mTOR: a pharmacologic target for autophagy regulation

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mTOR, a serine/threonine kinase, is a master regulator of cellular metabolism. mTOR regulates cell growth and proliferation in response to a wide range of cues, and its signaling pathway is deregulated in many human diseases. mTOR also plays a crucial role in regulating autophagy. This Review provides an overview of the mTOR signaling pathway, the mechanisms of mTOR in autophagy regulation, and the clinical implications of mTOR inhibitors in disease treatment.

Overview of mTOR signaling pathway

Nutrients, growth factors, and cellular energy levels are key determinants of cell growth and proliferation. mTOR, a serine/threonine kinase, is a master regulator of cellular metabolism and promotes cell growth in response to environmental cues. Deregulation of mTOR signaling has been implicated in many human diseases, including diabetes, neurodegenerative diseases, and cancer (1).

mTOR forms two distinct signaling complexes, mTOR complex 1 (mTORC1) and mTORC2, by binding with multiple companion proteins (Figure 1). mLST8, DEPTOR, and the Tti1/Tel2 complex exist in both mTORC1 and mTORC2 (2–5). On the other hand, RAPTOR and PRAS40 are specific to mTORC1 (6–11) whereas RICTOR, mSin1, and PROCTOR1/2 are specific to mTORC2 (10, 12–16). The two kinase complexes have specific substrate preferences and therefore elicit distinct downstream signaling events to modulate cellular function.

One of the well-established roles of mTORC1 is to promote anabolic cellular metabolism to supply the necessary building blocks for cell growth and proliferation. mTORC1 integrates various stimuli and signaling networks to stimulate synthesis of protein, lipid, and nucleotides and block catabolic processes such as autophagy at the post-translational and transcriptional levels (reviewed in refs. 17, 18). The tuberous sclerosis (TSC) tumor suppressor complex (TSC1/TSC2) is arguably the most important upstream negative regulator of mTORC1. Genetic mutations in hamartin or tuberin (encoding TSC1 and TSC2, respectively) cause tumor development in various tissues such as angiofibromas, angiomyolipomas, lymphangioleiomyomatosis, and renal cell carcinoma. Loss-of-function mutations in either TSC1 or TSC2 lead to constitutive mTORC1 activation, which contributes to uncontrolled growth and underlies the TSC disease (19). These findings provide the scientific basis of using mTORC1 inhibitors for the treatment of TSC and related diseases such as cancer.

A well-established upstream regulator of mTORC1 is the growth factor/PI3K/AKT signaling pathway. Growth factors such as insulin and IGF activate their cognate receptors (receptor tyrosine kinases [RTKs]) and subsequently activate the PI3K/AKT signaling axis. Activated AKT directly phosphorylates and thereby inhibits TSC1/2, a GTPase-activating protein (GAP) for the Ras homolog enriched in brain (Rheb) GTPase (19–23). The AKT-dependent phosphorylation results in dissociation of TSC1/2 from lysosome, where Rheb is localized, promoting Rheb activation (24). Since GTP-bound Rheb is a potent mTORC1 activator, inhibition of TSC1/2 by AKT-dependent phosphorylation results in mTORC1 activation (25, 26). Additionally, AKT directly phosphorylates and inhibits PRAS40, an mTORC1 component that negatively regulates the complex's kinase activity, leading to mTORC1 activation (8–11). Furthermore, the activated RTK also stimulates the Ras/Erk/p90 ribosomal S6 kinase 1 (RSK1) signaling axis, which directly phosphorylates TSC2 to inactivate its GAP activity (27, 28). In contrast, cellular stressors such as low cellular energy levels or hypoxia activate TSC1/2 to inhibit mTORC1 activation. AMPK is a sensor of cellular energy levels and is activated by a high AMP/ATP ratio. AMPK phosphorylates TSC2 and presumably increases the TSC1/2 GAP activity (29). Moreover, AMPK directly phosphorylates RAPTOR, resulting in decreased mTORC1 activity through allosteric inhibition (30). These studies show a critical role of AMPK in linking cellular energy level to mTORC1 regulation. Low cellular oxygen levels also inhibit mTORC1 by upregulating DNA damage response 1 (REDD1), which may modulate TSC2 activity to inhibit mTORC1 (31, 32). Current studies reveal the TSC1/2 tumor suppressor as a key signaling hub receiving a diverse array of signals to control mTORC1 activity and thus cell growth.

Amino acids, which are essential components for protein synthesis, are also crucial regulators of mTORC1 (reviewed in refs. 33, 34). A genetic screen using a shRNA library targeting small GTPases and biochemical analysis using immunoaffinity-purified mTORC1-interacting proteins independently identified Rag proteins (RagA/B/C/D), a family of Ras-like small GTPases, as key mediators of amino acid–induced mTORC1 activation (35, 36). RagA and RagB have high amino acid homology and are functionally redundant, and RagC and RagD are homologous and functionally redundant when they are expressed in the same cell. RagA (or RagB) forms a heterodimer with RagC (or RagD) (37),
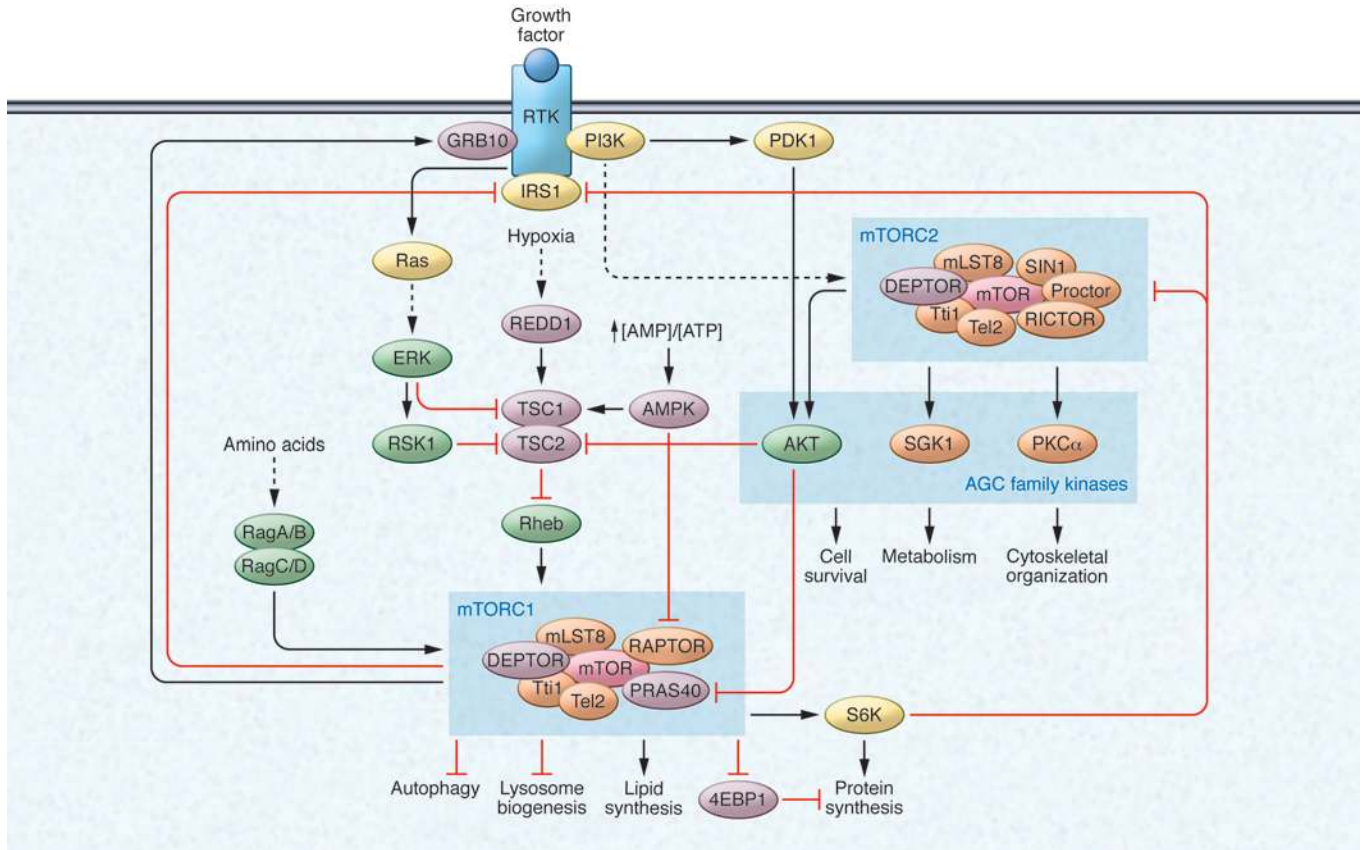
Figure 1. The mTOR signaling network. mTOR forms two distinct signaling complexes, mTORC1 and mTORC2. mTORC1 integrates nutrient and growth factor signaling to promote anabolic metabolism, such as protein synthesis and lipid synthesis, and to inhibit catabolic pathways, such as lysosome bio-genesis and autophagy. Growth factors activate RTKs and the downstream signaling cascades PI3K/AKT and Ras/Erk, resulting in inhibition of the TSC complex, which functions as a GAP for the mTORC1 upstream effector Rheb GTPase. In contrast, cellular stressors, such as low energy levels and hypoxia, activate the TSC complex via AMPK and REDD1. AMPK also directly inhibits mTORC1 activity by phosphorylating RAPTOR. In the presence of amino acids, Rag GTPases recruit mTORC1 to the lysosomal surface, where it can be activated by Rheb GTPase. Growth factor/RTK/PI3K signaling also activates mTORC2, which regulates cell survival, metabolism, and cytoskeletal organization via AGC family kinases. mTORC1 activation exerts feedback inhibition on RTK/PI3K/AKT signaling via the inhibition of IRS and activation of GRB10. S6K, an mTORC1 downstream effector, also inhibits IRS and mTORC2 via inhibitory phosphorylation.

and this dimer is tethered to the lysosome by the lysosomal protein complex Ragulator (38). In the presence of amino acids, the Rag GTPases are activated and recruit mTORC1 to the surface of the lysosome, where the kinase complex encounters its upstream effector Rheb, which activates the kinase complex through an undefined mechanism (38). Of note, only the GTP-bound form of Rheb is capable of activating mTORC1. Rag GTPases bind to RAPTOR when RagA/B and RagC/D are bound to GTP and GDP, respectively. Amino acids modulate Rag guanine nucleotide bind-ing, therefore controlling the interaction between Rag GTPases and mTORC1. In addition, it has been shown that the insulin/ AKT signaling axis regulates lysosomal localization of TSC1/2, demonstrating that the lysosome is the signaling hub of mTORC1 activation (24). Recent studies suggest that other subcellular com-partments such as stress granule and peroxisome also play impor-tant roles in mTORC1 signaling regulation in response to oxida-tive stress (39, 40), although the interplay of those subcellular compartments with lysosome in mTORC1 activation is not fully elucidated. Moreover, despite recent findings of a guanine nucle-otide exchange factor (GEF) and GAPs for the Rag GTPases and

the requirement of vacuolar H^+-ATPase (v-ATPase, a proton pump for lysosomal acidification) in mTORC1 activation (41–45), the signaling mechanism from amino acids to a Rag GEF and/or GAP is still unknown. A recent knockout mouse study confirms a role of Rag GTPases in mTORC1 activation during embryonic devel-opment (46). However, another study using conditional knockout mice shows that loss of RagA and RagB in cardiomyocytes causes lysosomal dysfunction due to deregulated lysosomal v-ATPase localization even though mTORC1 activation is not substantially affected (47). Further studies are needed to determine whether there is a Rag GTPase-independent mechanism in amino acid–induced mTORC1 activation.

The molecular mechanism of mTORC2 regulation by upstream effectors is largely unknown. The only known upstream activator is the growth factor/PI3K signaling axis. One study pro-poses that PI3K signaling activates mTORC2 by promoting the association of the kinase complex with ribosomes (48). Again, the mechanism of how ribosomal association activates mTORC2 has yet to be revealed. Moreover, this model needs to be verified in other systems. A subset of AGC family kinases (PKA, PKG, and

Table 1. mTOR inhibitors and examples of their use for autophagy induction

Drug	Type	Regimen[A]	Model system	Reference
Rapamycin	Allosteric	10~100 nM for 72 hr	Glioma cell lines	94
		200 nM for 24 hr	HD model cell line	95
		1 μM in food	HD model fly[B]	96
		1~10 nM for 24~48 hr	Mouse islet cells	98
		0.2 mg/kg BW i.p. daily	GFP-LC3 mouse	98
		2 mg/kg BW i.p. 3 times/wk	Mouse nervous system	99
CCI-779	Rapalog	20 mg/kg BW i.p. 3 times/wk	HD model mouse[B]	96
RAD001	Rapalog	20~200 nM for 48~72 hr	Cancer cell lines	107
Torin 1	ATP competitive	250 nM for 1~6 hr	Cell lines	106
KU-0063794	ATP competitive	3 μM for 18 hr	Cancer cell lines	107
WYE-354	ATP competitive	3 μM for 18 hr	Cancer cell lines	107
AZD8055	ATP competitive	1~3 μM for 48~72 hr	Cancer cell lines	108
Metformin	Biguanide	0.5~2 μM for 1 hr	p53^{+/+} cell lines	114
		250 mg/kg BW i.p. daily	Xenograft	114
		2~4 mg/kg BW i.p. daily	Xenograft (lymphoma)	115
		5~10 mM for 72 hr	Melanoma cell line	116
		2 mg/day i.p. daily	Xenograft	116
		200 mg/kg BW daily in drinking water	Diabetic mice	117
		10 mM for 48 hr	Esophageal squamous cancer cells	118

[A]The experimental conditions used for autophagy induction in the indicated references. [B]Ameliorated disease phenotypes observed.

PKC) has been shown to be phosphorylated by mTORC2, thereby regulating cell survival, metabolism, and cytoskeletal organization (reviewed in ref. 49). mTORC2 phosphorylates both the turn motif and hydrophobic motif of the AGC family kinases, thereby leading to stabilization and activation of these kinases, respectively. Of note, the hydrophobic motif of AKT (Ser473) is a well-characterized substrate of mTORC2, and its phosphorylation is critical for maximal activity of AKT (50). Therefore, AKT mediates positive crosstalk from mTORC2 to mTORC1 because AKT is an upstream stimulator of mTORC1 (as discussed above).

There are multiple negative feedback circuits from mTORC1 to mTORC2 via downregulation of the RTK/PI3K signaling pathway. Ribosomal protein S6 kinase 1 (S6K1), a downstream effector of mTORC1, negatively regulates the insulin receptor substrate 1 (IRS1), which is a positive mediator of RTK/PI3K signaling (51, 52). It has been also shown that S6K1 phosphorylates RICTOR, thereby affecting mTORC2 activity (53). Furthermore, mTORC1 directly inhibits RTK/PI3K signaling by phosphorylating IRS1 and growth factor–bound protein 10 (GRB10) (54–56). Therefore, the kinetics of mTORC1 and mTORC2 signaling are finely tuned to respond to dynamic changes in cellular metabolism and environmental cues.

Regulation of autophagy by mTOR

Autophagy is the major cellular digestion process that removes damaged macromolecules and organelles. In addition, autophagy is critical to providing energy and molecular building blocks by recycling macromolecules in response to nutrient and environmental stress (reviewed in ref. 57). The discovery by electron microscopy of vesicle structures containing amorphous materials and cytoplasmic organelles in the kidneys of newborn mice led to the introduction of autophagy in the late 1950s (58, 59). About 20 years after that initial discovery, it was observed that amino acid deprivation is a potent autophagy inducer in cultured mammalian cells and in perfused rat livers (60, 61). As previously mentioned, amino acids are key regulators of mTORC1 activation. Furthermore, most if not all autophagy induction conditions such as nutrient or growth factor deprivation and low cellular energy levels have been shown to inhibit mTORC1 activity. This suggests a tight, inverse coupling of autophagy induction and mTORC1 activation.

Autophagy induction by genetic or pharmacologic inhibition of mTORC1 (TORC1 in yeast) was first demonstrated in yeast (62) and later in Drosophila (63). However, the mechanistic understanding of how mTORC1 regulates autophagy in mammalian cells is fairly recent (Figure 2 and reviewed in refs. 64, 65). Three groups independently demonstrated that mTORC1 inhibits the autophagy-initiating UNC-5 like autophagy activating kinase (ULK) complex by phosphorylating complex components including autophagy related gene 13 (ATG13) and ULK1/2 (66–68). Inhibition of mTORC1 results in increased ULK1/2 kinase activity. ULK1/2 then phosphorylates ATG13 and FIP200, which are critical subunits of the ULK1/2 kinase complex (66–68). In yeast, TORC1 inhibits the autophagy-initiating kinase ATG1 (the mammalian ULK1/2 homolog) by phosphorylating ATG13 and disrupting the ATG1 and ATG13 interaction (69). In mammalian cells, mTORC1 phosphorylates Ser758 (Ser757 in mouse) of ULK1, preventing the interaction and phosphorylation of ULK1 by AMPK, which is essential for ULK1 activation (70). Thus, the initiation of autophagy by ULK is reciprocally regulated by mTORC1 and AMPK in response to dynamic changes in cellular nutrients and energy levels. In addition, another layer of ULK1 regulation by mTORC1 has been suggested in which mTORC1 inhibits ULK1 stability by inhibitory phosphorylation of autophagy/beclin 1 regulator 1 (AMBRA1) (71). Furthermore, AMPK and mTORC1
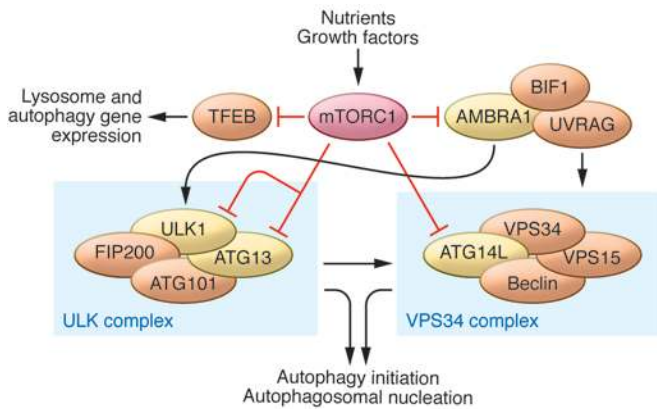
Figure 2. Regulation of autophagy by mTORC1. Activation of mTORC1 by nutrients and growth factors leads to inhibition of autophagy through the phosphorylation of multiple autophagy-related proteins, such as ULK1, ATG13, AMBRA1, and ATG14L, which promote autophagy initiation and autophagosome nucleation. mTORC1 also phosphorylates and prevents nuclear localization of the transcription factor TFEB, a master regulator of lysosomal and autophagy gene expression. Proper lysosome function is essential for autophagy completion.

also regulate the VPS34 complex, a class III PI3K whose activity is crucial for autophagosome formation. VPS34 forms multiple complexes and has critical roles in cellular vesicle trafficking and autophagy induction. The ATG14L-associated VPS34 complex is specifically involved in autophagy regulation. In response to nutrient stress, AMPK activates the proautophagy VPS34 complex by phosphorylating Beclin 1, whereas it simultaneously inhibits the nonautophagy VPS34 complex via phosphorylation of Thr163/Ser165 in VPS34 (72). In contrast, mTORC1 phosphorylates ATG14L in the VPS34 complex and inhibits the lipid kinase activity of VPS34, providing another mTORC1-mediated mechanism in autophagy inhibition (73).

mTORC1 also regulates autophagy at the transcriptional level by modulating localization of transcription factor EB (TFEB), a master transcriptional regulator of lysosomal and autophagy genes (reviewed in ref. 74). The transcriptional activity of TFEB is regulated by nutrient and phosphorylation-dependent cytoplasm-to-nucleus shuttling (75). Although other kinases may also phosphorylate TFEB, it has been shown that mTORC1 directly phosphorylates TFEB at Ser142 and Ser211, and these phosphorylation events result in cytoplasmic sequestration of TFEB (76, 77). As a key signal transducer of amino acids, the Rag GTPases can bind and sequester TFEB in the lysosome, thereby inhibiting TFEB activity (78). Thus, TFEB is constitutively activated regardless of nutrient availability in RagA and RagB deficient cells (47). In conclusion, mTORC1 coordinates both anabolism and catabolism to meet the needs of cell growth. In growing cells, high mTORC1 activity promotes biomolecule synthesis and simultaneously inhibits autophagy. mTORC1 tightly regulates autophagy by suppressing autophagy induction via phosphorylation-dependent inhibition of ULK1/2 and the VPS34 complex and by preventing global expression of lysosomal and autophagy genes through TFEB phosphorylation.

Autophagy flux denotes the sum total of autophagic molecular events, from the induction of autophagy and autophagosome formation to the autolysosomal degradation and reformation of lysosome. Interestingly, although mTORC1 is inactivated during autophagy initiation, the kinase complex is reactivated by energy supplies generated by the degradation of autolysosomal products at the end of autophagy flux. Its reactivation is required for the reformation of functional lysosomes, indicating the critical role of mTORC1 in the completion of autophagy flux (79).

As discussed above, it is not fully understood how mTORC2 activity is regulated. However, it was proposed that insulin/PI3K signaling activates mTORC2 by promoting its interaction with ribosome, and subsequently mTORC2 phosphorylates AKT at the turn motif site, Thr450, during translation (48, 80). In addition, mTORC2 phosphorylates AKT at the hydrophobic motif site, Ser473, which can lead to the activation of the AKT/mTORC1 signaling axis. Therefore, mTORC2 may indirectly suppress autophagy by activating mTORC1. Further studies are needed to determine whether mTORC2 can directly regulate autophagy.

Pharmacologic regulation of mTOR and autophagy

Autophagy is a cellular process essential for development and tissue homeostasis. Autophagy is implicated in various physiologic and pathologic processes (including exercise, metabolic adaptation, and disorders such as neurodegenerative diseases, infectious diseases, cardiovascular diseases, cancer, and aging), and thus pharmacologic modulation of autophagy is of great interest (reviewed in refs. 81, 82). As a master regulator of cellular metabolism and autophagy, mTORC1 is an appealing pharmacologic target to manipulate autophagy. In fact, deregulation of mTORC1 has been implicated in diseases that are associated with autophagy defects (1), and there are mTOR inhibitors already in clinical trials or approved for treatment of these diseases (reviewed in refs. 83–86). There are also pharmacologic molecules that can induce or inhibit autophagy via mTOR-independent mechanisms (81). For example, agents such as bafilomycin A1 and hydroxychloroquine that increase lysosomal pH can block autophagy flux by inhibiting autolysosomal formation. Such inhibitors could be combined with mTOR inhibitors to finely modulate autophagy flux. In this section we discuss currently available mTOR inhibitors and their effects on autophagy. We also summarize some mTOR inhibitors and their use for autophagy induction in preclinical studies (Table 1).

Rapamycin and rapalogs. Rapamycin was originally isolated from the soil bacterium *Streptomyces hygroscopicus* as an antifungal compound in 1975, and was later shown to be a strong immunosuppressant with broad anti-proliferative effects in mammalian cells (reviewed in ref. 84). About 16 years after the isolation of rapamycin, an elegant yeast genetic screen to identify rapamycin-resistant genes led to the discovery of TOR1 and TOR2 (87). mTOR was identified in the mid-1990s through biochemical isola-

tion using a rapamycin-FKBP12 complex and by yeast two-hybrid analysis (88–91). Rapamycin forms a complex with FKB12 in the cell, and this complex specifically binds to mTORC1 and allosterically inhibits its kinase activity. Although the rapamycin-FKB12 complex does not bind directly to mTORC2, it has been reported that long-term incubation with rapamycin can decrease mTORC2 activity (reviewed in ref. 92). Because of its anti-proliferation effects, rapamycin has been thoroughly evaluated as a therapeutic drug for cancer. In addition, rapamycin has been used as an immunosuppressant for organ transplantation and a cell growth inhibitor for preventing restenosis. In order to improve the pharmacokinetics of rapamycin, several derivative compounds (RAD001, CCI-779, and AP23573, collectively called rapalogs) have been developed. These rapalogs have a similar ability to inhibit mTORC1 with fewer immunosuppressive effects (93).

Induction of autophagy by rapamycin or rapalogs has been tested in various model systems. Treatment of rapamycin strongly induces autophagy in yeast even in the presence of nutrients (62); however, the effectiveness of rapamycin on inducing autophagy in mammalian cells is dependent on the cell type. Thus, in a panel of glioma cell lines, rapamycin effectively induces autophagy in U87-MG and T98G cells but is not sufficient to induce autophagy in U373-MG cells, although rapamycin in combination with a PI3K or AKT inhibitor sensitizes the cells to autophagy induction (94). In contrast, rapamycin or CCI-779 treatment reduces protein aggregates through autophagy induction, thereby ameliorating symptoms of Huntington's disease (HD) in both an in vitro cell culture model and in vivo fly and mouse models (95, 96). In addition, there are reports suggesting that rapamycin-induced autophagy may sensitize cancer cells to radiotherapy. Treatment of *PTEN*-null prostate cancer cells with the rapamycin derivative RAD001 (also called everolimus) induces autophagy and sensitizes the cells to radiotherapy. Moreover, prolonging autophagy with rapamycin causes radioresistant cancer cells to enter senescence and inhibits the growth of cancer cells in a xenograft model (97). Furthermore, through the use of in vivo reporter systems, rapamycin treatment has been shown to induce autophagy in both the nervous system and pancreatic β cells of mice (98, 99). Although further studies are necessary to determine whether the beneficial effects seen in these model systems can be attributed to increased autophagy, these studies demonstrate that rapamycin or rapalogs can induce autophagy in vitro and in vivo. Of note, a study using a mouse model of TSC showed that autophagy is prosurvival for TSC tumorigenesis, suggesting that rapamycin or rapalog treatment is not effective, but autophagy inhibition is beneficial for the treatment of TSC (100). Additionally, the rapalog RAD001 has been approved by FDA for the treatment of certain cancers such as subependymal giant cell astrocytoma, advanced hormone receptor–positive and HER2-negative breast cancer, progressive neuroendocrine tumors of pancreatic origin, and renal cell carcinoma (101).

ATP-competitive mTOR inhibitors (mTOR kinase inhibitors). Despite the high expectations for rapamycin and rapalogs as anticancer agents, the outcomes from clinical trials have not been uniformly positive except for certain types of cancers such as renal cell carcinoma and TSC-associated angiomyolipoma (83, 84). This is possibly due in part to the loss of the negative feedback circuit from mTORC1 to the PI3K/AKT/mTORC2 signaling axis, which

mediates cell survival. Thus, mTORC1 inhibition by rapamycin treatment may lead to activation of PI3K/AKT/mTORC2 signaling in cancer cells. Intriguingly, one report suggests that mTORC2 activity is essential for development and survival of prostate cancer cells, but not for normal prostate epithelium (102). Furthermore, rapamycin has differential effects on S6K and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), two major mTORC1 substrates that regulate protein synthesis (103). Additionally, a recent study showed that the sequence composition of substrates determines the substrate quality of mTORC1, thereby dictating the substrate's sensitivity to rapamycin (104). Phosphorylation of poor mTORC1 substrates is more sensitive to inhibition by rapamycin than is phosphorylation of good mTORC1 substrates. Interestingly, the autophagy-related mTORC1 substrates TFEB Ser142 and ULK1 Ser758 are less sensitive to rapamycin than the mTORC1 substrate S6K (104), which highlights the need for other types of mTORC1 inhibitors to efficiently regulate autophagy. For these reasons, several groups have independently developed mTOR inhibitors that directly target the kinase activity of both mTORC1 and mTORC2 (83–85, 105). These inhibitors (listed in Table 1) are ATP analogs, collectively called ATP-competitive mTOR inhibitors or mTOR kinase inhibitors (mTOR-KIs). Some of these inhibitors have dual inhibitory effects on mTOR and PI3K due to the similarity of their kinase domain structures (105).

As anticipated, treatment with mTOR-KIs induces autophagy and cytotoxicity in various cell types. The mTOR-KI torin 1 blocks the phosphorylation of all mTORC1 substrates more efficiently than does rapamycin, and as a result torin 1 elicits stronger autophagy induction in both mouse and human cell lines (106). Consistently, the mTOR-KIs Ku-0063794 and WYE-354 induce autophagy in rapamycin-resistant cancer cells, and a subefficacious dose of mTOR-KIs in combination with rapamycin can synergistically inhibit mTORC1 to induce autophagy (107). Additionally, another mTOR-KI, AZD8055, potently induces autophagy and inhibits proliferation in a broad range of cancer cell lines (108). Interestingly, it has been shown that AZD8055 treatment can be cytoprotective via autophagy induction in a cytotoxic chemotherapy setting (109). Some of these mTOR-KIs are undergoing or recently completed phase I or II clinical trials as anticancer agents (ClinicalTrials.gov identifiers NCT01316809, NCT00973076, NCT00999882, NCT00731263, NCT01545947, and NCT01177397.

Metformin. Metformin is a biguanide that is widely used for the treatment of type II diabetes. Metformin activates AMPK indirectly by inhibiting the mitochondrial respiratory chain complex I, thus increasing the cellular AMP/ATP ratio (110). As discussed above, AMPK is an upstream negative regulator of mTORC1. Thus, metformin treatment inhibits mTORC1-mediated protein synthesis in breast cancer cells (111). It has also been shown that metformin can inhibit mTORC1 in an AMPK-independent manner, possibly via Rag GTPases or REDD1 (112, 113). Therefore, since active AMPK promotes autophagy by phosphorylating key autophagy regulatory proteins such as ULK1, VPS34, and Beclin 1 (70, 72), metformin can induce autophagy by simultaneously activating AMPK and inhibiting mTORC1. Consequently, metformin-induced autophagy and its beneficial effects have been demonstrated in various cancer cells in vitro as well as in in vivo models (114–118).

Conclusion

mTOR promotes anabolic metabolism and inhibits autophagy induction. Therefore, the regulation of autophagy with mTOR inhibitors provides a new therapeutic strategy for a variety of diseases, including neurodegenerative diseases, diabetes, and cancer. Most available mTOR inhibitors that have been rigorously tested for clinical uses are rapamycin derivatives, and the majority of these tests have been focused on their anti-proliferation effects for cancer treatment (84). These compounds must be further evaluated in autophagy-related diseases such as neurodegeneration and cardiac myopathy, which are often associated with lysosomal and autophagy defects (82). One critical factor that must be considered is the potential side effects of mTOR inhibitors. mTOR-KIs are cytotoxic, likely due to the inhibition of mTORC2, whereas rapamycin is generally cytostatic with less toxicity (83). It might be advantageous to use mTOR-KIs for cancer treatment but not for chronic diseases such as neurodegeneration. Therefore, in the treatment of neurodegenerative or metabolic diseases, rapalogs are probably more desirable, as they have fewer side effects. Both rapalogs and mTOR-KIs have immunosuppressive effects that could also limit their potential application. Further pharmacokinetic studies are needed to determine the effective doses of mTOR inhibitors for inducing autophagy with minimal side effects.

Autophagy induction could be beneficial or detrimental depending on the type or stage of disease (82). For example, autophagy may promote survival during tumor initiation and development by providing nutrients to tumor cells when nutrients are limited. Thus inhibition of autophagy may sensitize cancer cells to metabolic stress conditions, leading to cell death. Autophagy can have growth-suppressive functions, and defects in autophagy may enhance genomic instability and promote cancer development. Given the potential dual functions of autophagy in tumor suppression and promotion, more studies are needed to elucidate the precise function of autophagy in individual cancer types before a therapeutic approach can be considered. In-depth discussions regarding the role of autophagy in tumor development and its implications in tumor therapy may be found in other articles in this Review series (119–121). For degenerative diseases, a mild induction of autophagy should protect cells from damaged proteins and organelles; thus, partial mTORC1 inhibition rather than a complete inhibition may be a possible therapeutic strategy.

Acknowledgments

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