Revisiting mTOR inhibitors as anticancer agents

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The mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine kinase that regulates a variety of cellular processes, influencing diverse pathological conditions including a variety of cancers. Accordingly, therapies that target mTOR as anticancer agents benefit patients in various clinical settings. It is therefore important to fully investigate mTOR signaling at a molecular level and corresponding mTOR inhibitors to identify additional clinical opportunities of targeting mTOR in cancers. In this review, we introduce the function and regulation of the mTOR signaling pathway and organize and summarize the different roles of mTOR in cancers and a variety of mTOR inhibitors that can be used as anticancer agents. This article aims to enlighten and guide the development of mTOR-targeted anticancer agents in the future.

Introduction

Owing to population growth and aging, along with the increasing prevalence of established risk factors such as smoking, physical inactivity and obesity, the occurrence of cancer has increased [1]. Although the cancer incidence rate and the cancer death rate have declined during the past decade, cancer is still the second leading cause of death in the USA [2]. Significant advances in the development of chemotherapeutic agents have been made in the past decade. However, several challenges, including the variation in cancer cell behavior and tumor progression among different patients, still hinder the further advance of cancer therapy.

Molecular-targeted therapy is defined as the use of a drug or other compound that is specific to a molecular target to prevent the proliferation and metastasis of cancer cells [3]. This technology, combined with new drug development, will accelerate the implementation of personalized medicine. Personalized therapy uses the concept of the genetic and environmental basis of disease to individualize prevention [4] and uses targeted therapy for specific disease-targeting enzymes, growth factor receptors and signal transducers to individualize diagnosis and treatment. The identification of ideal targets is the key to the successful development of molecular-targeted therapy. One of the ways in which cancer develops is via genetic changes that affect the expression and function of tumor suppressor genes and the proteins they encode. The other way is via mutations leading to changes in proteins and receptors that promote cell survival and proliferation. These two specific genetic alterations distinguish cancer cells from normal cells and can be used as molecular targets while serving as the focus of targeted therapy development [5]. By understanding the physiology and characteristics of specific molecular targets in cancer, researchers can identify potential molecular strategies to inhibit tumor growth and progression, leading to the development of effective drugs. Depending on whether the target is a cell surface antigen, growth factor, receptor or signal transduction pathway, molecular-targeted therapies for cancer treatment can exhibit different functions and features [6]. Molecular-targeted

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therapy represents an attempt to achieve anticancer effects by selectively modifying the differences in biological characteristics between normal and cancer cells. The best molecular-targeted therapeutic agent should satisfy the following three prerequisites: the treatment should be directed at the target; the treatment should have an anticancer effect; and the anticancer effect should be explicable in terms of modification of the target.

The mammalian target of the rapamycin (mTOR) pathway regulates cell growth and survival. Alterations in mTOR signaling have been associated with malignant neoplasia, including colorectal cancer, pancreatic carcinoma, gastric cancer, breast carcinoma, prostate cancer and non-small-cell lung cancer. Inhibition of mTOR signaling might therefore be a promising alternative for management of these cancers. The mTOR signaling pathway responds to many different intracellular (energy and stress) and extracellular (nutrient, growth factor, hormone) signals that comprise a wide variety of proteins. There are two mTOR protein complexes, defined by their different components: mTOR complex (mTORC)1 and mTORC2 [7]. Some components are identical for both complexes, including mammals with SEC13 protein 8 (mLST8) [8] and inhibitory protein DEP domains containing the mTOR-interacting protein (DEPTOR) [9]. By contrast, several specific components differ between these two complexes, including regulatory-associated protein of mTOR (RAPTOR), proline-rich AKT1 substrate 1 (PRAS40) of mTORC1, rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) and mammalian stress-activated protein kinase interacting protein 1 (mSIN-1) of mTORC2 [10], the latter accounting for differences in function between mTORC1 and mTORC2. mTORC1 responds to the availability of nutrients and growth factors and inhibits autophagy, a process whereby cellular stress leads to the circulation of cytoplasmic components (including organelles) to restore the energy needed for survival. mTORC1 has also recently been identified to play a part in cell growth by stimulating nucleotide synthesis via a pyrimidine synthesis pathway [11]. mTORC2 plays an important part in cell survival, metabolism, proliferation and cytoskeletal organization, because it phosphorylates protein kinase Cα (PKCα), serum/glucocorticoid-regulated kinase 1 (SGK1) and AKT to fully activated AKT [12,13]. Because the mTOR signaling pathway plays a crucial part in cancer biology and has become a prospective target for anticancer development, clinical interest in mTOR has increased during recent years. Some mTOR inhibitors have been investigated in preclinical trials to test their efficacy against different human cancers but have not been highly successful. However, with the synthesis of new compounds, the role of mTOR inhibitors in the treatment of cancer continues to develop. This review introduces and discusses the recent development of mTOR inhibitors as potential anticancer agents.

mTOR signaling pathway

mTOR is a complex serine/threonine kinase belonging to the phosphoinositide-kinase-related family of protein kinases (PIKKs) [14]. mTOR, in conjunction with other biological pathways such as JAK/STAT3 and PI3K/AKT, coordinates a variety of cellular processes such as growth, differentiation and metabolism (Fig. 1) [15]. mTOR consists of two functional multiprotein complexes: mTORC1 and mTORC2, which are characterized by the presence of distinct cellular proteins that regulate their functions [16]. Recently, a novel mTOR complex G-protein-coupled receptor (GPCR)-kinase-interacting protein 1 (GIT1) was identified in somatic tissues, highlighting the possible variation in mTOR composition in different tissues [17]. The distinct and overlapping

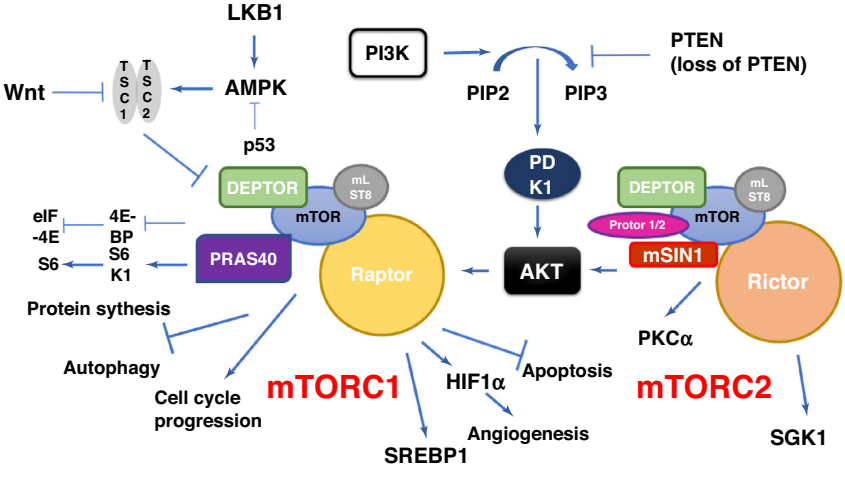

FIGURE 1

mTOR signaling pathways in cancer cells. mTOR signaling is activated via the PI3K/AKT signaling pathway. Abnormal upstream signaling in the PI3K/AKT/mTOR pathway (mutations in PTEN, PI3K, AKT and TSCs) lead to mTOR deregulation. AKT can also be a downstream effector of mTOR, because mTORC2 can activate AKT in a positive feedback loop. Abbreviations: mTOR, mammalian target of rapamycin; PI3K, phosphatidyl inositol 3 kinase; PTEN, phosphatase and tensin homolog; TSC, tuberous sclerosis complex; mTORC, mTOR complex; Raptor, regulatory-associated protein of mTOR; PRAS40, proline-rich AKT1 substrate 1; DEPTOR, DEP domains containing the mTOR-interacting protein; mLST8, mammals with SEC13 protein 8; Rictor, rapamycin-insensitive companion of mammalian target of rapamycin; mSIN1, mammalian stress-activated protein kinase interacting protein; PDK1, Phosphatidylinositol-dependent kinase-1; AMPK, AMP-activated protein kinase; TSC, tuberous sclerosis complex; HIF, hypoxia-inducible factor; PIP3, phosphoinositide-3,4,5-tri-phosphate; PKC, phosphorylates protein kinase C; SGK, serum/glucocorticoid-regulated kinase.

components of mTORC1 and mTORC2 are crucial for their unique functionalities; mTORC1 regulates cell growth, proliferation, motility, autophagy and protein synthesis, whereas mTORC2 regulates organization of actin and metabolic control [18].

The activation of the mTOR complex is essential for maintaining cellular function. mTORC1 is activated by its translocation to the lysosome through the action of Rag GTPases and regulators, such as the late endosomal/lysosomal adaptor and mTOR activator (LAMTOR/regulator). mTORC2 is activated in the ribosomes by insulin signaling, mediated through insulin-like growth factor receptor (IGFR) [18,19]. In addition, the mTOR complex is activated by exogenous factors such as nutrient and oxygen levels through the PI3K/AKT pathways [20]. mTORC1 is reported to be rapamycin-sensitive and targets proteins that are involved in mRNA translation, including p70S6K1 and 4EBP-1, resulting in initiation of specific cap-dependent translation events [21]. Moreover, mTORC1 regulates lipid synthesis through SREBP1 and angiogenesis through hypoxia-inducible factor (HIF)-1 [21,22]. By contrast, mTORC2 is rapamycin-insensitive and regulates phosphorylation of AKT, serum and glucocorticoid kinase (SGK), as well as protein kinase C (PKC) [22,23].

Alterations in signaling pathways either upstream or downstream of mTOR are known to promote tumor growth. A number of studies have reported the dysregulation of mTORC1 or mTORC2 as the major contributor to the development of solid tumors and hematological malignancies [14,18,20,24]. Considering the wide variety of parts that mTOR plays, it is important to develop novel mTOR inhibitors that can halt tumor growth and decrease mortality. Extensive effort has been directed toward the development of cancer therapy targeting the PI3K/AKT/mTOR pathway. Various drugs such as PI3K, AKT or mTOR kinase inhibitors are in clinical development, and allosteric inhibitors of mTORC1: temsirolimus and everolimus, have been approved by the FDA and the European Medicines Agency (EMA) for the treatment of advanced renal cell carcinoma [25], hormone receptor-positive Her2-negative breast cancer and hormone therapy [26], as well as pancreatic-derived neuroendocrine tumors [27].

mTOR and cancer development

mTOR is an important cell proliferation and protein translational regulator that plays a key part in human cancer. Activation of the PI3K/AKT/mTOR pathway, via receptor tyrosine kinase activation or amplification, phosphatase and tensin homolog (PTEN) deletion, or activation of mutations in PIK3CA or other downstream effectors [28,29], leads to inhibition of apoptosis and unlimited cell growth in a wide range of cancers. The PI3K pathway activates the mTOR complexes mTORC1 and mTORC2. It is worth noting that tumors containing this mutation are sensitive to mTOR inhibitors. In cancer cells, abnormal activation of the pathway can occur through increased signaling by growth factor receptors, mutation and/or amplification of the activation pathway kinase or loss of the tumor suppressor protein PTEN [30], and can be caused by aberrant activation of upstream signals, including protein kinase B/AKT and extracellular signal-regulated kinase (ERK). In addition to these kinases, AMP-activated protein kinase (AMPK) can influence mTOR signaling by promoting tuberous sclerosis complex (TSC)1 or TSC2 activation [10]. Upregulation of the PI3K/AKT pathway through amplification of the *PIK3CA* gene (encoding the p110a subunit of PI3K) constitutively activates the mTOR signal [23]. In addition, p53 and LKB1 can also cause mTOR activation [1,24] (Fig. 1).

Activation of the mTOR signaling pathway contributes to a variety of different cancers because the activated mTOR signaling pathway leads to the proliferation and migration of cancer cells by increasing the expression of cyclin D1 and c-Myc – two important oncogenes [31]. Moreover, β-catenin is closely related to the regulation of vascular endothelial growth factor (VEGF)-A expression [32] and has also been shown to induce cyclin D1 in cancer cells, which contributes to neoplastic transformation [33]. When β-catenin is inactivated in adenomatous polyposis coli (APC) the Wnt pathway is constitutively activated, which stimulates the TSC/mTOR pathway [34]. Activation of this signaling pathway is considered to be the initiating event in colorectal cancer and is also involved in thyroid cancer.

Phosphatidylinositol-dependent kinase-1 (PDK1) and mTORC2 are activated by the phosphorylation of phosphatidylinositol 4,5-diphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). PDK1 phosphorylates AKT on Thr308, whereas mTORC2 phosphorylates AKT on Ser473, and double phosphorylation completely activates AKT activity [35], which promotes oncogenesis [12,36–38]. Mutations in the PI3K/PTEN/AKT pathway are found in a large number of colorectal cancer cell lines [39–42] and PTEN ablation is associated with Cowden's syndrome, which increases the lifetime risk of colorectal cancer [43,44].

The p53 gene encodes a protein that regulates the cell cycle and has an important tumor suppressor function [45]. It also modulates the insulin growth factor (IGF)-1/AKT pathway, which in turn regulates mTOR. Moreover, p53 negatively regulates mTOR by activating AMPK and TSC2 phosphorylation [46], especially in colorectal cancer cell lines. In addition, *REDD1*, a gene affected by reactive oxygen species (ROS) and oxidative stress, is another target gene of p53 that regulates the mTOR pathway.

CXCL12 activates CXCR4, a signal transduced in the mTOR pathway of pancreatic and gastric cancer cells, as well as T cell leukemia cells, to initiate G protein signaling. CXCR4, in turn, activates the PI3K pathway and then induces mTORC2 phosphorylation and increases the expression of RhoA, Cdc42 and Rac1 [47,48], which might increase cell motility to promote cancer metastasis. CXCL12 stimulation does not enhance signal transduction downstream of mTOR1, and the inhibition of mTORC2 blocks angiogenesis *in vivo* and *in vitro* [47,49]. The mTOR inhibitor rapamycin could suppress the expression of CXCL12 and induce the autophagic cell death in gastric and pancreatic cancer [50].

Leukemia inhibitory factor (LIF) is a multifunctional protein that can contribute to several cancers, including medullary thyroid cancer, malignant melanoma, skin tumors, pancreatic carcinoma, breast carcinoma and rhabdomyosarcoma [51–57], by activating selective signaling pathways, including JAK/STAT3, PI3K/AKT/mTOR, MAPK and/or ERK1/2 pathways. Particularly in prostate cancer models, the combination of PI3K/mTOR inhibitors and chemotherapy has shown superior efficacy over monotherapy [58,59]. Activation of the PI3K/mTOR pathway is associated with a reduction in disease-free survival in cancer patients [60,61]. In prostate cancer, phosphatase and angiotensin homolog (PTEN) deletions and associated upregulation of the PI3K/mTOR pathway usually occur, contributing to tumorigenesis and invasion [59,62–64].

mTOR inhibitors as anticancer agents

It has long been believed that mTOR inhibitors can be effective in the treatment and prevention of tumor progression. Great efforts have been made to develop effective molecules to target the mTOR pathway [65].

Rapamycin and its derivatives

Based on the fact that mTORC1 promotes cell growth and is overactivated in a large number of cancers, small molecules that specifically target mTORC1, such as rapamycin (also known as sirolimus), have antineoplastic potential. Rapamycin is a natural allosteric inhibitor of the mTOR complex and is obtained from *Streptomyces hygroscopicus* which is found in the soil from Easter Island [66,67]. Originally introduced as an antimicrobial agent, it showed what was then considered an undesirable immunosuppressive effect [66]. The structure of rapamycin is comparable to a widely known immunosuppressant FK506, and both molecules were found to bind to the same receptor: FKBP12, a small cytosolic protein [66,68]. Even though both molecules have comparable structures and bind to the same receptor, they have different mechanisms of action by which they elicit their immunosuppressive effects [66]. Rapamycin inhibits B and T cell proliferation and thereby inhibits antibody formation [69]. This results from its interference with growth-advancing cytokine signaling [66]. Rapamycin has no effect on FKBP12, but its binding to the receptor enables its interaction with its target mTOR, through the formation of a ternary complex. The inhibition of mTOR is a result of indirect inhibition rather than the direct result of mTOR binding to the rapamycin–FKBP12 complex [68]. Some findings suggest that this complex inhibits mTOR by preventing the phosphorylation of the enzymes that promote protein synthesis and the proteins that are involved in transcription and translation and cell cycle regulation [67]. Of the two mTOR complexes, rapamycin exhibits inhibition of only mTORC1 activity [68]. This was contradicted in *in vitro* studies, however, where mTORC1 kinase activity demonstrated less sensitivity to rapamycin [66].

The anticancer property of rapamycin was recognized in the 1980s [68]. It was hypothesized to be because cancer cells have increased levels of AKT, which is related to increased mTOR signaling in some cancers, and it was later found that mTOR inhibition by rapamycin leads to suppressed expression of the hyperactive growth phenotype observed in some mouse cells that express activated AKT3 [70] when the cells are null for PTEN. Presumably, PTEN inactivation leads to hyperactivity of AKT, which is regulated by PI3K signaling. Increased PI3K leads to an increase in the production of PIP3, which is responsible for the activation of AKT signaling, which leads to cell growth, proliferation and survival. The dysfunction of these activities and their interaction with mTOR leads to alteration in cellular functions that are found in most cancers [69]. Thus, activation of mTOR–raptor encourages tumor growth, supporting the effectiveness of rapamycin as an anticancer agent for cancer cells that have high AKT activity. However, the relationship between the PTEN inactivation and rapamycin efficacy is not convincing and, thus, PTEN status cannot be a factor for determining rapamycin sensitivity [70].

Although rapamycin has the potential to inhibit tumor progression and is a promising anticancer therapy, the poor bioavailability and efficacy of rapamycin led to the development of several rapamycin derivatives [66,68,69]. To improve its pharmacokinetic properties and stability, analogs of rapamycin (named rapalogs) were developed in the late 1990s. After minor modifications, temsirolimus (CCI-779), an ester of dihydroxylmethyl propionic acid, everolimus (RAD001), which has a hydroxyethyl group, and ridaforolimus (AP23573), which has a dimethyl phosphate group, were the three analogs of rapamycin that were developed [68,69]. All three compounds have the same mechanism of action as that of rapamycin: binding to FKBP12 and inhibiting mTORC1. Despite having the same mechanism of action, however, they differ in their formulation and bioavailability [69].

Temsirolimus was the first rapalog approved by the FDA for the treatment of renal cell carcinoma [68,69]. It is a prodrug that is converted to rapamycin when injected, which is known for its activity [66,71]. Everolimus went on to be approved by the FDA for the treatment of renal cell carcinoma. Both these rapalogs have better pharmacodynamic and pharmacokinetic properties than rapamycin. Whereas temsirolimus is available as an injectable preparation, everolimus is an orally bioavailable treatment [71]. Ridaforolimus was developed after temsirolimus and everolimus, and it showed stability in water and organic solvents. Ridaforolimus is administered intravenously but, unlike temsirolimus, it is not a prodrug of rapamycin [67]. Temsirolimus and everolimus are the two rapalogs that have been approved by the FDA for the treatment of renal cell carcinoma and HER-2-negative breast cancer in combination with aromasin [66,69]. All these analogs work by binding to mTORC1 and thereby inhibiting cells in the G1 phase of the cell cycle [69]. Rapamycin and its derivatives are used in combination with chemotherapeutic drugs for the treatment of certain types of cancer [66–69,71,72]. Antitumor agents when given with rapalogs showed better efficacy than when given alone [69,72].

Even though rapalogs showed success in treating certain types of cancer, they are not very efficacious as treatments for several solid tumors and sporadic cancers [68,69,73]. Rapalogs prevented tumor proliferation but showed no success in tumor regression [68]. Recently, mutations in the PTEN genes, which cause drug resistance [73], have been reported. There is also a concern that long-term use of rapamycin leads to upregulation of AKT which enhances the survival of cells otherwise undergoing apoptosis. This could be a major reason for resistance to other chemotherapeutic agents [66]. Recent work showed that the dephosphorylation of Ser65 and Thr70 on 4E-BP1 was found to be sensitive to rapamycin treatment, whereas the other phosphorylating sites: Thr37 and Thr46, showed insensitivity to rapamycin treatment [74]. It was observed that the rapamycin-sensitive phosphorylation sites of 4E-BP1 became re-phosphorylated on long-term use [75]. This led to the conclusion that rapalogs only incompletely inhibit mTORC1. Because mTORC1 is a ubiquitously expressed protein complex, blocking its activity also causes nonspecific binding leading to side effects and toxicity. These effects are usually mild but can also be fatal in some cases. The toxicity and side effects of rapamycin further limit its use [73]. These limitations led to the development of second-generation mTOR inhibitors [75].

ATP-competitive mTOR inhibitors

To overcome the limitations of rapalogs, a new generation of mTOR inhibitors was developed called the ATP-competitive mTOR

inhibitors [73]. The mTOR kinase inhibitors showed greater anticancer effects compared with rapalogs, thus leading to a strong interest in developing these agents. They are known to act by targeting the ATP-binding site in the mTOR kinase domain which is responsible for the functioning of both mTOR complexes [65]. Therefore, unlike rapamycin, which blocks only mTORC1, these agents inhibit mTORC1 and mTORC2 [65,73,76,77], and are known as mTORC1/mTORC2 dual inhibitors [65]. Some of these compounds also target the PI3K pathway, which could be due to the close relationship between the kinase domains of mTOR and PI3K [65,77]. Thus, these compounds are called mTOR/PI3K dual inhibitors [65,78]. Targeting the PI3K pathway further regulates mTORC2, which is known to be activated by PI3K. The mechanism of this activation is not clearly understood [78]. These inhibitors also show robust inhibition of mTORC1 compared with rapalogs [73,76]. Because the phosphorylation of AKT is regulated by PI3K and mTOR, these inhibitors minimize the activation of AKT. This feature of these compounds can overcome the rapalog-induced hyperactivity of AKT signaling. As previously mentioned, PI3K/AKT/mTOR signaling is the prominent feature in the majority of cancers. These two factors have served as a rationale for using these inhibitors [65].

As the name suggests, these inhibitors work by competing with ATP to bind to the kinase domain of mTOR. ATP is responsible for phosphorylation of various mTOR proteins. Inhibiting the binding of ATP to these domains prevents the phosphorylation of the signaling proteins [77]. The difference in the IC50 s between mTOR/PI3K inhibitors and pan-mTOR inhibitors is the basis for selectivity of these compounds. The dual mTOR/PI3K inhibitors inhibit the kinases at similar concentrations, whereas the pan-mTOR inhibitors inhibit mTOR at much lower concentrations than those required for PI3K [77]. Most of the ATP-competitive inhibitors are in clinical trials alone or in combination therapy [65,76].

Dual PI3K/mTOR inhibitors

Several PI3K/mTOR inhibitors have been developed. The advantage of these compounds is the already existing PI3K inhibitors, which were found to inhibit the mTOR pathway and were in fact also PI3K/mTOR inhibitors [65]. PI-103, a PI3K inhibitor that showed tumor-suppressive activity in various human tumor models, formed the basis for the development of some of the first compounds from the PI3K/mTOR inhibitor class [65,79]. This parent compound showed poor *in vivo* pharmacokinetics and therefore was not evaluated as a PI3K inhibitor in humans [65]. The first compounds that were developed from PI-103 were PI-450 and PI-620, both of which showed improved pharmacokinetic properties [79]. After that, other derivatives, such as PI-540 and GDC-0541, were developed and showed better pharmacokinetic antitumor activities [80].

Although all these derivatives showed better efficacy along with pharmacokinetic and metabolic activities, the imidazoquinoline derivative NVP-BEZ235 became the first successful inhibitor in clinical trials from this class. When given in combination with inhibitors of other mitogenic pathways, this compound showed enhanced efficacy [79]. In breast cancer trials, this compound showed marked reduction in the phosphorylation of various mTOR signaling proteins including AKT and 4EBP1 and better antitumor activity compared with everolimus. It is also a reversible inhibitor and blocks the p110α subunit of PI3K and mTOR. It is an oral inhibitor and no dose-dependent toxicities have been reported with the use of this compound [65,76]. The high level of activity of this compound led to its use in clinical trials for breast cancer and other solid tumors [65]. GDC-0941 is another orally bioavailable PI3K inhibitor that showed success in clinical trials [81]. Being a PI-103 derivative, it showed some difference in the isoform selectivity but had comparable antiproliferative activity. XL765, another dual inhibitor, showed phosphorylation of various PI3K/mTOR pathways – AKT was the most significant. This led to a reduction in tumor proliferation, and the compound went on to become the first oral drug with successful results in clinical trials [65]. In contrast to NVP-BEZ235, XL765 is a potent pharmacodynamic inhibitor of PI3K and has stable pharmacokinetic properties [76]. SF1126, the vascular targeted conjugate of a pan-PI3K inhibitor, LY294002, has recently been developed as an inhibitor of mTOR catalytic activity. Its conjugate showed enhanced solubility and targeting to tumor sites. Owing to its vascular targeting nature and the ability to inhibit multiple kinases, it shows similar *in vivo* efficacy compared to the other inhibitors, even though the *in vitro* results showed less potency. This compound is known to inhibit tumor progression based on the tumor microenvironment and inhibition of cell signaling. It is being tested extensively in solid tumors, and the clinical trials for these drugs are also being extended to include hematopoietic malignancies, such as chronic lymphocytic leukemia and certain types of lymphoma [65]. GDC-0980, which is also an oral PI3K/mTOR inhibitor, showed success in Phase I clinical trials and is currently in Phase II clinical trials. The dose-limiting side effects of this compound are rash and hyperglycemia [79].

mTORC1/mTORC2 inhibitors

Nonselective inhibition of mTORC1/2 enhances antitumor activity by improved inhibition of PI3K/AKT pathways [79]. These compounds suppress the activity of 4E-BP1 and S6K1 and inhibit phosphorylation of AKT at Ser473 [76]. Several of these inhibitors have been recently developed, including Torin1, PP30, OSI-027, Ku-0063794, PP242, WYE-687, INK128, WAY-600, AZD2014 and AZD8055 [78]. Out of these, INK128, AZD8055, OSI-027 and AZD2014 are already in clinical trials [65]. In contrast to PI3K inhibitors, they inhibit mTORC1/2 without inhibiting other kinases and are efficacious at concentrations in the nanomolar range [78]. These inhibitors are in clinical trials for gynecological tumors, lung cancer, breast cancer and liver cancer. The first inhibitor to be developed was PP242 which could completely inhibits both mTOR complexes and inhibits dephosphorylation in the rapamycin-resistant sites of 4EBP1 [79]. This compound works by inducing apoptosis and thereby causing cytoreduction. In addition to being more effective than rapamycin in leukemia and multiple myeloma cells, the major advantage of this compound is its weak immunosuppressive activity. Rapamycin's immunosuppressive activity is a major drawback of rapamycin and thus this compound has a better potential to translate into a therapeutic drug [65]. This compound has already been tested in 25 clinical trials [79]. Along with PP242, PP30 is also a selective inhibitor of mTOR kinase. Some of the inhibitors were developed from the parent compound WAY001. These included WAY600, WYE687 and WYE354. They showed anticancer properties against various cancer cell lines but their clinical development ceased because of their unfavorable pharmacological properties [65] (Table 1).

TABLE 1

Current class and status of some mTOR-targeting anticancer drugs

Name	Class	Target	Status
Rapamycin (sirolimus)	mTOR inhibitor	mTORC1	Clinical application [66–69]
Temsirolimus (CCI-779)	mTOR inhibitor	mTORC1	Clinical application [66,68,69,71]
Everolimus (RAD001)	mTOR inhibitor	mTORC1	Clinical application [66,68,69,71]
Ridaforolimus (AP23573)	mTOR inhibitor	mTORC1	Clinical application [67–69]
PI-103	PI3K/mTOR inhibitors	PI3K pathway	Stop [65,79]
PI-450	PI3K/mTOR inhibitors	PI3K pathway	Stop [79]
PI-620	PI3K/mTOR inhibitors	PI3K pathway	Stop [79]
PI-540	PI3K/mTOR inhibitors	PI3K pathway	Stop [79]
GCD-0541	PI3K/mTOR inhibitors	PI3K pathway	Stop [80]
WYE354	PI3K/mTOR inhibitors	PI3K pathway	Stop [32]
WJD008	PI3K/mTOR inhibitors	PI3K pathway	Stop [32]
WAY600	PI3K/mTOR inhibitors	PI3K pathway	Stop [32]
Ku0063794	PI3K/mTOR inhibitors	PI3K pathway	Stop [32]
NVP-BEZ235	PI3K/mTOR inhibitors	PI3K/mTOR pathway	Clinical trial [65,76,78,79]
GCD-0941	PI3K/mTOR inhibitors	PI3K pathway	Clinical trial [81]
XL765	PI3K/mTOR inhibitors	PI3K/mTOR pathway	Stop [65,76]
SF1126	PI3K/mTOR inhibitors	pan-PI3K inhibitor	Stop [65]
LY294002	mTOR inhibitor	mTOR pathway	Stop [65]
GDC-0980	PI3K/mTOR inhibitors	PI3K/mTOR pathway	Phase II clinical trial [65,79]
Torin1	mTORC1/mTORC2 inhibitors	PI3K/AKT/mTOR pathway	Experimental development [78,79,82,83]
PP30	mTOR inhibitor	mTOR kinase inhibitor	Experimental development [78]
OSI-027	mTOR inhibitor	mTOR pathway	Clinical trial [65,78]
Ku-0063794	mTOR inhibitor	mTOR pathway	Experimental development [78]
PP242	mTOR inhibitor	mTOR pathway	Experimental development [78,79]
WYE-687	mTOR inhibitor	mTOR pathway	Stop [78]
INK-128	mTOR inhibitor	mTOR pathway	Clinical trial [65,78,79]
WAY-600	mTOR inhibitor	mTOR pathway	Stop [78]
AZD2014	mTOR inhibitor	mTOR pathway	Clinical trial [65,78,79]
AZD8055	mTOR inhibitor	mTOR pathway	Clinical trial [65,78,79]
Rapalink	mTOR inhibitor	mTOR pathway	Experimental development [73,79,82]
Everolimus + PLS-123	Combination	mTOR inhibitor + BTK inhibitor	Experimental development [87]
Everolimus + fulvestrant	Combination	mTOR inhibitor + anti-estrogens	Experimental development [94]
Everolimus + exemestane	Combination	mTOR inhibitor + anti-estrogens	Clinical application [94]
Rapamycin + STX-0119	Combination	mTOR inhibitor + STAT2 inhibitor	Experimental development [95]
Rapamycin + sorafenib	Combination	mTOR inhibitor + VEGFR inhibitor	Experimental development [81]

Abbreviations: BTK, Bruton's tyrosine kinase; STAT, signal transducers and activators of transcription; VEGFR, vascular endothelial growth factor receptor.

Torin1, a compound that showed inhibition of mTOR kinase during the screening of mTOR inhibitors, is a potent mTORC1/2 inhibitor [78,82]. Torin1 and its sister compound torin are quinolone derivatives [79]. *In vitro* studies showed that torin1 inhibited both mTOR complexes at a very low concentration, between 2 and 10 nM [78,83]. Torin1 is, at a minimum, 200-fold more selective toward mTOR compared with other kinases, including PI3K, which makes torin1 a highly selective mTOR inhibitor [78]. The advantage of torin1 over rapamycin is that it is more effective at inhibiting tumor proliferation and it has no effect on the stability of mTORC1 or mTORC2 [78,80]. A recent study suggests that the effects of torin1 are caused by the suppression of the rapamycin-insensitive function of mTORC1 and not the inhibition of mTORC2 [80]. This is an indication that these inhibitors can be used for revealing the rapamycin-insensitive domains of mTORC1 without investigating mTORC2 [78].

AZD8055 and AZD2014, which are currently in clinical trials, are orally bioavailable inhibitors. AZD8055 is in clinical trials for advanced solid tumors such as hepatocellular carcinoma [65]. It showed a 1000-fold difference in potency for mTOR compared with all the classes of PI3K isoforms [78]. AZD2014 is currently being tested in combination with other chemotherapeutic drugs for lymphoma, breast cancer and lung cancer [79]. INK128 is another orally bioavailable potent and selective mTORC1/2 inhibitor [65]. It inhibits mTOR at a subnanomolar level and it has selectivity for mTOR among >400 kinases [78]. INK128 alone or in combination inhibits angiogenesis and tumor proliferation [78]. It is also efficacious in rapamycin- and PI3K-resistant tumors [65].

Even though ATP-competitive inhibitors of mTOR are promising agents for cancer, they have certain limitations. They show efficacy in some of the rapamycin-insensitive tumors but are not efficacious in other cancer cells such as colon cancer cells [65]. Treatment of the breast cancer cell line MCF-7 with these inhibitors leads to development of resistance because of mutations in the kinase domain of mTOR [73]. The emergence of this mutation hyperactivates mTORC1 and mTORC2. Interestingly, in some tumors, the mutations are found without treatment with these inhibitors. This suggests that many tumors are innately resistant to these inhibitors [73]. The efficacy of these compounds in tumors with specific genetic lesions is still obscure. Development of biomarkers is required for predicting the efficacy of these agents [65]. The restoration of AKT by PI3K-independent pathways has been reported upon long-term use of these inhibitors [73]. These compounds inhibit multiple kinases, where inhibition of PI3K can be useful for antitumor activity, but in other cases it can cause adverse effects. PI3K has various roles in cell differentiation and

proliferation, such as glucose metabolism and regulation of hematopoietic stem cells. Inhibiting its activity can lead to hyperglycemia and hematological insufficiency [65].

New inhibitors are needed to overcome these limitations. Thus, a third generation of inhibitors was developed [73,82]. RapaLink was developed in 2016 [79]. It is a bivalent mTOR inhibitor that shows efficacy in tumors that are resistant to first- and second-generation inhibitors [73,79,82]. It has two drug-binding sites: an allosteric and a kinase site. Along with being effective in tumors having mutations, it also shows inhibition of mTOR-activated tumors [82]. It has a potential as an anticancer agent but is still in experimental development [73]. The ability of these compounds to overcome resistance caused by first- and second-generation inhibitors can guide the exploration of new bivalent kinase inhibitors and assure further clinical development [82].

Combinational therapy

The use of mTOR inhibitors as monotherapy seems to be insufficient for most tumors. Considering the complex crosstalk between the signaling pathways of cancer onset and progression, mTOR inhibitors might not be sufficient to produce the desired clinical response as single agents. The combination of mTOR inhibitors and other therapies is needed to produce synthetic lethality and achieve the best effect. Currently, multiple clinical studies evaluating mTOR inhibitors combined with chemotherapy are in progress. Some studies are investigating mTOR inhibitors and other targeted drugs such as VEGFR and EGFR inhibitors, tyrosine kinase inhibitors or cytotoxic chemotherapy drugs. In addition, another key consideration is the RAS/RAF/MAPK connection to multiple mTOR channels leading to cross-inhibition, cross-activation and pathway convergence [84].

The combined inhibition of both mTOR complexes with novel mTOR inhibitors and inhibition of activated receptor tyrosine kinase completely abolishes AKT signaling, leading to cancer cell death and tumor regression *in vivo* [85]. Another resistance mechanism to dual mTORC1/mTORC2 inhibitors was observed in the PP242 study. The feedback activation of ERK in the MAPK pathway after mTOR inhibition was partially overcome by mTORC1/mTORC2 and MEK blockade [86]. Multi-level feedback and crosstalk among different signaling pathways emphasize the need to further evaluate combined inhibition methods in targeted anticancer therapies.

Rapalogs have antineoplastic activity in various tumors and are frequently combined with other anticancer agents. The efficacy of rapalogs combined with endocrine therapy for advanced breast cancer was evident in the Bolero-2 trial, which showed a median progression-free survival (PFS) of 6.9 months for everolimus and exemestane, whereas the PFS was only 2.8 months with exemestane alone [26]. A novel irreversible Bruton's tyrosine kinase (BTK) inhibitor: PLS-123, which has potent and selective antitumor activity, exerts synergistic activity in relieving mantle cell lymphoma (MCL) proliferation and metastasis [87] when used in combination with the mTOR inhibitor everolimus.

The antiproliferative activity of LY2835219, a selective cyclin-dependent kinase (CDK)4/6 inhibitor [88,89] only has a limited effect in head and neck squamous cell carcinoma (HNSCC) as a single-agent treatment [90]. By having investigated that LY2835219 could inhibit the activation of AKT and ERK [91], it is possible that the combination of LY2835219 with an mTOR inhibitor might be more effective than either drug alone, and this postulation has been verified *in vivo* and *in vitro*, suggesting that the combinational treatment with LY2835219 and an mTOR inhibitor could be a practicable clinical therapy for HNSCC [92,93].

The combination of anti-estrogen fulvestrant and the mTOR inhibitor everolimus (which has already been approved for combination with exemestane and vistusertib – a novel mTOR inhibitor) increases PFS [94]. In addition, fulvestrant when used in combination with CDK4/6 inhibitors or mTOR inhibitors provides the greatest promise regarding the balance of benefit and toxicity [94]. The mTOR pathway associated with the STAT3 pathway, via downstream YKL-40 protein along with combination therapy of the STAT3 inhibitor STX-0119 and rapamycin, could elevate the effect of both drugs significantly. This combination is being developed as a novel therapy against TMZ-resistant relapsed gliomas [95]. Inhibiting the mTOR signaling pathway and VEGFR pathway at the same time by combining the mTOR inhibitor rapamycin and the VEGFR inhibitor sorafenib has a potential therapeutic effect on the rare perivascular epithelioid cell tumor (PEComa) [81].

Concerns related to the toxicity of mTOR inhibitors

A growing body of evidence suggests that mTOR inhibitors exhibit rare to severe side effects, often related to their immunosuppressive properties [96,97]. Some common dose-limiting toxicity mechanisms have already been investigated, including oral ulcers caused by the direct toxic effects of mTOR inhibitors on the oral and nasal mucosa [98], dermatologic disorders such as stomatitis and rash (possibly caused by the blockade of the EGF pathway and can be resolved spontaneously) [99], metabolic disorders such as diabetes and hyperlipidemia (which increase in a dose-dependent manner and could be effectively controlled by dose interruption or reduction) [100] and impaired wound healing, which results from blocking the growth signals required for endothelial and fibroblast

TABLE 2

Commonly encountered adverse and toxic effects associated with mTOR inhibitors

mTOR inhibitory class	Adverse/toxic effect
Rapamycin and its analogs	Nausea/vomiting, fatigue, hyperglycemia, anemia, pulmonary and metabolic toxicities, stomatitis, mucositis and mood alterations [97,100,104]
ATP-competitive mTOR inhibitors	Nausea/vomiting, fatigue, hyperglycemia, diarrhea, rash, liver dysfunction, pneumonia, pyrexia and hematologic toxicities [97,100,104]
Dual (mTOR/PI3K inhibitors)	Nausea/vomiting, fatigue, hyperglycemia, decreased appetite, diarrhea, rash and mucositis [100,104]
Combinational therapy/agents	Nausea/vomiting, fatigue, hyperglycemia, rash, liver dysfunction, mood alterations and hematologic toxicities, and impaired wound healing [100]

Abbreviations: mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3 kinase.

proliferation, thereby limiting fibrosis and also inhibiting VEGF and nitric oxide, which are mediators of vascular, inflammatory and immune functions in skin wounds [101,102]. Although some mechanisms of adverse effects are not understood yet, including renal dysfunction such as proteinuria and delayed graft function, hematological abnormalities like anemia and microcytosis, as well as hormonal problems, such as impaired gonadal function and ovarian toxicity [100,103,104], rarely lead to discontinuation of mTOR inhibitor therapies because they can be overcome by either adjusting the dose or giving other medications at the same time. The various classes of mTOR inhibitors vary in their toxicity profile. Table 2 summarizes the major adverse and toxic effects associated with the various classes of mTOR inhibitors.

Concluding remarks and future perspectives

Understanding the relevant signaling pathway, including the feedback loop, is a requirement for understanding the biological consequences of interfering with this intricate system. Identifying the components of the signaling pathway that initiates tumor formation is crucial to developing an intervention strategy. Because mTOR inhibitors can exert antitumor effects through a variety of mechanisms of action, it is still challenging to determine biological factors that predict the efficacy of anti-mTOR inhibitors. In addition, crosstalk between the mTOR pathway and other pathways can cause compensatory circuits for tumor cells to escape antitumor actions. Drug combinations for multiple pathways have been used to overcome this resistance, but it is unclear which strategy will yield greater therapeutic benefits. In addition, the use of multiple drugs might increase side effects. Further comprehension of the molecular mechanisms of the mTOR signaling network is necessary for designing safe and effective treatment therapy.

However, the overall response rate of major solid tumors treated with a single-agent rapalog is not sufficient, especially in advanced metastatic tumors. The existence of a therapeutic window for mTOR inhibitors and the side effects of dual PI3K/mTOR inhibitors and of ATP-competitive mTORC1/mTORC2 inhibitors are at present not well known and must be clearly defined. Regarding mTOR inhibitors, could it be possible to specifically target mTOR signaling in cancers without affecting the functions of healthy stem cells? It is important to emphasize that there are subtle differences in how healthy stem cells and cancer cells utilize the same signaling pathways.

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