levels are low, TSC1/2 associates with Rheb, leading to reversion of Rheb to an inactive GDP-bound state [42]. In this study, it was found that arginine was sufficient to prevent the RhebGAP function of TSC1/TSC2, which indicates an additional signaling mechanism that is dependent on arginine. Recent work also showed that Cytosolic Arginine Sensor for MTORC1 (CASTOR) subunit 1 interacts with GATOR2 when cells are deprived of arginine and leads to mTORC1 inhibition. When arginine is resupplied, arginine binds to CASTOR1 and disrupts the inhibited CASTOR1-GATOR2 complex that then allows GATOR2 to turn off GATOR1 to sequentially activate mTORC1 [43]. Collectively, mTORC1 signaling is highly complex with multiple inputs from nutrients, energy and receptor-mediated growth signals that ensures that the growth status of the cell is efficiently managed.

Autophagy regulation by mTORC1 and nutrient and energy homeostasis

While the mTORC1 signaling nexus is lysosomal localized, the main function of lysosomes is to conduct autophagy. Autophagy is a catabolic process whereby damaged or unwanted

organelles and macromolecules are degraded via sequestration in membrane-bound autophagosomes that fuse with lysosomes to allow enzymatic break down of its contents. Autophagy recycles components through catabolic degradation to generate energy and components for biosynthetic reactions [44]. 'Self-eating' was first observed in 1963 by Christian de Duve [45], whose subsequent research led to the detection of both macro- and microautophagy [46]. Chaperone-mediated autophagy was discovered much later in 1981 [47]. Microautophagy is concerned with maintenance of organelle size, membrane homeostasis and survival. It is triggered in conditions of nitrogen starvation (but also when mTORC1 is inhibited). In microautophagy, lysosomes directly engulf malfunctioning or damaged organelles [48]. Chaperone-mediated autophagy uses a translocation protein complex to deliver single, soluble proteins to the lysosome where they must be unfolded before entry. With chaperone-mediated autophagy, the high degree of selectivity may help to prevent degradation of essential structures whilst still acquiring amino acids [47]. Ultimately, all forms of autophagy shuttle macromolecules into lysosomes for digestion and recycling of components. During macroautophagy the cytoplasm, proteins and organelles are internalized within double-membrane vesicles termed autophagosomes. In this review, we will now refer to macroautophagy as autophagy.

Autophagy is activated within the first few hours of starvation, increasing in activity until up to about 6 hours before slowly declining [49]. Autophagy is tightly regulated by the kinase ULK1 as an active complex with autophagy-related (ATG)-13, focal adhesion kinase family interacting protein of 200 kDa (FIP200) and ATG101. This protein kinase complex with ULK1 is essential for autophagy, as loss of ULK1, ATG13 or ATG101 expression impairs autophagy [50-53]. Nutrient withdrawal stimulates ULK1 complex activation via ULK1 autophosphorylation and phosphorylation of the ATG13 and FIP200 binding partners [51], which leads to relocalization of this protein complex to autophagic isolation membranes to initiate autophagy [54].

To coordinate the growth status of the cell depending on the availability of nutrients and energy, mTORC1 relies on the signaling input of ULK1 to help regenerate energy and amino acids from the cells. Furthermore, there is signaling interplay from AMPK, the energy sensing kinase that is turned on when energy supply becomes limited. Homeostatic balance is tightly maintained by this mTORC1-AMPK-ULK1 kinase triad. The activities of mTORC1, AMPK and ULK1 are tightly regulated on lysosomal membranes. Over the last two decades, research has delineated a complex web of signaling mechanisms that regulates the kinase activities of mTORC1, AMPK and ULK1, which essentially coordinate cell growth, energy status, and cell regeneration, respectively. A normal cell can efficiently switch between growth and growth arrested states, which is necessary for cells to maintain energy and amino acid homeostasis (Figure 3). In a growth arrested state, when energy or nutrients become limiting, cells employ a series of negative feedback mechanisms from AMPK and ULK1 that rapidly switch off mTORC1 and potently induce autophagy. The cell will then regenerate their amino acid and energy levels, leading to AMPK and ULK1 inactivation and allowing mTORC1 to be switched on again to promote cell growth. During the growth phase, mTORC1 potently down-regulates autophagy and ensures that a steady anabolic rate of growth is maintained. mTORC1 phosphorylates ULK1, which slows down autophagosomal maturation and lysosomal activity [51,53]. However, it should be noted that even when mTORC1 is active, a basal level of lysosomal activity is still present to maintain a constant

low level of protein degradation. Cancer cells with constitutive mTORC1 activation have less signaling flexibility to relay the appropriate signaling input from AMPK and ULK1 depending on energy and nutrient supply. Such dysregulated signaling can lead to metabolic transformation.

As another mechanism of regulation within this kinase triad, AMPK indirectly inactivates mTORC1 via ULK1. AMPK phosphorylates ULK1, which is necessary for ULK1 stabilization and its full activity [55-59]. ULK1 then sequentially phosphorylates Raptor, which prevents Raptor associating with mTORC1 substrates [60]. Raptor is considered a scaffold protein of mTORC1 that is necessary for recruiting and delivering substrates to the kinase active site of mTORC1. As ULK1-phosphorylated Raptor can no longer bind substrates and present them to the mTOR kinase, mTORC1 signaling is dramatically down-regulated. AMPK also phosphorylates Raptor and is thought to be inhibitory [61]. Therefore, both AMPK and ULK1 work together to down-regulate mTORC1 when energy levels are low.

There are additional signaling mechanisms that ensure that autophagy is tightly regulated. For instance, ULK1 was also found to directly inhibit AMPK by phosphorylation [62]. Presumably, this signaling feedback mechanism from ULK1 towards AMPK ensures that autophagy is not constitutively turned on for long periods of time and would allow mTORC1-mediated signaling to become more dominant after the cell has recovered its levels of nutrients and energy. mTORC1 becomes the dominant kinase when nutrient supply is sufficient and this is because phosphorylation of ULK1 by mTORC1 blocks the ability of AMPK to phosphorylate and activate ULK1 [55,56]. Another mechanism by how mTORC1 inhibits autophagy is via phosphorylation of Autophagy/Beclin-1 regulator 1 (AMBRA1). mTORC1 inhibits ULK1 indirectly through phosphorylation of AMBRA1 at Ser52 [63]. Phosphorylation of AMBRA1 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 by TRAF6 causes ULK1 to dimerize, leading to its enhanced stability and sequential activation. During conditions that favor mTORC1 activation, AMBRA1 is kept in an inactive state and is tethered to intracellular vesicles as part of a dynein motor complex. However, upon conditions when mTORC1 is inactivated, autophagy is rapidly promoted through Lys-63-ubiquitination of ULK1 by the AMBRA1-TRAF6 complex that leads to ULK1 stabilization.

Longer-term regulation of autophagy by mTORC1 is managed by a set of transcription factors that are responsible for lysosomal biogenesis; transcription factor EB (TFEB) [64] and transcription factor binding to IGHM enhancer 3 (TFE3) [65]. These TFEB and TFE3 transcription factors are retained to lysosomes via the active Rag heterodimers when nutrients are sufficient and mTORC1 phosphorylates TFEB at SerS142 [64] and TFE3 at Ser311 [65] to keep them inactive. When nutrients become limiting, TFEB and TFE3 are dephosphorylated and are then released, allowing them to translocate to the nucleus to drive gene-expression of lysosomal genes.

Therapeutic avenues to treat cancer involving mTORC1, autophagy and endoplasmic reticulum stress

While targeting autophagy has potential as an anti-cancer therapy, there are some current challenges. One issue is that not all cancer cells require autophagy for cell survival, so autophagy inhibitors are unlikely to be broadly effective. While autophagy can function as a key survival pathway in some cancer cells, restoration of autophagy in other cancer cells can instead enhance cell death [66]. At early stages of cancer progression, autophagy is often involved in cancer cell survival. However, at later stages of cancer progression, autophagy is often heavily compromised through genetic instability, causing loss of or mutation of autophagy genes. As an example, Beclin 1 (BECN1) was shown to be monoallelically deleted in 40-75% of sporadic human breast cancers and ovarian cancers [67].

Autophagy can either promote or antagonize cancer development. Such variation in the dependency of autophagy makes it technically tricky when considering autophagy as a therapy for cancer (reviewed in detail [68]). If a tumor was initially screened for autophagy activity and dependence for survival, would stratifying cancer patients improve efficacy when considering autophagy as a therapeutic target? Patient stratification would certainly help, however, there is a current lack of molecular tools or biomarkers to robustly ascertain whether autophagy is either potentially activated or severely compromised in patient material. Immunohistochemistry of LC3 in patient tumor tissue is not particularly informative as it can be misleading. This is because an accumulation of LC3 (which indicates that there are more autophagosomes in the cell) could mean that autophagy is either enhanced or impaired. Indeed, enhanced autophagy could lead to an accumulation of LC3. Or conversely, LC3 accumulation could also mean that there is a build-up of autophagosomes due to an inhibition of autophagosomal flux. A robust method to determine autophagy within tumor tissue is clearly needed to accurately stratify patients. If patient stratification regarding dependency of autophagy for cancer cell survival was improved, targeting autophagy as a therapy would become much more viable a strategy.

mTORC1 inhibitors have had much clinical interest for the treatment of cancer. The first-generation inhibitors of mTORC1 are called rapalogues; rapamycin analogues with improved pharmacokinetics that bind allosterically and inhibit mTORC1 as a FKBP12-rapamycin protein-drug complex. In 2007, the rapalogue called temsirolimus (and later, everolimus) was approved for advanced-stage renal cell carcinoma and was the first mTORC1 inhibitor that was approved for cancer [69]. The median overall survival of patients with renal cell carcinoma treated with temsirolimius was 10.9 months. While with everolimus, survival was observed to be increased by 5.9 months in advanced renal cell carcinoma patients who previously failed with treatment with either of the receptor tyrosine kinase inhibitors, sorafenib or sunitinib [70]. Later, temsirolimus was approved for the treatment of mantle cell lymphoma [71]. Given the involvement of mTORC1 in cell growth control and cancer progression it is surprising that mTORC1 inhibitors have not been more successful in treating a wider range of cancers. However, there is still much clinical interest; there is over 400 registered trials within clinicaltrials.gov using mTOR inhibitors (both rapalogues and the 2nd generation ATP-competitive mTOR kinase inhibitors) as a mono agent or when combined with other drugs to treat many cancer types, including melanoma, myeloma, breast, renal, gynecological and brain cancers.

One reason why rapalogues have had limited clinical use to treat cancer is that their drug action is cytostatic rather than cytotoxic, i.e., rapalogues arrest cancer cell growth instead of

killing them. The cytostatic property of rapalogues is partly through the promotion of autophagy. However, it is important to note that mTORC1 signaling towards autophagy is partially rapamycin resistant and the level of resistance varies depending on cell-type. The variation in the ability of rapamycin to induce autophagy could be due to the stability of mTORC1. It was postulated that cells with a more unstable mTORC1 conformation would have heightened sensitivity to rapamycin, which would fully inhibit mTORC1 and induce autophagy [72]. In this study, it was found that rapamycin-resistant cells became sensitized to rapamycin when combined with a non-efficacious, low concentration of an ATP-competitive mTOR inhibitor (i.e., at concentrations that is unable to inhibit mTORC1 or mTORC2 as a mono agent). It is therefore feasible that combination treatment of ATP-competitive mTOR inhibitors with rapalogues would more completely block mTORC1 and restore autophagy in cancer cells where the autophagy pathway is compromised. ATP-competitive mTOR inhibitors when used at higher concentrations to inhibit both mTORC1 and mTORC2 would potently induce autophagy initially. However, after longer periods of mTORC2 inhibition the cytoskeleton would be reorganized and would lead to impaired autophagosomal flux.

While directly targeting mTORC1 and autophagy has potential issues and caveats when considering them as an anticancer therapy, these pathways still have much potential for a targeted approach to selectively kill cancer cells. For instance, the imbalance to cell signaling at the level of the lysosome, i.e., mTOR, AMPK and ULK1, in many cancers often leaves the cancer cell with vulnerabilities that could be exploited. One critical cancer vulnerability is a lack of homeostatic flexibility within the mTOR, AMPK and ULK1 signaling pathways that is caused by constitutively high levels of mTORC1 signaling. Cancer cells with hyperactive mTORC1 are unable to efficiently restore homeostatic balance during periods of cell stress. For instance, a cancer cell will still maintain a high level of cell growth in the presence of cell stress, where these conflicting signals of cell growth and chronic stress will trigger cell death. In contrast, a non-cancer cell will become growth arrested under cell stress, which will allow these normal cells to better tolerate the stress input and effectively restore homeostatic balance.

A good example of a stress pathway that is intrinsically linked to mTORC1 and autophagy and is necessary for maintaining cellular homeostasis is endoplasmic reticulum (ER) stress. One consequence of hyperactive mTORC1 and repression of autophagy is that ER stress becomes markedly elevated. This is because autophagy is normally utilized by a cell to remove unfolded protein aggregates in the ER to efficiently restore the protein folding environment [73]. To further help maintain protein turn-over via degradation while autophagy is down-regulated, mTORC1 upregulates the proteasome [74]. When autophagy is compromised, the proteasome becomes the primary proteolytic pathway to clear the cell of unfolded protein aggregates, thereby restoring ER homeostasis and preventing cell death [75]. Given this higher dependency on the proteasome to lessen the burden of ER stress, a potential therapeutic strategy to target mTORC1-driven cancer cells with down-regulated autophagy could be through proteasomal inhibition, with the aim to push the levels of ER stress beyond a tolerated survival threshold. In support of this therapeutic concept, selective cytotoxicity using proteasome inhibitors has been shown towards cancer cell lines with heightened signal transduction through mTORC1 [76-78]. Regarding anti-cancer therapy, proteasome inhibitors are currently being used in the clinic for the treatment of multiple myeloma, recurrent multiple myeloma and mantle cell lymphoma.

Concluding remarks

Much progress has been made regarding the signaling interplay between mTORC1 and autophagy and how the nutrient and energy status of the cell is tightly regulated at the level of lysosomes. During the late stages of cancer, it is common that mTORC1 is upregulated and autophagy becomes heavily compromised, leading to an inflexibility in mTORC1/AMPK/ULK1 signaling to maintain homeostasis. While targeting autophagy has promise as an anti-cancer therapy, there is a real need to better stratify patients regarding the dependence of cancer cells to autophagy to make such therapies more effective. Targeting the vulnerabilities of mTORC1-hyperactive cancer cells through promoting stress pathways, such as ER stress, has the potential to impact a wider range of cancers when compared to the current mTORC1 inhibitors that are limited due to their cytostatic properties. Future work is needed to better understand how we can further exploit the signaling inflexibility that cancer cells when mTORC1 and autophagy signaling is dysregulated. Such research would allow for better targeted therapies and would improve patient survival.

Acknowledgements
We would like to give special thanks to Cancer Research Wales who provided funding to CEJ and ART. This work was also supported by Health and Care Research Wales (the Wales Cancer Research Centre), the Tuberous Sclerosis Association and the Tuberous Sclerosis Alliance (to ART).

Conflict of interest statement
The authors have no conflict of interest that should be disclose.

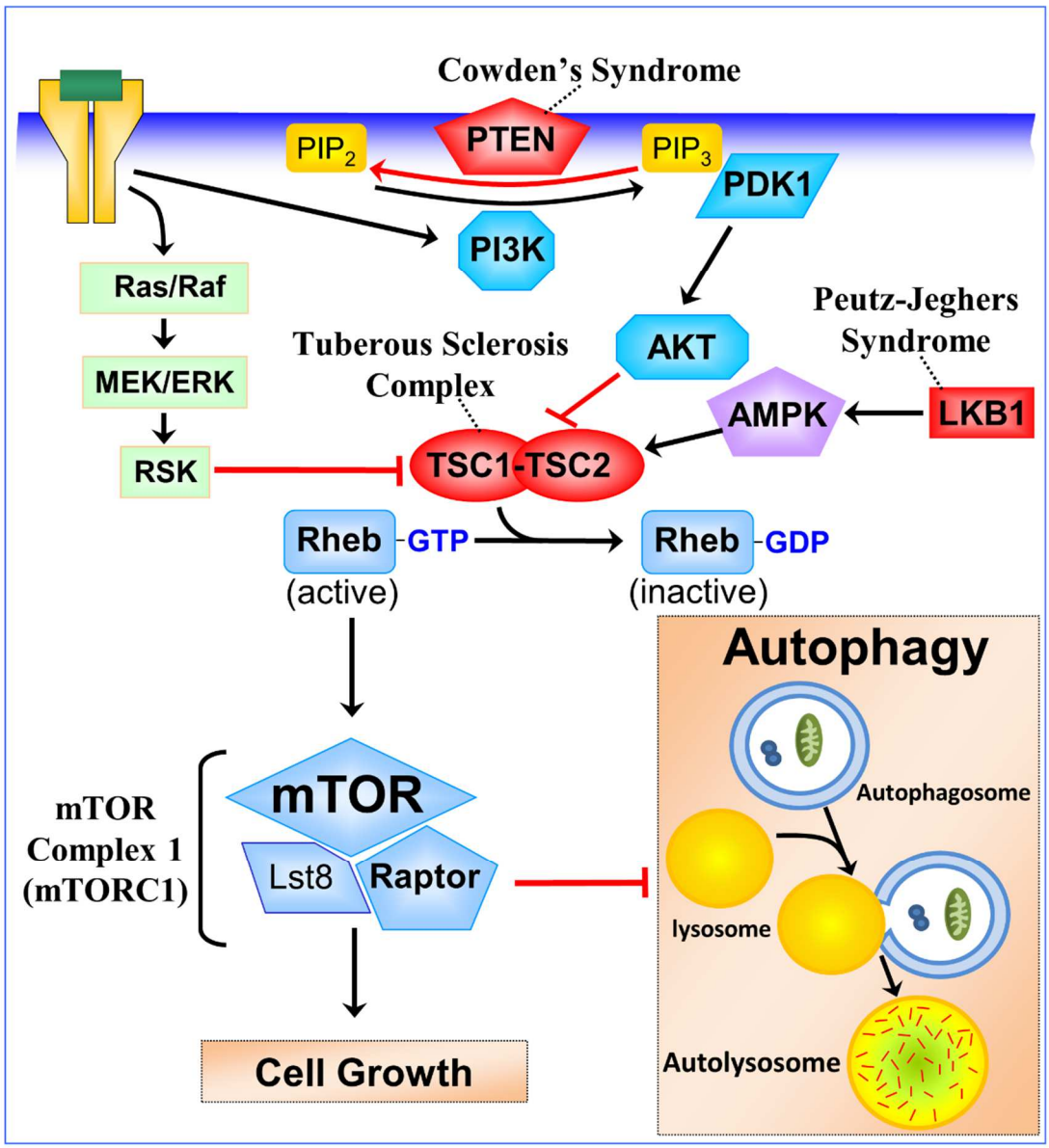

Figure 1: mTORC1 signal transduction and tumor suppressors. Growth factor signal transduction via receptors on the plasma membrane switches off the TSC1/TSC2 tumor suppressor complex (where loss of function mutations causes Tuberous Sclerosis Complex). These include the Ras/Raf/MAPK/ERK/RSK and PI3K/AKT signaling pathways. Tumor suppressors upstream of TSC1/TSC2 include PTEN and LKB1, where mutations in these genes can cause Cowden's syndrome and Peutz-Jeghers syndrome, respectively. Upon growth factor stimulation and inactivation of TSC1/TSC2, Rheb is converted to an active GTP-bound state that enhances mTORC1 activation and cell growth control. mTORC1 activation also potently inhibits autophagy.

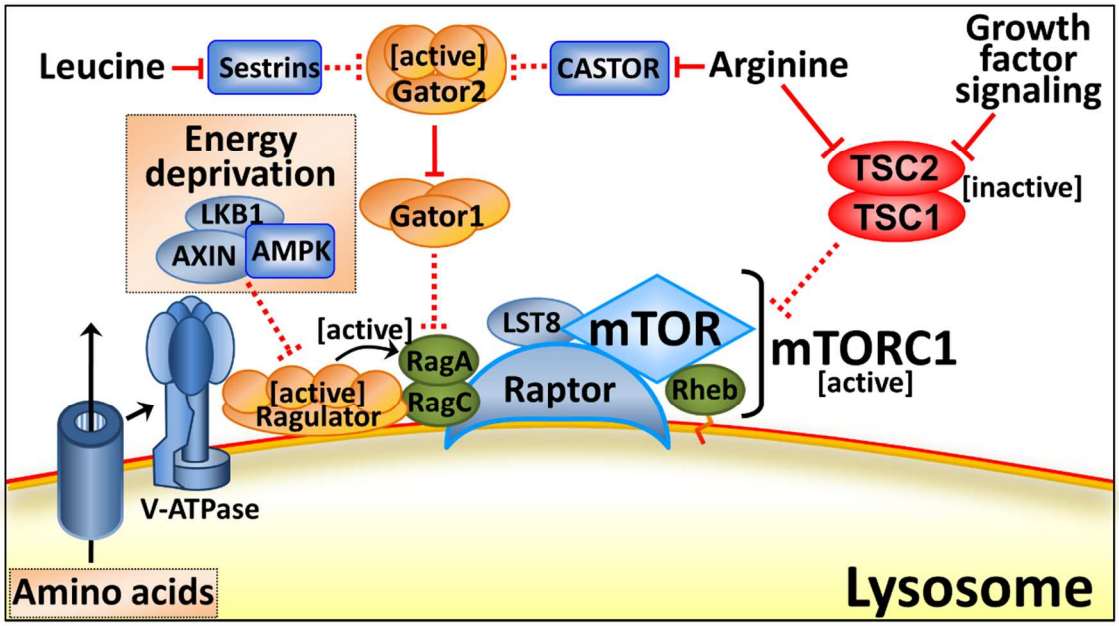

Figure 2: mTORC1 signaling nexus at the lysosome. Amino acids within the lumen of the lysosome signal through to the Ragulator complex (coined as the 'inside-out signal'). Amino acid transporters (such as PAT1 and SLC38A9) transport amino acids into the cytoplasm and are required for mTORC1 activation. Through the Ragulator complex, the Rag GTPase heterodimers are switched to an active state to recruit mTORC1 to the lysosome. As a mechanism to sense amino acids, arginine inhibits both TSC1/TSC2 and CASTOR, negative regulators of Rheb and GATOR2, respectively. Leucine positively switches on GATOR 2 via Sestrins. Dotted red inhibitory lines between Sestrins-Gator2, CASTOR-Gator2, and Gator1-Rag are not inhibitory when nutrients (leucine and arginine) are present. Signal transduction via growth factors inactivates TSC1/TSC2 (the dotted red inhibitory line between TSC1/2 and Rheb is not inhibitory in the presence of a growth signal input), which converts Rheb to an active GTP-bound state to induce mTORC1 kinase activity. Under energy deprivation, LKB1/AMPK docks to the Ragulator complex through AXIN to switch off mTORC1. When energy is sufficient, the dotted red inhibitory line between AMPK and the regulator complex is not inhibitory.

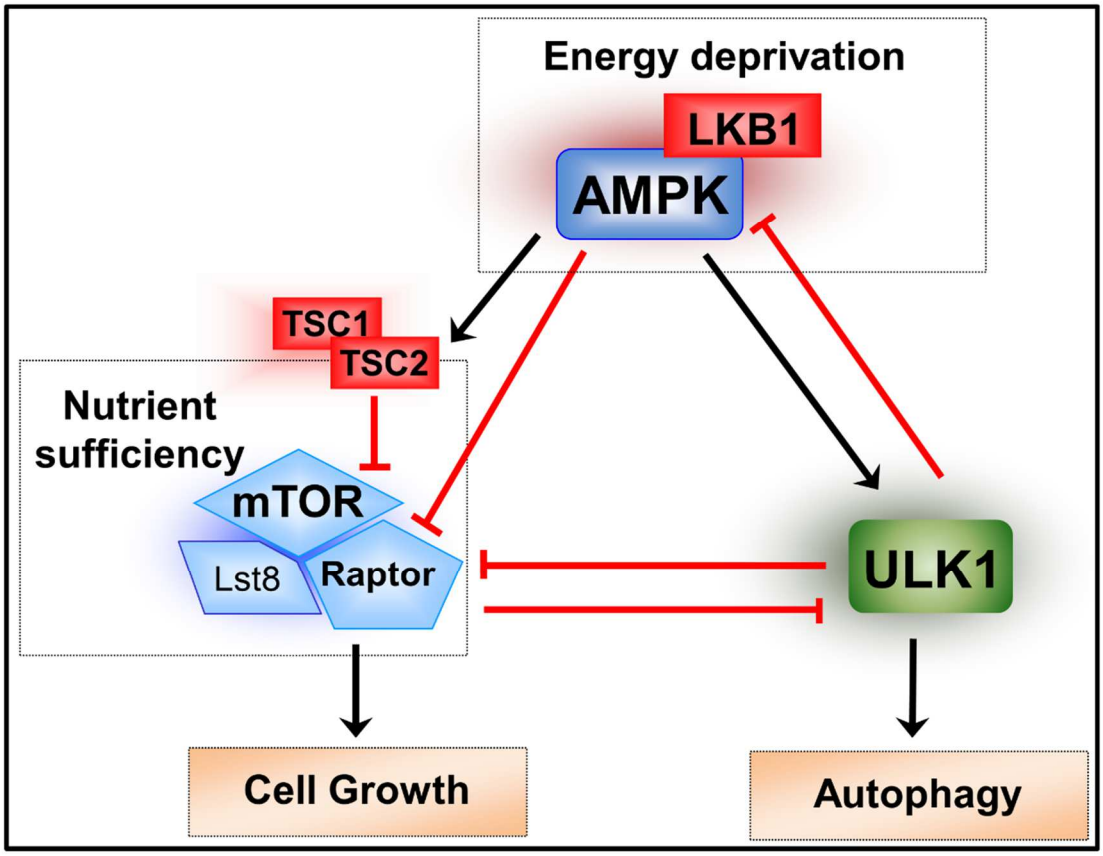

Figure 3: The mTORC1-AMPK-ULK1 kinase triad. Signaling interplay and feedback mechanisms between mTORC1, AMPK and ULK1 is essential for energy and nutrient homeostasis. Under energy deprivation, AMPK activates ULK1 and switches off mTORC1 via phosphorylation of both TSC2 and Raptor to down-regulate cell growth. When nutrients and energy are in supply, mTORC1 becomes the dominant kinase, which turns off ULK1. However, under nutrient deprivation, ULK1 is gradually enhanced as mTORC1 becomes inactivated. Reduced phosphorylation of ULK1 by mTORC1 causes ULK1 stabilization and sequential activation to drive autophagy. ULK1 also directly phosphorylates Raptor to further turn off mTORC1. To restrict the duration of ULK1 activity and to prevent constitutive ULK1 activation, ULK1 phosphorylates and inactivates AMPK. Both inputs of AMPK activation (via energy starvation) and mTORC1 inhibition (via nutrient starvation) are required for a robust level of ULK1 activation.

References

1. Iadevaia V., Liu R., Proud C.G. (2014) mTORC1 signaling controls multiple steps in ribosome biogenesis. *Semin Cell Dev Biol* **36**, 113–120
2. Saxton R.A., Sabatini D.M. (2017) mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **168,** 960–976
3. Jiang B.H., Liu L.Z. (2008) Role of mTOR in anticancer drug resistance: perspectives for improved drug treatment. *Drug Resist. Updat.* **11,** 63–76
4. Kohrman M.H. (2012) Emerging treatments in the management of tuberous sclerosis complex. *Pediatr Neurol.* **46**, 267–275
5. Krymskaya V.P., Goncharova E.A.. (2009) PI3K/mTORC1 activation in hamartoma syndromes: therapeutic prospects. *Cell Cycle.* **8**, 403–413
6. Tee A.R., Manning B.D., Roux P.P., Cantley L.C., Blenis J. (2003) Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol.* **13**, 1259–1268
7. Dibble C.C., Elis W., Menon S., Qin W., Klekota J., Asara J.M., (2012) TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. *Mol Cell.* **47**, 535–546
8. Platt, F.M., Hurst, C.D., Taylor, C.F., Gregory, W.M., Harnden, P., Knowles, M.A., (2009) Spectrum of phosphatidylinositol 3-kinase pathway gene alterations in bladder cancer. *Clin. Cancer Res*. **15**, 6008e6017
9. Sjödahl G., Lauss M., Gudjonsson S., Liedberg F., Halldén C., Chebil G., (2011) A systematic study of gene mutations in urothelial carcinoma; inactivating mutations in TSC2 and PIK3R1. *PLoS One.* **6**, e18583
10. Jiao, Y., Shi, C., Edil, B.H., de Wilde, R.F., Klimstra, D.S., Maitra, A., (2011) DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* **331**, 1199e1203
11. Grabiner B.C., Nardi V., Birsoy K., Possemato R., Shen K., Sinha S., (2014) A diverse array of cancer-associated MTOR mutations are hyperactivating and can predict rapamycin sensitivity. *Cancer Discov.* **4**, 554–563
12. Sato, Y., Yoshizato, T., Shiraishi, Y., Maekawa, S., Okuno, Y., Kamura, T., (2013) Integrated molecular analysis of clear-cell renal cell carcinoma. *Nat. Genet.* **45**, 860e867
13. Liao, Y.M., Kim, C., Yen, Y. (2011) Mammalian target of rapamycin and head and neck squamous cell carcinoma. *Head Neck Oncol.* **3**, 22
14. Fonseca BD, Smith EM, Lee VH, MacKintosh C, Proud CG. (2007) PRAS40 is a target for mammalian target of rapamycin complex 1 and is required for signaling downstream of this complex. *J. Biol. Chem.* 282, 24514–24524
15. Peterson T.R., Laplante M., Thoreen C.C., Sancak Y., Kang S.A., Kuehl W.M., (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell* **137**, 873–886
16. Jacinto E., Loewith R., Schmidt A., Lin S., Rüegg M.A., Hall A., (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.* **6**, 1122–1128

17. Sarbassov D.D., Guertin D.A., Ali S.M., Sabatini D.M. (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**, 1098–1101

18. Manning B.D., Tee A.R., Logsdon M.N., Blenis J., Cantley L.C. (2002) Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol. Cell* **10**, 151–162
19. Ballif B.A., Roux P.P., Gerber S.A., MacKeigan J.P., Blenis J., Gygi S.P. (2005) Quantitative phosphorylation profiling of the ERK/p90 ribosomal S6 kinase-signaling cassette and its targets, the tuberous sclerosis tumor suppressors. *Proc. Natl. Acad. Sci. USA.* **102**, 667–672
20. Inoki K., Zhu T., Guan K.L. (2003) TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577–590
21. Hawley S.A., Pan D.A., Mustard K.J., Ross L., Bain J., Edelman A.M., Frenguelli B.G., Hardie DG. (2005) Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab. 2,* 9–19
22. Hong S.P., Leiper F.C., Woods A., Carling D., Carlson M. (2003) Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc. Natl. Acad. Sci. USA.* **100**, 8839–8843
23. Hemminki A., Markie D., Tomlinson I., Avizienyte E., Roth S., Loukola A., (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* **391**, 184–187
24. Sancak Y., Peterson T.R., Shaul Y.D., Lindquist R.A., Thoreen C.C., Bar-Peled L., (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* **320**,1496–1501
25. Kim E., Goraksha-Hicks P., Li L., Neufeld T.P., Guan K.L. (2008) Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol.* **10**, 935–945
26. Sancak Y., Bar-Peled L., Zoncu R., Markhard A.L., Nada S., Sabatini D.M. (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* **141**, 290–303
27. Zoncu R., Bar-Peled L., Efeyan A., Wang S., Sancak Y., Sabatini D.M. (2011) mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. *Science* **334**, 678–683
28. Menon S., Dibble C.C., Talbott G., Hoxhaj G., Valvezan A.J, Takahashi H. (2014) Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. *Cell* **156**, 771–785
29. Bar-Peled L., Schweitzer L.D., Zoncu R., Sabatini D.M. (2012) Ragulator is a GEF for the Rag GTPases that signal amino acid to mTORC1. *Cell* **150**, 1196–1208
30. Petit C.S., Roczniak-Ferguson A., Ferguson S.M. (2013) Recruitment of folliculin to lysosomes supports the amino acid-dependent activation of Rag GTPases. *J. Cell Biol.* **202**, 1107–1122
31. Tsun Z.Y., Bar-Peled L., Chantranupong L., Zoncu R., Wang T., Kim C. (2013) The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. *Mol. Cell* **52**, 495–505
32. Bar-Peled L., Chantranupong L., Cherniack A.D., Chen W.W., Ottina K.A., Gra-biner B.C., (2013) A tumor suppressor complex with GAP activity for the RagGTPases that signal amino acid sufficiency to mTORC1. *Science* **340**, 1100–1106

33. Peng M., Yin N., Li M.O. (2017) SZT2 dictates GATOR control of mTORC1 signalling *Nature* **543,** 433–437
34. Wolfson R.L., Chantranupong L., Wyant G.A., Gu X., Orozco J.M., Shen K., (2017) KICSTOR recruits GATOR1 to the lysosome and is necessary for nutrients to regulate mTORC1. *Nature* **543,** 438–442
35. Chantranupong L., Wolfson R.L., Orozco J.M., Saxton R.A., Scaria S.M., Bar-Peled L., (2014) The Sestrins interact with GATOR2 to negatively regulate the amino-acid-sensing pathway upstream of mTORC1. Cell Rep. 9, 1–8.
36. Parmigiani A., Nourbakhsh A., Ding B., Wang W., Kim Y.C., Akopiants K. (2014) Sestrins inhibit mTORC1 kinase activation through the GATOR complex. Cell Rep. 9, 1281–1291
37. Jung J., Genau H.M., Behrends C. (2015) Amino Acid-Dependent mTORC1 Regulation by the Lysosomal Membrane Protein SLC38A9. *Mol. Cell Biol.* **35,** 2479–2494
38. Rebsamen M., Pochini L., Stasyk T., de Araújo M.E., Galluccio M., Kandasamy R.K., (2015) SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. *Nature* **519,** 477–481
39. Wang S., Tsun Z.Y., Wolfson R.L., Shen K., Wyant G.A., Plovanich M.E., (2015) Metabolism. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. *Science* **347,** 188–194
40. Zhang C.S., Jiang B., Li M., Zhu M., Peng Y., Zhang Y.L., (2014) The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism. *Cell Metab.* **20**, 526–540
41. Korolchuk V.I., Saiki S., Lichtenberg M., Siddiqi F.H., Roberts E.A., Imarisio S., (2011) Lysosomal positioning coordinates cellular nutrient responses. *Nat. Cell Biol.* **13**, 453–460
42. Carroll B., Maetzel D., Maddocks O.D., Otten G., Ratcliff M., Smith G.R., (2016) Control of TSC2-Rheb signaling axis by arginine regulates mTORC1 activity. *Elife* **5**, e11058
43. Chantranupong L., Scaria S.M., Saxton R.A., Gygi M.P., Shen K., Wyant G.A., (2016) The CASTOR Proteins Are Arginine Sensors for the mTORC1 Pathway. *Cell* **165**, 153–164
44. Puissant, A., Robert, G., Auberger, P. (2010) Targeting autophagy to fight hematopoietic malignancies. *Cell Cycle* **17**, 3470–3478
45. De Duve, C. (1963) The lysosome. *Scientific American* **208**, 64–72
46. De Duve, C., Wattiaux, R. (1966) Functions of lysosomes. *Annu. Rev. Physiol.* **28**, 435–492
47. Neff, N.T., Bourret, L., Miao, P., Dice, J.F. (1981) Degradation of proteins microinjected into IMR-90 human diploid fibroblasts. *J. Cell Biol.* **1**, 184–194
48. Li, W.W., Li, J., Bao, J.K. (2012) Microautophagy: lesser-known self-eating. *Cel.l Mol. Life Sci.* **7**, 1125–1136
49. Cuervo A.M. (2010) The plasma membrane brings autophagosomes to life. *Nat. Cell Biol.* **8**, 747–757
50. Chan E.Y., Kir S., Tooze S.A. (2007) siRNA screening of the kinome identifies ULK1 as a multidomain modulator of autophagy. *J. Biol. Chem.* **282**, 25464–25474
51. Jung C.H., Jun C.B., Ro S.H., Kim Y.M., Otto N.M., Cao J., (2009) ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell.* **20**, 1992–2003

52. Mercer C.A., Kaliappan A., Dennis P.B. (2009) A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. *Autophagy* **5**, 649–662
53. Hosokawa N., Hara T., Kaizuka T., Kishi C., Takamura A., Miura Y., (2009) Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell* **20**, 1981–1991
54. Hara T., Takamura A., Kishi C., Iemura S., Natsume T., Guan J.L., (2008) FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. *J. Cell Biol.* **181**, 497–510
55. Kim J., Kundu M., Viollet B., Guan K.L. (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132–141
56. Shang L., Chen S., Du F., Li S., Zhao L., Wang X. (2011) Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. *Proc. Natl. Acad. Sci. USA.* **108**, 4788–4793
57. Lee J.W., Park S., Takahashi Y., Wang H.G. (2010) The association of AMPK with ULK1 regulates autophagy. *PLoS ONE* **3**, e15394
58. Egan D.F., Shackelford D.B., Mihaylova M.M., Gelino S., Kohnz R.A., Mair W., (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* **331**, 456–461
59. Bach M., Larance M., James D.E., Ramm G. (2011) The serine/threonine kinase ULK1 is a target of multiple phosphorylation events. *Biochem. J.* **440**, 283–291
60. Dunlop E.A., Hunt D.K., Acosta-Jaquez H.A., Fingar D.C. and Tee A.R. (2011) ULK1 inhibits mTORC1 signaling, promotes multisite Raptor phosphorylation and hinders substrate binding. *Autophagy* **7**, 737–747
61. Gwinn D.M., Shackelford D.B., Egan D.F., Mihaylova M.M., Mery A., Vasquez D.S., (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* **30,** 214–226
62. Löffler A.S., Alers S., Dieterle A.M., Keppeler H., Franz-Wachtel M., Kundu M., (2011) Ulk1-mediated phosphorylation of AMPK constitutes a negative regulatory feedback loop. *Autophagy* **7**, 696–706
63. Nazio F., Strappazzon F., Antonioli M., Bielli P., Cianfanelli V., Bordi M., (2013) mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat. Cell Biol.* **15**, 406–416
64. Settembre C., Zoncu R., Medina D.L., Vetrini F., Erdin S., Erdin S., (2012) A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J.* **31**, 1095–1108.
65. Martina J.A., Diab H.I., Lishu L., Jeong-A L., Patange S., Raben N., (2014) The nutrient-responsive transcription factor TFE3 promotes autophagy, lyso- somal biogenesis, and clearance of cellular debris. *Sci. Signal* **7**, ra9
66. Glick D., Barth S., Macleod K.F. (2010) Autophagy: cellular and molecular mechanisms. *J. Pathol.* **221**, 3–12
67. Liang X.H., Jackson S., Seaman M., Brown K., Kempkes B., Hibshoosh H., (1999) Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* **402**, 672–676
68. White E. (2012) Deconvoluting the context-dependent role for autophagy in cancer. *Nat. Rev. Cancer* **12**, 401–410

69. Hudes G., Carducci M., Tomczak P., Dutcher J., Figlin R., Kapoor A., (2007) Temsirolimus, interferon α, or both for advanced renal-cell carcinoma. *N. Engl. J. Med.* **356,** 2271–2281
70. Motzer R.J., Escudier B., Oudard S., Hutson T.E., Porta C., Bracarda S., (2010) Phase 3 trial of everolimus for metastatic renal cell carcinoma: final results and analysis of prognostic factors. *Cancer* **116,** 4256–4265
71. Hess G., Herbrecht R., Romaguera J., Verhoef G., Crump M., Gisselbrecht C., (2009) Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J. Clin. Oncol.* **27,** 3822–3829
72. Nyfeler B., Bergman P., Triantafellow E., Wilson C.J., Zhu Y., Radetich B., (2011) Relieving autophagy and 4EBP1 from rapamycin resistance. *Mol. Cell Biol.* **31**, 2867–2876
73. Høyer-Hansen M., Jäättelä M. (2007) Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ.* **14**, 1576–1582
74. Zhang Y., Nicholatos J., Dreier J.R., Ricoult S.J., Widenmaier S.B., Hotamisligil G.S., (2014) Coordinated regulation of protein synthesis and degradation by mTORC1. *Nature* **513**, 440–443
75. Rubinsztein D.C. (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature* **443**, 780–786
76. Auricchio N., Malinowska I., Shaw R., Manning B.D., Kwiatkowski D.J. (2012) Therapeutic trial of metformin and bortezomib in a mouse model of tuberous sclerosis complex (TSC). *PLoS One* **7**, e31900
77. Siroky B.J, Yin H., Babcock J.T., Lu L., Hellmann A.R., Dixon B.P., (2012) Human TSC-associated renal angiomyolipoma cells are hypersensitive to ER stress. *Am. J. Physiol. Renal Physiol.* **303**, F831–844
78. Babcock J.T., Nguyen H.B., He Y., Hendricks J.W., Wek R.C., Quilliam L.A. (2013) Mammalian target of rapamycin complex 1 (mTORC1) enhances bortezomib-induced death in tuberous sclerosis complex (TSC)-null cells by a c-MYC-dependent induction of the unfolded protein response. *J. Biol. Chem.* **288**, 15687–15698