Targ Oncol (2011) 6:17–27
DOI 10.1007/s11523-011-0167-8

REVIEW

Mechanisms of mTOR inhibitor resistance in cancer therapy

Jennifer S. Carew · Kevin R. Kelly · Steffan T. Nawrocki

Received: 20 January 2011 / Accepted: 22 February 2011 / Published online: 9 March 2011

Abstract Mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase that regulates cell cycle progression, protein translation, metabolism, and cellular proliferation. The mTOR pathway promotes cell proliferation under energy or nutrient-rich conditions by increasing ribosomal biogenesis and protein synthesis. Since enhanced activity of the mTOR pathway is frequently observed in malignant cells, inhibition of this kinase has become an attractive strategy to treat cancer. Rapamycin and its analogs temsirolimus, everolimus, and ridaforolimus referred to as "rapalogs" have demonstrated promising efficacy against renal cell carcinoma and are under investigation for the treatment of other malignancies. However, the emergence of drug resistance may ultimately limit the utility of rapalog therapy. Here we summarize the known mechanisms of resistance to mTOR-inhibitor therapy and describe potential strategies to overcome these for the current agents that target this pathway.

Keywords mTOR Inhibitor · Rapamycin · Drug resistance · Apoptosis · Autophagy · PIM kinase

This work was supported by funding from The Institute for Drug Development, Cancer Therapy and Research Center at The University of Texas Health Science Center at San Antonio

J. S. Carew · K. R. Kelly · S. T. Nawrocki (✉)
Department of Medicine and Institute for Drug Development, Cancer Therapy and Research Center at The University of Texas Health Science Center,
14960 Omicron Drive,
San Antonio, TX 78245, USA
e-mail: Nawrocki@uthscsa.edu

Introduction

The serine/threonine kinase mammalian target of rapamycin (mTOR) is a critical regulator of cell proliferation, metabolism, and protein synthesis. mTOR is ubiquitously expressed and is activated downstream of the phosphoinositide 3-kinase (PI3K) pathway. It functions as a sensor of nutritional/metabolic stress during cell development and promotes protein synthesis and cell growth during nutrient or energy rich periods. Inhibition of mTOR signaling can abrogate the cellular response to growth factor receptor activation. Consistent with its essential role in cell growth, aberrant activity of the mTOR pathway is frequently observed in many types of cancer. Therefore, targeting mTOR activation is an attractive approach for cancer therapy.

Recent work has determined that mTOR exists in two multi-protein complexes, mTORC1 and mTORC2, which differ in their binding partners and their sensitivity to rapamycin [1] (Fig. 1). mTORC1 forms a complex with raptor (regulatory-associated protein of mTOR), GβL (mLST8), the proline-rich AKT substrate 40 kDa (PRAS40), disheveled, egl-10, and pleckstrin (DEP)-domain containing mTOR-interacting protein (deptor) [2–6]. Raptor appears to be critical for mTORC1 function as it acts as a scaffold to recruit mTORC1 targets [2, 3]. mTORC1 activity is inhibited by rapamycin (sirolimus) and associated analogs (temsirolimus/CCI-779, everolimus/RAD001, and ridaforolimus/AP23573), which are collectively termed rapalogs. These agents suppress mTORC1 activity through their association with FK506 binding protein 12 (FKBP-12) [7]. However, mTORC2 is considered to be largely insensitive to rapalogs, although prolonged treatment may be able to reduce mTORC2 activity in some cell types [8–10].

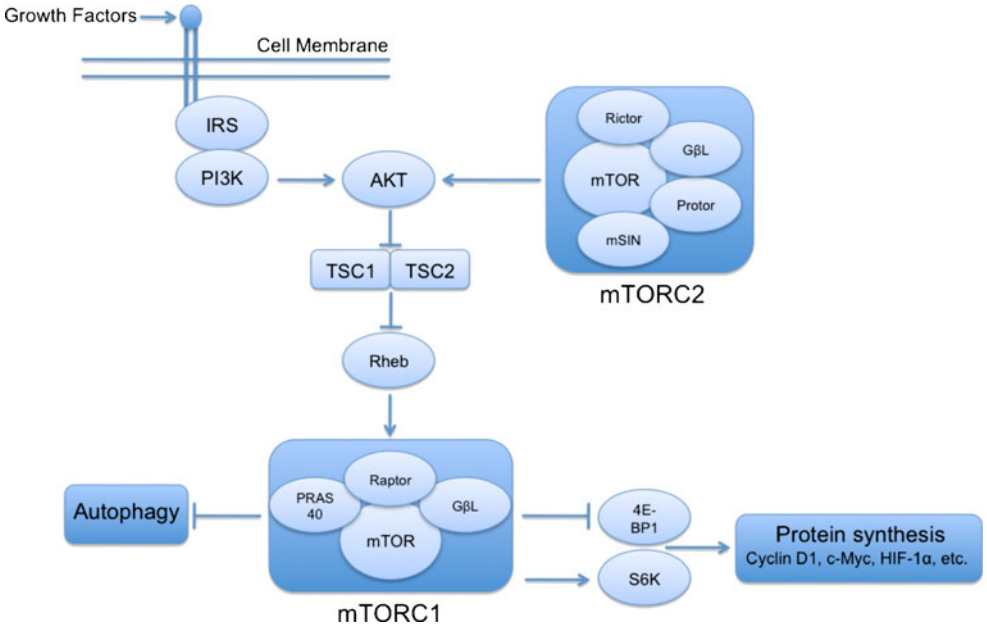

Fig. 1 The mTOR signaling pathway. The functional mTOR signaling complex exists in two forms: mTORC1 and mTORC2. Growth factor stimulation signals through PI3K to activate AKT leading to mTORC1 activation. mTORC2 may also activate AKT via phosphorylation of Ser473. Two major substrates of mTORC1 are 4E-BP1 and S6K, whose phosphorylation promotes the translation of key cell cycle regulators and transcription factors. Since mTORC1 activity is also controlled by the cellular environment (presence of nutrients and energy), it is also a critical regulator of autophagy

The mTORC1 pathway can be stimulated by growth factor receptor signaling through the tuberous sclerosis complex (TSC1/2) with Rheb or under conditions of metabolic stress [11]. Downstream of mTORC1 are the S6 kinases (S6K1 and S6K2) and 4E-BP1, which modulate cap-dependent translation [12]. 4E-BP1 binds to eIF-4E and blocks the formation of a functional eIF-4F complex leading to translational inhibition. S6K1 also regulates protein synthesis initiation by phosphorylating the S6 protein of the 40S ribosomal subunit and by inducing eIF4A helicase activity [13, 14]. Therefore, rapalogs decrease mTOR-associated protein synthesis of key proteins involved in cell cycle progression, such as cyclin D1, c-MYC, and hypoxia inducible factor 1α (HIF-1α) [15]. In agreement with the mTOR pathway playing a critical role in cell proliferation and survival, inhibition of mTOR signaling blocks tumor cell proliferation, disrupts angiogenesis, and induces apoptosis and autophagy [16–18].

The frequent hyperactivation of the mTOR pathway in cancer provided a rationale for the clinical evaluation of rapalogs as potential anticancer agents. The US Food and Drug Administration (FDA) approved temsirolimus and everolimus in 2007 and 2009, respectively, for the treatment of patients with metastatic renal cell carcinoma (RCC) [19, 20]. Ridaforolimus is currently being evaluated in a phase III trial in patients with metastatic soft-tissue and bone sarcomas. Numerous other clinical trials are underway to investigate the safety and efficacy of mTOR inhibitors in additional tumor types.

Despite the promising anticancer activity of mTOR inhibitors observed in preclinical and clinical studies, the molecular basis of sensitivity and resistance to these agents remains largely unknown and drug resistance is an emerging problem. This review focuses on potential mechanisms of mTOR inhibitor resistance and outlines strategies to improve mTOR inhibitor-based therapy.

Molecular determinants of mTOR inhibitor sensitivity

Although mTOR inhibitors have activity against a number of cancer types, only a portion of patients treated with these agents exhibit substantial clinical benefit. Considering this, it is imperative to identify which patients may benefit most from mTOR inhibitor therapy in order to optimize their utility and improve clinical outcomes. Several associations have been made between the molecular characteristics of tumors and sensitivity to mTOR inhibitors (Table 1). While sensitive tumors may be responsive to low doses of rapalogs, higher doses or rapalogs given concurrently with other targeted therapeutic agents may be required to achieve activity in the majority of patients due to the presence of redundant signaling pathways that abrogate rapalog efficacy. Future studies are needed to establish detailed molecular profiles that predict sensitivity to mTOR inhibitor therapy.

Constitutive PI3K activity

Early studies suggested that dysregulated PI3K signaling induced by loss of phosphatase and tension homologue deleted on chromosome ten (PTEN) function may predict the anticancer efficacy of mTOR inhibition [21, 22]. PTEN functions as a major negative regulator of the PI3K/AKT pathway. Accordingly, loss of PTEN or activation of AKT increased the sensitivity of cancer cells to mTOR inhibitor treatment in preclinical models [21]. However, clinical

Table 1 Molecular characteristics of mTOR inhibitors

Sensitive tumors	Resistant tumors
• PTEN deficient	• Redundant signaling pathways
• Constitutive P13K/AKT/mTOR activity	• Activation of feedback loops
• VHL deficient	• Loss of PP2A regulatory subunit B22β
• Cyclin D1 overexpressed	• Presence of oncogenic *KRAS* or *BRAF* mutation
• Functional apoptotic pathways	• Non-functional apoptotic pathways
	• Low p27 levels
	• Decreased expression of 4E-BP1
	• Overexpression of elF4E

studies in tumor types with frequent PTEN mutation such as melanoma and glioblastoma have not demonstrated significant responses to mTOR inhibitor treatment, suggesting that patient selection on the basis of PTEN status alone will not define an mTOR inhibitor-sensitive population [23, 24]. A possible explanation is that activation of the RAS pathway can bypass mTORC1 inhibition. A recent study found that cancer cells harboring oncogenic phosphoinositide-3-kinase catalytic α polypeptide (PIK3CA) mutations or loss of PTEN function were consistently sensitive to mTOR inhibitor treatment, except when KRAS or BRAF mutations were also present [25]. Given the prevalence of RAS pathway activation in cancers, this observation may explain, in part, why all tumors with constitutive PI3K activity do not respond to mTOR inhibitors.

Hypoxia inducible factor 1α (HIF-1α) activity

The mTOR inhibitors temsirolimus and everolimus are approved for use in advanced RCC. The majority of clear cell RCCs are characterized by a loss of function of the von Hippel-Lindau (VHL) tumor suppressor gene [26]. VHL is an E3 ubiquitin ligase that promotes the proteasomal degradation of HIF-1α and HIF-2α [27]. Abnormal stabilization of these transcription factors promotes the transcription of pro-angiogeneic cytokines and glycolytic enzymes. Since mTOR inhibitors decrease HIF-1α levels, tumors that are dependent upon its activity may be hypersensitive to rapalog therapy [17, 28]. The reported efficacy of rapalogs in patients with RCC is in agreement with this hypothesis.

Cyclin D1 expression

mTOR inhibitors have recently demonstrated therapeutic activity against mantle cell lymphoma (MCL) [29]. This cancer type is characterized by a chromosomal t:11–14 translocation that induces overexpression of cyclin D1, a phenomenon that drives the pathogenesis of this disease. The correlation between inhibition of mTOR activity and a dramatic reduction in cyclin D1 expression may explain the efficacy of mTOR inhibitors in MCL and provides a rationale for their use in MCL therapy. However, it is possible that mTOR inhibitors possess other mechanisms of action that account for the activity of this drug class against this disease.

Mechanisms of resistance to mTOR inhibitors

Mutations in FKBP-12 or mTOR

A number of potential mechanisms that may lead to resistance to mTOR inhibitors have been proposed (Fig. 2). Rapalogs bind to FKBP-12 and thus mutations in FKBP-12 or the FKB domain of mTOR could reduce binding affinity and result in rapalog resistance [30–32]. To overcome this potential resistance mechanism, second-generation active site mTOR inhibitors are being developed. These agents termed "TORKinhibs" inhibit the serine/threonine kinase activity of mTOR in an FKBP-12-independent manner and could theoretically circumvent this resistance mechanism [33–35].

PI3K/AKT pathway activation

Inhibition of mTORC1 has been reported to induce AKT activation in numerous cell types [36–39]. In most cell types, rapalogs block mTORC1 activity but do not alter mTORC2 assembly. Thus, it is possible that inhibition of mTORC1 shifts the balance to increased mTORC2 activity, which has been shown to directly phosphorylate AKT at Ser 473 [40]. It is also possible that inhibition of mTORC1 leads to AKT activation via upregulation of receptor tyrosine kinases such as insulin-like growth factor-1 receptor (IGF-R1) [39]. Increases in AKT activation have been shown to be major contributors to diminished rapalog anticancer activity [41]. This data provides a strong rationale for dual targeting of mTORC1 and PI3K activity. Consistent with this idea, several dual inhibitors of PI3K and mTOR activity have been developed including PI-103

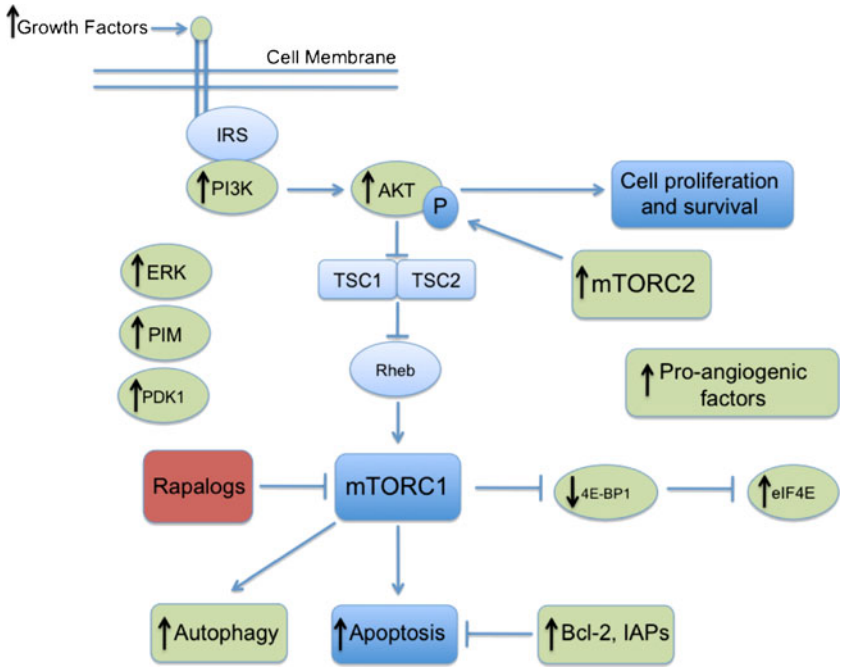

Fig. 2 Mechanisms of rapalog resistance. Many mechanisms of resistance to rapalog therapy have been identified and are labeled in green. Following rapalog treatment, numerous feedback loops have been reported to promote the activation of pro-survival signaling cascades, including PI3K/AKT, ERK, PIM, and PDK1. One mechanism of increased AKT activation is via enhanced phosphorylation of Ser473 by mTORC2. Alterations in protein translation (decreased 4E-BP1 or increased eIF4E) have also been demonstrated to interfere with the effects of rapalogs on protein synthesis. The stimulation of autophagy and increased levels of anti-apoptotic molecules, such as Bcl-2 represent additional mechanisms of resistance. Since mTOR inhibitors induce apoptosis in some tumor types, non-functional apoptotic pathways can also confer resistance to rapalogs

and NVP-BEZ235. These agents have shown greater anticancer efficacy compared with agents that inhibit either mTOR or PI3K pathways alone [42–46]. Another potential strategy to target PI3K/AKT activation induced by mTORC1 inhibition is to target IGF-1/IGF-1R signaling. IGF-1 production has been shown to promote constitutive IGF-1R signaling and high PI3K activity. Similar to blocking PI3K directly, inhibition of IGF-1R also was determined to enhance the efficacy of mTOR inhibitor therapy [39, 47–49].

Previous investigations have established that upregulation of mTORC2 leads to increased activation of AKT [40]. TORKinhibs such as PP242 bind directly to the ATP site of mTOR and therefore inhibit both mTORC1 and mTORC2 [33]. Importantly, these agents suppress 4E-BP1 phosphorylation and block the phosphorylation of the mTORC2 substrate AKT [50]. As expected, TORKinhibs displayed more potent anticancer activity compared to rapalogs in preclinical studies [33, 50].

Increases in ERK/MAPK signaling

In addition to activating the PI3K pathway, treatment with rapalogs has been reported to increase signaling of the ERK/MAPK pathway in both in vitro and in vivo preclinical models and in biopsies from patients with cancer [51]. Functional Ras and MEK1/2 activity were required for the phosphorylation of ERK in these studies. Notably, inhibition of PI3K decreased ERK activation following rapalog treatment. These results suggest that activation of ERK induced by rapalog treatment requires signaling via the PI3K and Ras pathways. Consistent with this hypothesis regarding a feedback loop promoting resistance to rapalogs, administration of the MEK1/2 inhibitor U0126 augmented the anticancer activity of everolimus [51]. However, additional studies are needed to more finely dissect the mechanism(s) by which PI3K signals to the Ras-ERK pathway following mTORC1 inhibition.

Activation of PIM kinases

The PIM kinases are another family of kinases that have been reported to be hyperactivated following rapalog treatment [52]. There are three isoforms of the serine/threonine PIM kinase (PIM-1, PIM-2, and PIM-3) that play a role in cancer growth and progression [53]. PIM kinase substrates with important roles in cancer biology include c-MYC, p27, CDC25A, and BAD. In addition to these targets, recent studies demonstrate that PIM kinases also stimulate mTORC1 activity via phosphorylation of 4E-BP1, eIF4E, and PRAS40 [52, 54, 55]. Consistent with this

observation, two recent reports have established a link between PIM kinase activity and resistance to rapalogs. The potential therapeutic implications of this correlative relationship are highlighted by the fact that combined therapy of rapalogs with PIM kinase inhibitors led to enhanced anticancer activity in preclinical investigations [56, 57].

Functional status of PP2A phosphatases and PDK1 activity

Activation of PDK1 was recently reported to contribute to rapamycin resistance [58]. Loss of *PPP2R2B*, which encodes for the B55β subunit of the serine/threonine protein phosphatase PP2A results in PDK1-dependent phosphorylation of MYC following treatment with rapamycin. PDK1-mediated MYC phosphorylation was independent of PI3K and AKT and promoted resistance to rapamycin [58]. This data is in agreement with other studies showing that alterations in phosphatase activity can stimulate rapamycin resistance [59–61].

Altered expression levels of eIF4E and 4E-BP1

One of the primary downstream substrates of mTOR is 4E-BP1, which suppresses eIF4E activity. Low levels of 4E-BP1 confer resistance to rapamycin as this translates into an inability to inhibit the activity of eIF4E [62]. Similarly, overexpression of eIF4E also results in resistance to rapamycin. eIF4E plays a critical role in translation and its increased expression levels have been associated with tumor progression [63]. Interestingly, cells with high 4E-BP1 expression are hypersensitive to rapamycin, suggesting that dysregulation of translation may be an important determinant for rapamycin sensitivity [62].

Dysregulation of $p27^{Kip1}$ levels

In an earlier study, rapamycin resistance was reported in fibroblasts and T lymphocytes that were deficient in *p27* [64]. p27 is a cyclin-dependent kinase (cdk) inhibitor that is decreased following serum stimulation, which facilitates cell proliferation. Rapamycin treatment prevents p27 downregulation and this effect may contribute to the anti-proliferative properties of this agent [64]. Accordingly, cells with low levels of p27 may be less responsive to rapalog-mediated growth inhibition.

Oxidative stress

A recent study conducted in yeast demonstrated that elevated superoxide levels induced rapamycin resistance [65]. The authors found that increased oxidative stress triggered mTORC1 modification and abrogated its ability to bind to the FKBP-12/rapamycin complex. Intrinsic oxidative stress is a hallmark feature of many cancer types and promotes cell proliferation, pro-survival signaling, genomic instability, and reduced sensitivity to conventional anticancer therapies. This report suggests that high levels of reactive oxygen species may also confer rapalog resistance [66]. Agents that bind directly to mTORC1 such as the TORKinhibs may be able to overcome this resistance mechanism since they inhibit mTOR activity in an FKBP-12-independent manner and would likely not be affected by redox-related changes in binding affinity.

Modulation of apoptotic regulators

The two main pathways of apoptosis induction are the "extrinsic" or death-receptor pathway and the "intrinsic" or mitochondrial pathway. Several studies have demonstrated that rapalogs induce apoptosis via activation of the mitochondrial apoptotic cascade [17, 67–69]. This pathway can be activated by stress stimuli that disrupt the mitochondrial membrane leading to the release of cytochrome c and SMAC (second mitochondria-derived activator of caspases) into the cytosol. The release of cytochrome c results in apoptosome formation and activation of caspase-9 and eventually caspase-3 culminating in apoptosis. The majority of conventional cancer therapeutics cause apoptotic cell death in this manner. The intrinsic susceptibility of cancer cells to apoptosis induction is thought to be regulated in a rheostat fashion by the balance between the levels of various pro- (Bax, Bak, Bim, Bid, etc.) and anti-apoptotic (Bcl-2, Bcl-X_L, Mcl-1, etc.) proteins. Consistent with this hypothesis, a preclinical study conducted in cells with enforced Bcl-2 overexpression demonstrated that rapalog treatment blocked mTOR signaling and suppressed proliferation, but the presence of Bcl-2 inhibited apoptosis and caused partial drug resistance [17].

Another family of proteins with anti-apoptotic functions is the inhibitor of apoptosis (IAP) proteins, including c-IAP1, c-IAP2, XIAP, and survivin. IAPs can block apoptosis via inhibition of caspases or through modulation of pro-survival signaling cascades, such as the nuclear factor kappa B (NF-κB) pathway [70]. Given their functional homology to Bcl-2, overexpression of IAPs may also diminish sensitivity to rapalog-induced apoptosis. Indeed, overexpression of survivin was recently demonstrated to blunt apoptosis stimulated by temsirolimus [28].

Enhanced angiogenesis

Angiogenesis is defined as the generation of new blood vessels from the pre-existing vasculature. This process plays an essential role in cancer progression by promoting tumor growth and metastasis. Accordingly, increased

production of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) have been reported in many types of cancer. One of the major mechanisms proposed to underlie the anticancer activity of mTOR inhibitor therapy is the inhibition of angiogenesis [71]. Rapalogs have been reported to have direct effects on endothelial cells, resulting in blunted tumor angiogenesis [71]. As mentioned earlier, the mTOR pathway also regulates cap-dependent translation and rapalog treatment has been shown to decrease HIF-1α expression [17]. Since HIF-1α controls the transcription of many pro-angiogenic genes, such as VEGF, reduced HIF-1α levels strongly suppresses angiogenesis. The ability of rapalogs to potently disrupt angiogenesis may explain why these agents are effective for the treatment VHL-deficient RCC. Considering this mechanism of action, resistance to rapalogs could emerge in tumors that upregulate pro-angiogenic factor secretion via mTOR-independent pathways or through heightened HIF-1α activity.

Stimulation of autophagy

The role that autophagy plays following chemotherapy remains highly controversial as its induction has been proposed to both mediate and provide protection against cell death [72]. It is likely that autophagy has dual roles that may be variable, cell type specific, and treatment-dependent. Stimulation of autophagy has been reported following treatment with many anticancer agents, including arsenic trioxide, histone deacetylase (HDAC) inhibitors, etoposide, tamoxifen, temozolomide, bortezomib, and imatinib [75–81]. Inhibition of autophagy has been reported to enhance the efficacy of many of these agents [82–86].

mTORC1 is a critical inhibitor of autophagy that is regulated by various upstream signals, including glucose, oxygen, amino acids, and growth factors [73]. Rapalogs are potent inducers of autophagy due to their ability to inhibit mTORC1 activity. It is not clear whether rapalog-induced autophagy contributes to its anticancer activity or is an undesired side effect that may limit drug efficacy. Consistent with this idea, a recent study found that a dual mTORC1 and mTORC2 inhibitor induced autophagy and disruption of this pathway with the autophagy inhibitor chloroquine promoted apoptotic cell death [74]. The authors concluded that induction of autophagy reduced the efficacy an mTORC1/mTORC2 inhibitor and suggested combination studies with autophagy inhibitors to overcome this resistance mechanism. Further investigation of the significance of autophagy induction associated with mTOR inhibition should diminish some of the current controversy.

Overcoming resistance to mTOR inhibitors

The many positive aspects of the anticancer mechanism of action of mTOR inhibitors have prompted extensive efforts aimed to elucidate effective strategies to circumvent drug resistance and to maximize clinical benefit. As discussed earlier, one possible strategy to overcome some of the limitations of rapalogs is to use ATP competitive active site mTOR inhibitors, which have been coined TORKinhibs. In contrast to rapalogs, TORKinhibs inhibit the kinase activity of mTOR directly rather than via FKBP-12. In addition, several reports have demonstrated that TORKinhibs, such as PP242 block both mTORC1 and mTORC2 activity [33, 50, 87, 88]. Thus, it is likely that enhanced anticancer activity can be obtained using TORKinhibs via dual mTORC1/mTORC2 inhibition, which prevents mTORC2-mediated AKT activation. Another approach to block rapalog-induced PI3K/AKT activation is to use dual mTOR and PI3K inhibitors. Several compounds have already been developed including PI-103 and NVP-BEZ235, which have demonstrated superior efficacy compared to rapalogs in various models [36, 42, 43, 89, 90]. Similarly, drug combination strategies to inhibit mTOR and AKT, such as rapalogs in combination with the PI3K/AKT inhibitor perifosine have yielded synergistic anticancer activity [91]. Of these two strategies, it is not clear which one will yield the greater therapeutic benefit. It is likely that dual inhibition of mTOR and PI3K will be more efficacious due to the numerous downstream targets of PI3K. However, it is also possible that these agents may cause greater toxicity compared to the TORKinhibs [50].

In addition to activating the PI3K/AKT pathway, rapalog treatment results in the stimulation of multiple other pro-survival feedback loops, including the MEK/ERK, PIM, and PDK1 pathways. Accordingly, inhibition of each of these pathways has promoted sensitization to rapalog therapy [51, 56, 58]. It is possible that activation of certain feedback loops by rapalog therapy may be cell type-dependent and thus, specific pathway inhibitors may be selected based on tumor response to improve clinical outcome.

mTOR inhibitors have been reported to simultaneously inhibit cell proliferation and induce apoptosis in various cancer cell types [67–69]. Induction of apoptosis by rapalogs may be related to their ability to decrease the expression levels of various anti-apoptotic proteins, including Bcl-2, Bcl-xL, Mcl-1, and survivin [28, 92–95]. However, defects in the apoptotic pathway have been known to cause resistance to a large number of stress stimuli and chemotherapeutic agents, including rapalogs. Consistent with this observation, cells with defective p53 or high levels of the anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-1, or survivin has resulted in rapalog resistance [17,

28, 96, 97]. To overcome this protective mechanism, rapalogs have been given in combination with Bcl-2 inhibitors (ABT-737, ABT-263) or other agents that decrease the expression of anti-apoptotic proteins [96, 98, 99]. For example, treatment with the HDAC inhibitor vorinostat or the multi-tyrosine kinase inhibitor sorafenib has been reported to decrease anti-apoptotic protein expression levels and sensitize cancer cells to rapalog-mediated apoptosis [28, 100]. Considering that most chemotherapeutic agents kill cancer cells by inducing apoptosis, modulation of this cell death pathway is likely to be an effective approach to improve the efficacy of many cancer therapeutics.

The mTOR pathway promotes VEGF production via upregulation of HIF-1α expression. Accordingly, mTOR inhibition has displayed potent anti-angiogenic activity in multiple cancer types [71, 101–105]. Inhibition of angiogenesis may be a major contributor to rapalog activity given that many cancer cell types that are resistant to rapalogs in vitro are very sensitive to the same cells in vivo [71]. Therefore, high levels of HIF-1α or activation of pro-angiogenic pathways that are mTOR-independent are likely to promote rapalog resistance. Several studies have been conducted to address this issue by testing the ability of various angiogenesis inhibitors including agents that target epidermal growth factor receptor (EGFR), VEGF, IGF-1R, and multi-tyrosine kinase inhibitors (i.e. sorafenib) to enhance rapalog activity [100, 106–108]. In addition, other agents such as MEK and HDAC inhibitors that have displayed anti-angiogenic activity have been shown to augment the efficacy of rapalogs [28, 41, 109–111]. Considering that angiogenesis can be initiated through several signaling cascades, a combination approach that simultaneously inhibits multiple pro-angiogenic regulators is likely to be the most effective approach.

Autophagy acts as a bulk protein degradation system that mediates the turnover of long-lived cytosolic proteins, aggregated proteins, and defective organelles. Persistent autophagy has been proposed to induce a necrotic-type death, suggesting that its activation may be an alternative cell death pathway [112]. However, autophagy has also been implicated as a survival mechanism used to recycle cellular components to produce nutrients and energy during periods of metabolic or hypoxic stress or following chemotherapeutic agent treatment [82–86]. Recent evidence suggests that rapalog-mediated induction of autophagy may represent an important pro-survival mechanism that limits the therapeutic efficacy of this class of agents in some tumor types [72]. Inhibition of mTOR signaling with rapalogs strongly induces autophagy, thus disrupting this process may enhance apoptosis. Consistent with this notion, blocking autophagy with chloroquine enhanced apoptosis induced by a novel mTORC1/mTORC2 inhibitor [74]. Further investigation aimed to critically evaluate the therapeutic potential of targeting autophagy to maximize the anticancer effects of mTOR inhibition is warranted.

Conclusions

Inhibition of mTOR activity in malignant cells results in growth inhibition and apoptosis. Since this kinase functions as a critical regulator of several hallmark features of tumorigenesis including cellular proliferation, translation, and angiogenesis, mTOR inhibitors have great therapeutic potential. Indeed, temsirolimus and everolimus were recently FDA approved for the treatment of advanced/metastatic RCC and have demonstrated preclinical and clinical therapeutic efficacy in several other cancer types. In spite of these promising data, the collective studies conducted to date have established that single agent rapalog therapy is not likely to be sufficiently efficacious for most tumor types. Drug resistance is clearly an important emerging problem. mTOR inhibitors have been shown to restore sensitivity to many standard chemotherapeutic agents, suggesting that rapalogs may be best utilized in combination chemotherapy. Second generation mTOR inhibitors that target both mTORC1 and mTORC2 (TORKinhibs) as well as dual pathway inhibitors (PI3K and mTOR) have showed improved efficacy in early studies and appear to have the potential to circumvent rapalog resistance in some cancer types. The ultimate success of agents that target this essential pathway in combination with other chemotherapeutic agents will likely vary between tumor types based on the functional status of known or yet to be elucidated resistance mechanisms. Future studies that establish optimal mTOR inhibitor-based drug combinations that target essential resistance mechanisms hold significant promise and will hopefully translate into direct patient benefit.

Conflict of interest statement None of the authors of this article have conflicts of interest to declare.

References

1. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell 10:457–468
2. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell 110:177–189
3. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 110:163–175

4. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS, Sabatini DM (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell 137:873–886
5. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA, Sabatini DM (2007) PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell 25:903–915
6. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH (2007) Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol 9:316–323
7. Choi J, Chen J, Schreiber SL, Clardy J (1996) Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science 273:239–242
8. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol 6:1122–1128
9. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 14:1296–1302
10. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22:159–168
11. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J (2005) Rheb binds and regulates the mTOR kinase. Curr Biol 15:702–713
12. Jefferies HB, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G (1997) Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. EMBO J 16:3693–3704
13. Holz MK, Ballif BA, Gygi SP, Blenis J (2005) mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell 123:569–580
14. Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, Polakiewicz RD, Sonenberg N, Hershey JW (2004) Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. EMBO J 23:1761–1769
15. Faivre S, Kroemer G, Raymond E (2006) Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 5:671–688
16. Bjornsti MA, Houghton PJ (2004) The TOR pathway: a target for cancer therapy. Nat Rev Cancer 4:335–348
17. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR (2004) mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 10:594–601
18. Mita MM, Mita A, Rowinsky EK (2003) The molecular target of rapamycin (mTOR) as a therapeutic target against cancer. Cancer Biol Ther 2:S169–177
19. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ (2007) Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 356:2271–2281
20. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grunwald V, Thompson JA, Figlin RA, Hollaender N, Urbanowitz G, Berg WJ, Kay A, Lebwohl D, Ravaud A (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372:449–456
21. Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H, Sawyers CL (2001) Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci USA 98:10314–10319
22. Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J, Neshat M, Wang H, Yang L, Gibbons J, Frost P, Dreisbach V, Blenis J, Gaciong Z, Fisher P, Sawyers C, Hedrick-Ellenson L, Parsons R (2001) An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/- mice. Proc Natl Acad Sci USA 98:10320–10325
23. Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, Peralba JM, Jenkins RB, Dakhil SR, Morton RF, Jaeckle KA, Scheithauer BW, Dancey J, Hidalgo M, Walsh DJ (2005) Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. J Clin Oncol 23:5294–5304
24. Margolin K, Longmate J, Baratta T, Synold T, Christensen S, Weber J, Gajewski T, Quirt I, Doroshow JH (2005) CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. Cancer 104:1045–1048
25. Di Nicolantonio F, Arena S, Tabernero J, Grosso S, Molinari F, Macarulla T, Russo M, Cancelliere C, Zecchin D, Mazzucchelli L, Sasazuki T, Shirasawa S, Geuna M, Frattini M, Baselga J, Gallicchio M, Biffo S, Bardelli A (2010) Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. J Clin Invest 120:2858–2866
26. Kaelin WG Jr (2007) The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. Clin Cancer Res 13:680s–684s
27. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG (2000) Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. Nat Cell Biol 2:423–427
28. Mahalingam D, Medina EC, Esquivel JA 2nd, Espitia CM, Smith S, Oberheu K, Swords R, Kelly KR, Mita MM, Mita AC, Carew JS, Giles FJ, Nawrocki ST (2010) Vorinostat enhances the activity of temsirolimus in renal cell carcinoma through suppression of survivin levels. Clin Cancer Res 16:141–153
29. Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, Ansell SM, Luyun R, Flynn PJ, Morton RF, Dakhil SR, Gross H, Kaufmann SH (2005) Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol 23:5347–5356
30. Dumont FJ, Staruch MJ, Grammer T, Blenis J, Kastner CA, Rupprecht KM (1995) Dominant mutations confer resistance to the immunosuppressant, rapamycin, in variants of a T cell lymphoma. Cell Immunol 163:70–79
31. Fruman DA, Wood MA, Gjertson CK, Katz HR, Burakoff SJ, Bierer BE (1995) FK506 binding protein 12 mediates sensitivity to both FK506 and rapamycin in murine mast cells. Eur J Immunol 25:563–571
32. Lorenz MC, Heitman J (1995) TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. J Biol Chem 270:27531–27537
33. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol 7:e38
34. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem 284:8023–8032
35. Yu K, Toral-Barza L, Shi C, Zhang WG, Lucas J, Shor B, Kim J, Verheijen J, Curran K, Malwitz DJ, Cole DC,

Ellingboe J, Ayral-Kaloustian S, Mansour TS, Gibbons JJ, Abraham RT, Nowak P, Zask A (2009) Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. Cancer Res 69:6232–6240
36. Bhatt AP, Bhende PM, Sin SH, Roy D, Dittmer DP, Damania B (2010) Dual inhibition of PI3K and mTOR inhibits autocrine and paracrine proliferative loops in PI3K/Akt/mTOR-addicted lymphomas. Blood 115:4455–4463
37. Kharas MG, Janes MR, Scarfone VM, Lilly MB, Knight ZA, Shokat KM, Fruman DA (2008) Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR-ABL+ leukemia cells. J Clin Invest 118:3038–3050
38. Shi Y, Yan H, Frost P, Gera J, Lichtenstein A (2005) Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. Mol Cancer Ther 4:1533–1540
39. Tamburini J, Chapuis N, Bardet V, Park S, Sujobert P, Willems L, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2008) Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/Akt by up-regulating insulin-like growth factor-1 receptor signaling in acute myeloid leukemia: rationale for therapeutic inhibition of both pathways. Blood 111:379–382
40. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307:1098–1101
41. Gupta M, Ansell SM, Novak AJ, Kumar S, Kaufmann SH, Witzig TE (2009) Inhibition of histone deacetylase overcomes rapamycin-mediated resistance in diffuse large B-cell lymphoma by inhibiting Akt signaling through mTORC2. Blood 114:2926–2935
42. Chiarini F, Grimaldi C, Ricci F, Tazzari PL, Evangelisti C, Ognibene A, Battistelli M, Falcieri E, Melchionda F, Pession A, Pagliaro P, McCubrey JA, Martelli AM (2010) Activity of the novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 against T-cell acute lymphoblastic leukemia. Cancer Res 70:8097–8107
43. Cho DC, Cohen MB, Panka DJ, Collins M, Ghebremichael M, Atkins MB, Signoretti S, Mier JW (2010) The efficacy of the novel dual PI3-kinase/mTOR inhibitor NVP-BEZ235 compared with rapamycin in renal cell carcinoma. Clin Cancer Res 16:3628–3638
44. Lopez-Fauqued M, Gil R, Grueso J, Hernandez-Losa J, Pujol A, Moline T, Recio JA (2010) The dual PI3K/mTOR inhibitor PI-103 promotes immunosuppression, in vivo tumor growth and increases survival of sorafenib-treated melanoma cells. Int J Cancer 126:1549–1561
45. Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia-Echeverria C (2008) Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. Mol Cancer Ther 7:1851–1863
46. McMillin DW, Ooi M, Delmore J, Negri J, Hayden P, Mitsiades N, Jakubikova J, Maira SM, Garcia-Echeverria C, Schlossman R, Munshi NC, Richardson PG, Anderson KC, Mitsiades CS (2009) Antimyeloma activity of the orally bioavailable dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. Cancer Res 69:5835–5842
47. Dilling MB, Dias P, Shapiro DN, Germain GS, Johnson RK, Houghton PJ (1994) Rapamycin selectively inhibits the growth of childhood rhabdomyosarcoma cells through inhibition of signaling via the type I insulin-like growth factor receptor. Cancer Res 54:903–907
48. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ (2007) Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. Oncogene 26:1932–1940
49. Wang X, Yue P, Chan CB, Ye K, Ueda T, Watanabe-Fukunaga R, Fukunaga R, Fu H, Khuri FR, Sun SY (2007) Inhibition of mammalian target of rapamycin induces phosphatidylinositol 3-kinase-dependent and Mnk-mediated eukaryotic translation initiation factor 4E phosphorylation. Mol Cell Biol 27:7405–7413
50. Janes MR, Limon JJ, So L, Chen J, Lim RJ, Chavez MA, Vu C, Lilly MB, Mallya S, Ong ST, Konopleva M, Martin MB, Ren P, Liu Y, Rommel C, Fruman DA (2010) Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. Nat Med 16:205–213
51. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 118:3065–3074
52. Hammerman PS, Fox CJ, Birnbaum MJ, Thompson CB (2005) Pim and Akt oncogenes are independent regulators of hematopoietic cell growth and survival. Blood 105:4477–4483
53. Beharry Z, Mahajan S, Zemskova M, Lin YW, Tholanikunnel BG, Xia Z, Smith CD, Kraft AS (2010) The Pim protein kinases regulate energy metabolism and cell growth. Proc Natl Acad Sci USA. doi:10.1073/pnas.1013214108
54. Lilly M, Kraft A (1997) Enforced expression of the Mr 33,000 Pim-1 kinase enhances factor-independent survival and inhibits apoptosis in murine myeloid cells. Cancer Res 57:5348–5355
55. Zhang F, Beharry ZM, Harris TE, Lilly MB, Smith CD, Mahajan S, Kraft AS (2009) PIM1 protein kinase regulates PRAS40 phosphorylation and mTOR activity in FDCP1 cells. Cancer Biol Ther 8:846–853
56. Beharry Z, Zemskova M, Mahajan S, Zhang F, Ma J, Xia Z, Lilly M, Smith CD, Kraft AS (2009) Novel benzylidene-thiazolidine-2,4-diones inhibit Pim protein kinase activity and induce cell cycle arrest in leukemia and prostate cancer cells. Mol Cancer Ther 8:1473–1483
57. Fox CJ, Hammerman PS, Thompson CB (2005) The Pim kinases control rapamycin-resistant T cell survival and activation. J Exp Med 201:259–266
58. Tan J, Lee PL, Li Z, Jiang X, Lim YC, Hooi SC, Yu Q (2010) B55beta-associated PP2A complex controls PDK1-directed myc signaling and modulates rapamycin sensitivity in colorectal cancer. Cancer Cell 18:459–471
59. Dennis PB, Fumagalli S, Thomas G (1999) Target of rapamycin (TOR): balancing the opposing forces of protein synthesis and degradation. Curr Opin Genet Dev 9:49–54
60. Liu J, Stevens PD, Gao T (2010) mTOR-dependent regulation of PHLPP expression controls the rapamycin sensitivity in cancer cells. J Biol Chem. doi:10.1074/jbc.M110.183087
61. Murata K, Wu J, Brautigan DL (1997) B cell receptor-associated protein alpha4 displays rapamycin-sensitive binding directly to the catalytic subunit of protein phosphatase 2A. Proc Natl Acad Sci USA 94:10624–10629
62. Dilling MB, Germain GS, Dudkin L, Jayaraman AL, Zhang X, Harwood FC, Houghton PJ (2002) 4E-binding proteins, the suppressors of eukaryotic initiation factor 4E, are down-regulated in cells with acquired or intrinsic resistance to rapamycin. J Biol Chem 277:13907–13917
63. Graff JR, Konicek BW, Carter JH, Marcusson EG (2008) Targeting the eukaryotic translation initiation factor 4E for cancer therapy. Cancer Res 68:631–634

64. Luo Y, Marx SO, Kiyokawa H, Koff A, Massague J, Marks AR (1996) Rapamycin resistance tied to defective regulation of p27Kip1. Mol Cell Biol 16:6744–6751
65. Neklesa TK, Davis RW (2008) Superoxide anions regulate TORC1 and its ability to bind Fpr1: rapamycin complex. Proc Natl Acad Sci USA 105:15166–15171
66. Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov 8:579–591
67. Hosoi H, Dilling MB, Shikata T, Liu LN, Shu L, Ashmun RA, Germain GS, Abraham RT, Houghton PJ (1999) Rapamycin causes poorly reversible inhibition of mTOR and induces p53-independent apoptosis in human rhabdomyosarcoma cells. Cancer Res 59:886–894
68. Huang S, Shu L, Dilling MB, Easton J, Harwood FC, Ichijo H, Houghton PJ (2003) Sustained activation of the JNK cascade and rapamycin-induced apoptosis are suppressed by p53/p21(Cip1). Mol Cell 11:1491–1501
69. Shi Y, Frankel A, Radvanyi LG, Penn LZ, Miller RG, Mills GB (1995) Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro. Cancer Res 55:1982–1988
70. Gyrd-Hansen M, Meier P (2010) IAPs: from caspase inhibitors to modulators of NF-kappaB, inflammation and cancer. Nat Rev Cancer 10:561–574
71. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK (2002) Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 8:128–135
72. Carew JS, Nawrocki ST, Cleveland JL (2007) Modulating autophagy for therapeutic benefit. Autophagy 3:464–467
73. Crazzolara R, Bradstock KF, Bendall LJ (2009) RAD001 (Everolimus) induces autophagy in acute lymphoblastic leukemia. Autophagy 5:727–728
74. Carayol N, Vakana E, Sassano A, Kaur S, Goussetis DJ, Glaser H, Druker BJ, Donato NJ, Altman JK, Barr S, Platanias LC (2010) Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells. Proc Natl Acad Sci USA 107:12469–12474
75. Bursch W, Ellinger A, Kienzl H, Torok L, Pandey S, Sikorska M, Walker R, Hermann RS (1996) Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. Carcinogenesis 17:1595–1607
76. Kanzawa T, Germano IM, Komata T, Ito H, Kondo Y, Kondo S (2004) Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. Cell Death Differ 11:448–457
77. Kanzawa T, Kondo Y, Ito H, Kondo S, Germano I (2003) Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. Cancer Res 63:2103–2108
78. Paglin S, Hollister T, Delohery T, Hackett N, McMahill M, Sphicas E, Domingo D, Yahalom J (2001) A novel response of cancer cells to radiation involves autophagy and formation of acidic vesicles. Cancer Res 61:439–444
79. Shao Y, Gao Z, Marks PA, Jiang X (2004) Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. Proc Natl Acad Sci USA 101:18030–18035
80. Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB, Kondo S (2005) Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. Cancer Res 65:3336–3346
81. Zhu K, Dunner K Jr, McConkey DJ (2010) Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. Oncogene 29:451–462
82. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, Thomas-Tikhonenko A, Thompson CB (2007) Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 117:326–336
83. Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, Galavotti S, Young KW, Selmi T, Yacobi R, Van Etten RA, Donato N, Hunter A, Dinsdale D, Tirro E, Vigneri P, Nicotera P, Dyer MJ, Holyoake T, Salomoni P, Calabretta B (2009) Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. J Clin Invest 119:1109–1123
84. Carew JS, Espitia CM, Esquivel JA 2nd, Mahalingam D, Kelly KR, Reddy G, Giles FJ, Nawrocki ST (2010) Lucanthone: a novel inhibitor of autophagy that induces cathepsin D-mediated apoptosis. J Biol Chem. doi:10.1074/jbc.M110.151324
85. Carew JS, Medina EC, Esquivel JA 2nd, Mahalingam D, Swords R, Kelly K, Zhang H, Huang P, Mita AC, Mita MM, Giles FJ, Nawrocki ST (2010) Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation. J Cell Mol Med 14:2448–2459
86. Carew JS, Nawrocki ST, Kahue CN, Zhang H, Yang C, Chung L, Houghton JA, Huang P, Giles FJ, Cleveland JL (2007) Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. Blood 110:313–322
87. Hoang B, Frost P, Shi Y, Belanger E, Benavides A, Pezeshkpour G, Cappia S, Guglielmelli T, Gera J, Lichtenstein A (2010) Targeting TORC2 in multiple myeloma with a new mTOR kinase inhibitor. Blood 116:4560–4568
88. Hsieh AC, Costa M, Zollo O, Davis C, Feldman ME, Testa JR, Meyuhas O, Shokat KM, Ruggero D (2010) Genetic dissection of the oncogenic mTOR pathway reveals druggable addiction to translational control via 4EBP-eIF4E. Cancer Cell 17:249–261
89. Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, Shokat KM, Weiss WA (2006) A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. Cancer Cell 9:341–349
90. Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, Botero ML, Llonch E, Atzori F, Di Cosimo S, Maira M, Garcia-Echeverria C, Parra JL, Arribas J, Baselga J (2008) NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Cancer Res 68:8022–8030
91. Cirstea D, Hideshima T, Rodig S, Santo L, Pozzi S, Vallet S, Ikeda H, Perrone G, Gorgun G, Patel K, Desai N, Sportelli P, Kapoor S, Vali S, Mukherjee S, Munshi NC, Anderson KC, Raje N (2010) Dual inhibition of akt/mammalian target of rapamycin pathway by nanoparticle albumin-bound-rapamycin and perifosine induces antitumor activity in multiple myeloma. Mol Cancer Ther 9:963–975
92. Hahn M, Li W, Yu C, Rahmani M, Dent P, Grant S (2005) Rapamycin and UCN-01 synergistically induce apoptosis in human leukemia cells through a process that is regulated by the Raf-1/MEK/ERK, Akt, and JNK signal transduction pathways. Mol Cancer Ther 4:457–470
93. Mills JR, Hippo Y, Robert F, Chen SM, Malina A, Lin CJ, Trojahn U, Wendel HG, Charest A, Bronson RT, Kogan SC, Nadon R, Housman DE, Lowe SW, Pelletier J (2008) mTORC1 promotes survival through translational control of Mcl-1. Proc Natl Acad Sci USA 105:10853–10858
94. Wangpaichitr M, Wu C, You M, Kuo MT, Feun L, Lampidis T, Savaraj N (2008) Inhibition of mTOR restores cisplatin sensitivity through down-regulation of growth and anti-apoptotic proteins. Eur J Pharmacol 591:124–127
95. Wei G, Twomey D, Lamb J, Schlis K, Agarwal J, Stam RW, Opferman JT, Sallan SE, den Boer ML, Pieters R, Golub TR,

Armstrong SA (2006) Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. Cancer Cell 10:331–342
96. Marinov M, Ziogas A, Pardo OE, Tan LT, Dhillon T, Mauri FA, Lane HA, Lemoine NR, Zangemeister-Wittke U, Seckl MJ, Arcaro A (2009) AKT/mTOR pathway activation and BCL-2 family proteins modulate the sensitivity of human small cell lung cancer cells to RAD001. Clin Cancer Res 15:1277–1287
97. Wendel HG, Malina A, Zhao Z, Zender L, Kogan SC, Cordon-Cardo C, Pelletier J, Lowe SW (2006) Determinants of sensitivity and resistance to rapamycin-chemotherapy drug combinations in vivo. Cancer Res 66:7639–7646
98. Ackler S, Xiao Y, Mitten MJ, Foster K, Oleksijew A, Refici M, Schlessinger S, Wang B, Chemburkar SR, Bauch J, Tse C, Frost DJ, Fesik SW, Rosenberg SH, Elmore SW, Shoemaker AR (2008) ABT-263 and rapamycin act cooperatively to kill lymphoma cells in vitro and in vivo. Mol Cancer Ther 7:3265–3274
99. Kim KW, Moretti L, Mitchell LR, Jung DK, Lu B (2009) Combined Bcl-2/mammalian target of rapamycin inhibition leads to enhanced radiosensitization via induction of apoptosis and autophagy in non-small cell lung tumor xenograft model. Clin Cancer Res 15:6096–6105
100. Huynh H, Ngo VC, Koong HN, Poon D, Choo SP, Thng CH, Chow P, Ong HS, Chung A, Soo KC (2009) Sorafenib and rapamycin induce growth suppression in mouse models of hepatocellular carcinoma. J Cell Mol Med 13:2673–2683
101. Bruns CJ, Koehl GE, Guba M, Yezhelyev M, Steinbauer M, Seeliger H, Schwend A, Hoehn A, Jauch KW, Geissler EK (2004) Rapamycin-induced endothelial cell death and tumor vessel thrombosis potentiate cytotoxic therapy against pancreatic cancer. Clin Cancer Res 10:2109–2119
102. Del Bufalo D, Ciuffreda L, Trisciuoglio D, Desideri M, Cognetti F, Zupi G, Milella M (2006) Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus. Cancer Res 66:5549–5554
103. Frost P, Shi Y, Hoang B, Lichtenstein A (2007) AKT activity regulates the ability of mTOR inhibitors to prevent angiogenesis and VEGF expression in multiple myeloma cells. Oncogene 26:2255–2262
104. Phung TL, Ziv K, Dabydeen D, Eyiah-Mensah G, Riveros M, Perruzzi C, Sun J, Monahan-Earley RA, Shiojima I, Nagy JA, Lin MI, Walsh K, Dvorak AM, Briscoe DM, Neeman M, Sessa WC, Dvorak HF, Benjamin LE (2006) Pathological angiogenesis is induced by sustained Akt signaling and inhibited by rapamycin. Cancer Cell 10:159–170
105. Stephan S, Datta K, Wang E, Li J, Brekken RA, Parangi S, Thorpe PE, Mukhopadhyay D (2004) Effect of rapamycin alone and in combination with antiangiogenesis therapy in an orthotopic model of human pancreatic cancer. Clin Cancer Res 10:6993–7000
106. Chiu CW, Nozawa H, Hanahan D (2010) Survival benefit with proapoptotic molecular and pathologic responses from dual targeting of mammalian target of rapamycin and epidermal growth factor receptor in a preclinical model of pancreatic neuroendocrine carcinogenesis. J Clin Oncol 28:4425–4433
107. Hainsworth JD, Spigel DR, Burris HA 3rd, Waterhouse D, Clark BL, Whorf R (2010) Phase II trial of bevacizumab and everolimus in patients with advanced renal cell carcinoma. J Clin Oncol 28:2131–2136
108. Wedel S, Hudak L, Seibel JM, Juengel E, Tsaur I, Haferkamp A, Blaheta RA (2011) Combined targeting of the VEGFr/EGFr and the mammalian target of rapamycin (mTOR) signaling pathway delays cell cycle progression and alters adhesion behavior of prostate carcinoma cells. Cancer Lett 301:17–28
109. Chang Q, Chen E, Hedley DW (2009) Effects of combined inhibition of MEK and mTOR on downstream signaling and tumor growth in pancreatic cancer xenograft models. Cancer Biol Ther 8:1893–1901
110. Kinkade CW, Castillo-Martin M, Puzio-Kuter A, Yan J, Foster TH, Gao H, Sun Y, Ouyang X, Gerald WL, Cordon-Cardo C, Abate-Shen C (2008) Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a preclinical mouse model. J Clin Invest 118:3051–3064
111. Verheul HM, Salumbides B, Van Erp K, Hammers H, Qian DZ, Sanni T, Atadja P, Pili R (2008) Combination strategy targeting the hypoxia inducible factor-1 alpha with mammalian target of rapamycin and histone deacetylase inhibitors. Clin Cancer Res 14:3589–3597
112. Yuan J, Kroemer G (2010) Alternative cell death mechanisms in development and beyond. Genes Dev 24:2592–2602