REVIEW
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Novel insights into mTOR signalling pathways: A paradigm for targeted tumor therapy

Oivind Riis[1], Andreas Stensvold[1], Helge Stene-Johansen[2], Frank Westad[2], Rabia Mehmod[2*]

[1]Industrial Biotechnology, Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad 44090, Pakistan. [2]Shifa College of Pharmaceutical Sciences, Shifa Tameer e Millat University, Islamabad 04403, Pakistan.

***Corresponding to:** Rabia Mehmod, Shifa College of Pharmaceutical Sciences, Shifa Tameer e Millat University, H-12 Islamabad, Islamabad 04403, Pakistan. E-mail: rrabiagul2022@gmail.com.

Author contributions

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Abbreviations

mTOR, mammalian target of rapamycin; N-HEAT, NH2-terminal HEAT; M-HEAT, middle HEAT; FAT, FRAP-ATM-TTRAP; FRB, FKBP12-rapamycin-binding; PH, pleckstrin homology; PKC, protein kinase C; SGK, serum and glucocorticoid-induced protein kinase 1; EGFR, epidermal growth factor receptor; IGFR, insulin-like growth factor receptor; PI3K, phosphatidylinositol-3-kinase; PDK1, pyruvate dehydrogenase kinase 1; Akt, protein kinase B; MAPK, mitogen-activated protein kinase; GPCR, G protein-coupled receptor; AMPK, AMP-dependent kinase; ATF6, activating transcription factor 6; REDD1, regulated in development and DNA damage response 1; AMPKβ, the regulatory subunit of AMPK; GATOR1, GAP activity toward the Rag GTPases 1; RNF152, ring finger protein 152; SKP2, S-phase kinase-associated protein 2; TRAF6, TNF receptor-associated factor 6; IKKα, IkappaB kinase-alpha; RTKs, receptor tyrosine kinases; PDGFR, platelet-derived growth factor receptor alpha; FGFR, fibroblast growth factor receptor; PIPK, phosphatidylinositol phosphate kinase; ABC, ATP-binding cassette; TSC, Tore stem cells; TP53, tumor suppressor p53; BCL2, B-cell lymphoma 2.

Citation

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Abstract

As a crucial protein kinase, the mammalian target of rapamycin (mTOR) intimately controls essential cellular processes like cell development, proliferation, metabolism, and other crucial activities. Different cancers and disorders have been linked to imbalances in mTOR's regulatory systems. Multiple mTOR inhibitor therapy has recently acquired popularity as a method of treating cancers brought on by abnormal signal transduction pathways. We also explore potential processes behind tumor cell resistance to mTOR inhibitors and suggest workarounds to overcome this challenge. We hold the potential to pioneer cutting-edge methods for tumor therapy by methodically examining the complex mTOR signaling system and its regulatory complexity. Increasing our knowledge of mTOR-related mechanisms not only creates opportunities for cutting-edge methods to target and treat cancers but also has the potential to improve patient outcomes and general quality of life significantly. This review paper explores the most recent developments in understanding mTOR signaling pathways and the use of mTOR inhibitors in treating tumors.

Keywords: mTOR signal transduction pathway; tumor; drug resistance; targeted therapy

Introduction

The mammalian target of rapamycin (mTOR) is a highly conserved protein kinase that phosphorylates the serine/threonine and tyrosine residues of its downstream substrate [1]. Various life processes, including cell development, metabolism, aging, and regeneration, are regulated by mTOR in response to environmental signals such as amino acids, nutrition, and growth hormones [1]. The MTORC1/2 complex contains mTOR; mTORC1 primarily governs cell growth and metabolism, whilst mTORC2 regulates cell proliferation and survival [2]. The mTOR signaling pathway is essential for controlling mammals' physiological processes and metabolism, and abnormal regulation contributes to several pathophysiological conditions, including aging, Alzheimer's disease, diabetes, obesity, and tumors, particularly during the tumorigenesis process [3]. mTOR inhibitors are commonly used as immunosuppressants and have been approved for treating human malignancies. Rapamycin and its homologues can inhibit mTORC1 activity but do not inhibit mTORC2 activity in the short term [4].

mTOR activation can promote tumor growth in various ways, including growth factor receptor signaling activation, angiogenesis, glycolytic metabolism, lipid metabolism, and tumor cell migration [5]. Therefore, mTOR is a promising target for tumor therapy, especially mTOR inhibitors, which are widely used in the research of tumor-targeted therapy, organ transplantation, and rheumatoid arthritis. Clinical studies of these inhibitors have confirmed their effectiveness in inhibiting cell growth and proliferation. However, the potential toxicity of mTOR inhibitors has made the results of clinical trials less impressive, and improved treatment strategies are needed to benefit from such small molecule compounds. The study of mTOR inhibitors reveals the metabolic plasticity of tumors, which can trigger other mechanisms independent of mTOR to compensate for the obstruction of mTOR activity, thereby allowing tumor cells to receive nutrients to promote their growth and proliferation. This review briefly introduces mTOR's structure and biological function, the potential mechanism of tumor cell resistance to mTOR inhibitors, and practical strategies to deal with drug resistance.

mTOR and the assembly of its complexes

Structural Characteristics of the mTOR Protein

The protein encoded by the human mTOR gene contains 2,549 amino acids and has multiple domains; it mainly includes N-HEAT (NH2-terminal HEAT), M-HEAT (middle HEAT), FAT (FRAP-ATM-TTRAP), FRB (FKBP12-rapamycin-binding) and kinase domains [6]. Rapamycin binds to the FRB on mTOR, preventing the downstream substrate from entering the active site, thereby inhibiting mTOR kinase activity. At the same time, the ATP-competitive mTOR inhibitor Torin 1 can directly target the mTOR catalytic site and inhibit mTOR kinase activity [7].

The formation of mTOR complexes

According to research so far, the mTORC1 complex is primarily made up of five subunits, including the regulatory subunit Raptor (regulatory-associated protein of mTOR), the mammalian lethal with mLST8 SEC13 protein 8, which is made up of PRAS40 (a 40 kDa proline-rich Akt substrate), and Debtor (a DEP domain-containing protein 6) [8]. Eukaryotic translation initiation factor 4E binding proteins and ribosomal protein S6 kinase are downstream targets of mTORC1. One indicator of its action for 1) is its amount of phosphorylation [9]. Several phosphorylation sites exist on the catalytic component mTOR and the regulatory subunit Raptor, including S1261, T2164 for mTOR and S696, T908 for Raptor. The phosphorylation of these locations may have an impact on mTORC1 activity. The harmful regulatory subunits of mTOR, PRAS40 and Deptor, are recruited to the mTORC1 complex when the activity of mTORC1 is down-regulated, thereby reducing the activity of mTORC1 [10]. When mTORC1 activity is increased, it can phosphorylate the harmful regulatory subunits PRAS40 and Debtor using its kinase activity. This reduces the negative regulatory subunit's inhibitory function and increases mTORC1 activity [11].

The mTORC2 complex consists of six subunits (catalytic subunit mTOR, fundamental subunit Rictor (rapamycin-insensitive companion of mTOR), and beneficial regulatory subunit mSin1 (mammalian stress-activated) proteins kinase-interacting protein 1), mLST8 (mammalian lethal with SEC13 protein 8), and Protor1/2 (protein observed with) Rictor 1/2), and the adverse regulatory subunit Deptor [12]. AGC kinase (cAMP-dependent protein kinase A/cGMP-dependent protein kinase G/protein kinase C) is the downstream substrate of mTORC2. The activation of mTORC2 depends on the PH (pleckstrin homology) domain of mSIN1 binding to phosphatidylinositol 3,4,5-triphosphate on the plasma membrane, and there are also multiple phosphorylation sites on Rictor and mLST8 to regulate the activity of mTORC2 [13].

mTORC1 and mTORC2 complex interaction

The catalytic component known as mTOR plays a crucial role in two separate complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). These complexes are distinguished by their unique composition and functions. The mTORC1 complex comprises three primary components, mTOR, mLST8, and Raptor, which play crucial roles in its functioning. Similarly, the mTORC2 complex consists of mTOR and mLST8, where Raptor is substituted by Rictor, a rapamycin-insensitive partner of mTOR (Rictor) [14, 15]. The component mLST8 can assist mTOR in establishing a stable kinase activation loop.

In contrast, Raptor and Rictor have a role in facilitating the recruitment of substrates to the mechanistic target of rapamycin (mTOR). Inhibitory subunits PRAS40 and DEPTOR, which contain the DEP domain and interact with mTOR, round out mTORC1's key components. DEPTOR, as well as the regulatory subunits Protor1/2 and mSin1, are involved in the functioning of mTORC2. Due to the pivotal role played by the mTOR pathway in many disorders, notably cancer, there has been substantial advancement in therapeutic strategies aimed at modulating critical components of this circuit. The initial iteration of mTOR inhibitors, including rapamycin and its analogues (rapalogs), bind to the FRB domain of mTOR through the protein FKBP12 [16]. The interaction impedes the catalytic crevice inside the mTOR kinase domain, impeding the entry of substrates. Rapamycin mainly suppresses the activity of mTORC1, with minimal impact on mTORC2. Significantly, rapamycin has diverse levels of efficacy in inhibiting various mTORC1 substrates. The second-generation inhibitors, namely PP242, INK128, and Torin-1, act as ATP analogues by competitively binding to the kinase domain of mTOR. This binding effectively inhibits mTORC1 and mTORC2. The compound known as RapaLINK, which is a third-generation inhibitor, has been developed to address drug resistance in mTOR-mutated malignancies. This inhibitor combines the properties of rapamycin and ATP analogues, resulting in a synergistic effect that helps to attenuate drug resistance [17].

In contrast to the relatively restricted comprehension of mTORC2 stemming from the lack of precise inhibitors, it is well-established that growth stimuli, such as insulin, elicit activation of mTORC2. Upon activation, the mTORC2 complex initiates the phosphorylation of Ser473 residue on AKT1 protein, hence playing a significant role in various essential physiological processes such as apoptosis, glucose metabolism, and other pathways regulated by AKT. Additionally, mTORC2 controls PKC (protein kinase C), a crucial component of cytoskeleton rearrangement, and SGK (serum and glucocorticoid-induced protein kinase 1), a crucial component of ion transport (Figure 1).

Controlling the regulation of the mTOR signaling pathway

Managing the activity of mTORC1

Various signals, such as growth factors, cellular energy, stress, and nutrients, regulate mTORC1 activity. These signals mainly regulate

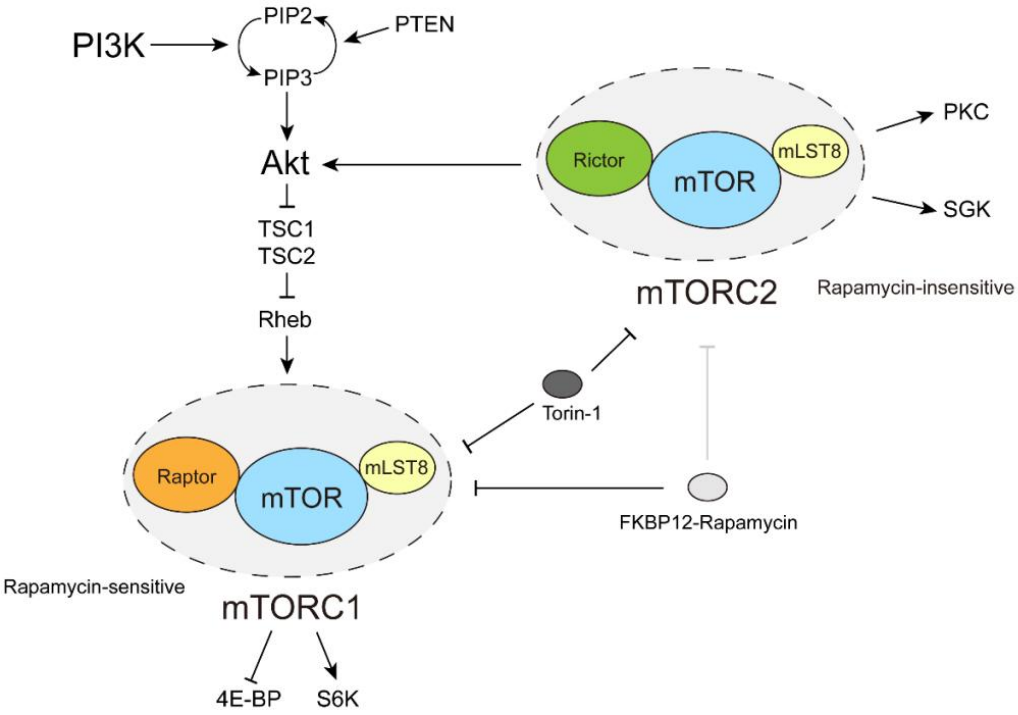

Figure 1 The mTOR pathway. The main mTORC1 and mTORC2 signaling pathways. Reproduced with permission. Yang M, Lu Y, Piao W, Jin H. The translational regulation in mTOR pathway. *Biomolecules* 2022;12(6):802. Copyright 2022, MDPI.

the activity of mTORC1 in two ways. One is that the small GTPase Rheb (Ras homolog enriched in the brain) located on the membrane surface responds to upstream signals such as growth factors, cell energy, and stress. Combining GTP or GDP in an activated or deactivated state and then interacting with mTORC1 to regulate its activity, the tuberous sclerosis protein complex (TSC) plays an essential regulatory role. As the Rheb enzyme activating protein, it promotes the hydrolysis of GTP bound to Rheb and inactivates Rheb to achieve regulation of mTORC1 activity. One is that when nutrients such as amino acids are sufficient, the small G protein Rags is activated, binds to mTORC1 and helps it locate on the surface of the lysosomal membrane, thus regulating its activity. According to recent research, this mechanism is controlled by the heterodimers RagA/RagB and RagC/D. Regulators interact with Raptor to stabilize the GTP-bound RagA/RagB and GDP-bound RagC/D complexes on lysosomal membranes. To control its activity, mTORC1 is attracted to the lysosomal membrane's outermost layer. The link between Rheb and mTOR on lysosomes is necessary for growth factor-induced activation of mTORC1. Growth factors EGF and IGF are recognized by growth factor receptors like EGFR (epidermal growth factor receptor) and IGFR (insulin-like growth factor receptor). The signaling pathway consisting of PI3K (phosphatidylinositol-3-kinase), PDK1 (pyruvate dehydrogenase kinase 1), and Akt (protein kinase B) can activate them and further activate them, increasing the activity of mTORC1 [18]. The activity of mTORC1 can also be increased by the mitogen-activated protein kinase (MAPK), which is located laterally of the G protein-coupled receptor (GPCR) [19]. In addition to detecting growth stimuli, mTORC1 also detects variations in cellular energy and reacts to them. When cells are low on energy, the AMP/ATP ratio rises, activating the energy sensor AMPK (AMP-dependent kinase), phosphorylating TSC, and downregulating mTORC1 activity [20]. ATF6 (activating transcription factor 6) can increase mTORC1 activity by stimulating the expression of Rheb, hence improving cell survival when cellular energy deficit causes endoplasmic reticulum stress [21]. In addition, mTORC1 can sense and respond to stress signals such as intracellular oxygen levels and DNA damage. In hypoxia, the decrease in ATP level will activate AMPK, and the activated AMPK will promote the activation of TSC2, thus inhibiting the activity of mTORC1 [22]. Hypoxia can also activate TSC through REDD1 (regulated in development and DNA damage response 1), thereby blocking the activation of mTORC1 [23]. The DNA damage response pathway can induce the expression of p53 target gene AMPKβ (the regulatory subunit of AMPK) and TSC, resulting in the enhancement of the activity of the TSC complex, thereby inhibiting the activity of mTORC1 [24].

The lysosomal regulatory protein Rags mediate amino acid control of mTORC1 activity. Two complexes, GATOR1 (GAP activity toward the Rag GTPases 1) and GATOR2, control the activity of Rags. GATOR1 inhibits RagA/B's GTPase activity to block the activity of mTORC1, while DEPDC5's degradation by GATOR2 adversely impacts GATOR1 [25]. As a leucine sensor, SESN2 (sestrin 2) controls the activity of mTORC1. Leucine binds to SESN2 directly, causing SESN2 to separate from GATOR2 and release GATOR2, which increases the activity of mTORC1 [26]. E3 ubiquitin ligases RNF152 (ring finger protein 152) and SKP2 (S-phase kinase-associated protein 2) stimulate RagA ubiquitination and enhance RagA binding to GATOR1 to prevent the overactivation of mTORC1 by amino acids. MTORC1 activity is thereby inhibited [27]. The ubiquitin ligase TRAF6 (TNF receptor-associated factor 6) also catalyzes Akt and mTOR's K63 ubiquitination to support Akt and mTORC1's amino acid activation [28].

Regulating the activity of mTORC2

mTORC2 activation is primarily regulated by growth factors (Figure 2). PI3K activated by extracellular signals is converted into PIP3, which binds to the PH domain of mSIN1 to block the inhibition of mSIN1 on mTOR activity, thereby activating mTORC2 [29]. Meanwhile, PI3K can also promote the binding of mTORC2 and ribosome. To up-regulate mTORC2 activity [30]. Activated mTORC2 is in the plasma membrane, mitochondria and endosomal vesicles [31]. In addition, IKKα (IkappaB kinase-alpha) can interact with mTORC2 and enhance Akt kinase activity [32]. mTORC2 activity is also regulated by cellular energy and nutrition. The energy sensor AMPK inhibits the activity of mTORC1 and further reduces the

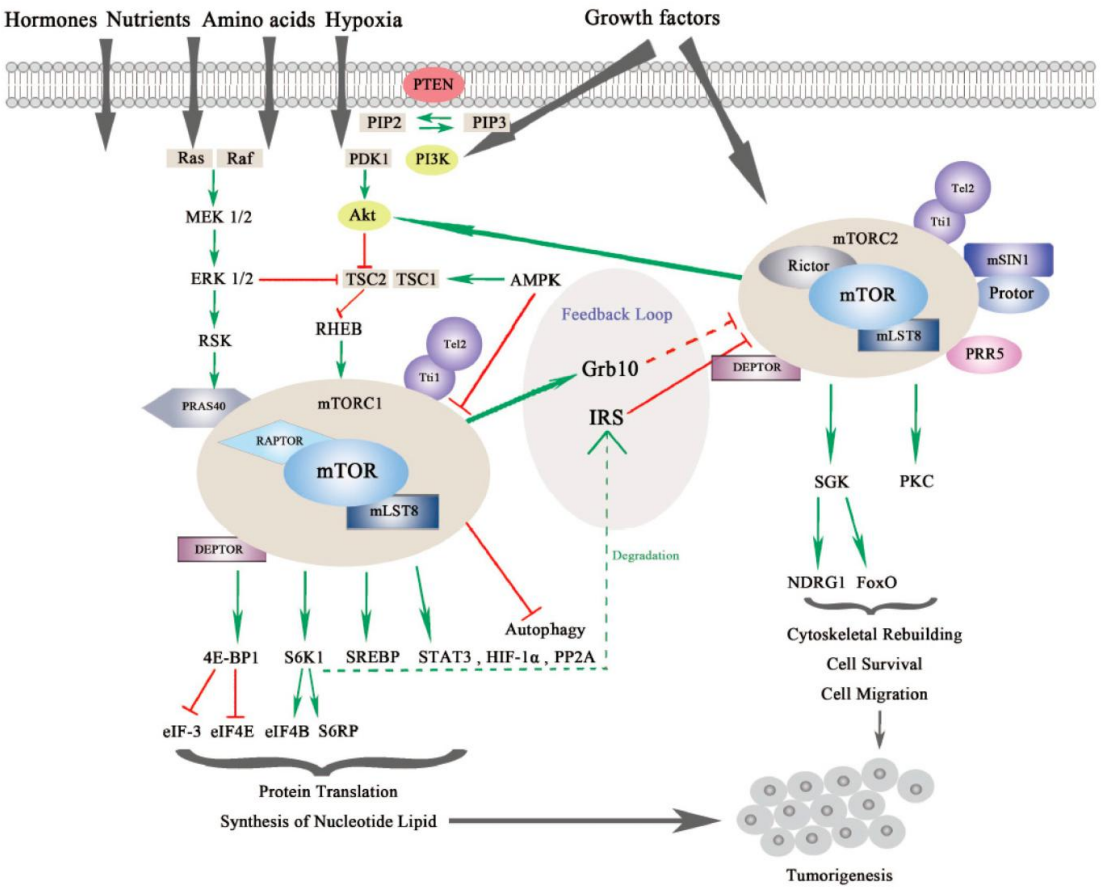

Figure 2 The mTOR signal transduction pathway. Reproduced with permission. Tian T, Li XY, Zhang JH. mTOR Signaling in Cancer and mTOR Inhibitors in Solid Tumor Targeting Therapy. *Int J Mol Sci* 2019;20(3):755. Copyright 2019, MDPI.

inhibition of mTORC1 to mTORC2, thereby activating mTORC2, so mTORC2 may help cells adapt to low energy levels, and glutamine starvation treatment promotes the activation of mTORC2 [33]. The structure of the mTORC2 complex is regulated by phosphatidic acid, and PA can maintain its structural stability [34].

mTORC2 activity is also regulated by mLST8 ubiquitination. tumor necrosis factor receptor associated factor 2 (TRAF2) positively regulates the ubiquitination modification of mLST8, weakens the interaction between mLST8 and mSIN1, and damages the integrity of mTORC2. Thus inhibiting the activity of mTORC2 [35].

Exploring the link between mTOR and tumor diseases

A mTOR signaling pathway is involved in many physiological activities in the cell, and its imbalance will affect the normal physiological function of the cell, damage human immune function, and cause tumors. Mutations in the mTOR pathway, mTOR complex component amplification or overexpression, and mutations or deletion of mTOR's negative regulators are more likely responsible for abnormal mTOR activation in human tumors.

Dysregulated activation of upstream signaling pathways in mTOR

RTKs (receptor tyrosine kinases) use PI3K to control mTORC1 and mTORC2 activity. RTKs or other mTOR upstream members, such as PIK3CA, the RAS, Akt, and PTEN (phosphatase and tensin homologue deleted on chromosome), abnormally activated 10) mTOR can be inappropriately activated by both mutation and loss [36]. The EGFR gene frequently exhibits aberrant expression and mutation in human tumors. In 25% to 82% of colorectal tumors, 30% to 50% of glioblastomas, and between 5 and 20% of non-small cell lung cancers, EGFR mutations are more prevalent [37]. About 5% of gastrointestinal cancers have platelet-derived growth factor receptor alpha (PDGFR) mutations, and 5% to 10% of oesophageal cancers, brain tumors, and endarteriosarcomas have PDGFR amplification [38]. FGFR (fibroblast growth factor receptor) mutations are widely present in human tumors. It has been reported that FGFR1/2/3 mutations frequently occur in head and neck, uterine, and colon cancers, and FGFR1/2 is abnormally amplified in breast cancer [39]. Translocation of FGFR gene fragments is also associated with cancer (including multiple myeloma) [40]. Tumor suppressor gene PTEN can negatively regulate signaling pathways such as PI3K, MAPK and FAK, and loss of function or mutation of PTEN often occurs in human malignant tumors (such as gastric cancer, cervical cancer and glioma, etc.). In addition, the mTOR gene is often misregulated, leading to various malignant tumors (such as adrenal cancer, etc.) [41].

Activation of downstream substrates in the mTOR signaling pathway

By controlling protein synthesis, cell survival, and growth, S6K1, 4EBP1, and eIF4 [42] are crucial mTOR downstream effectors in the formation of tumors. S6K1 was significantly overexpressed in breast cancer cell lines and primary tumors [43]. S6K1 was amplified in roughly 8% of primary breast tumors compared to normal tissues, showing that it is essential for carcinogenesis and patient prediction. S6K1 has been linked to cancer and glial cell transformation. A critical mechanism of migration and invasion is the phosphorylation of PIPK (phosphatidylinositol phosphate kinase) by S6K1, essential for developing tumour adhesion plaques and invasive pseudopodia [44]. S6K1 is a critical player in cell motility [45]. Clinical trials of recently

created S6K1 small molecule inhibitors revealed that these drugs may prevent breast cancer cells from colonizing and growing without adhesion, which suggests that using S6K1 inhibitors may prevent breast cancer from spreading locally and metastasizing. The analysis of p-4EBP1 shows that its overexpression is directly linked to the development of malignant tumors, and p-4EBP1 may be used as a prediction of carcinogenic signal and a critical molecular marker of tumour malignancy, especially in the areas of carcinogenic ability and signaling that promotes proliferation [46]. Additionally, it has been discovered that 4EBP1 levels affect whether tumour cells are sensitive to or resistant to inhibitors of the PI3K/Akt signaling pathway. The early and late relapse of renal cell carcinoma might be predicted based on the amount of 4EBP1/eIF4E activation [47]. Additionally, current research indicates that p-4EBP1 can be a promising biomarker for prognostic classification and treatment selection in ovarian cancer patients [48].

Harnessing mTOR inhibitors in tumor therapy

MTOR inhibitors are anticipated to be employed in treating conditions like tumors because mTOR is essential to the progression of tumors. Many mTOR inhibitors with various modes of action have been produced (Table 1), some of which are being tested in clinical studies in various neoplastic illnesses. Rapamycin and its derivatives (rapalogs) have been licensed in clinical treatment [49].

Exploring rapamycin and its analogues in tumor therapy

Rapamycin was initially identified as an antifungal agent, immunosuppressant, and antiproliferant. Rapamycin is unsuitable for treating human tumors due to its poor solubility and pharmacokinetics. Several water-soluble rapamycin analogues have been developed, such as Sirolimus and Everolimus, which show tumor-suppressive effects and have been clinically used in treating renal cell carcinoma. Moreover, clinical trials have shown that rapalogs have considerable efficacy in treating gastric cancer, non-small cell lung cancer and endometrial cancer [50]. In clinical trials, only rapamycin analogues are adjunctive in treating solid tumors. However, the tumour inhibition effect of rapamycin analogues is limited, and its incomplete inhibition of mTOR may lead to a low clinical trial success rate.

Inhibitors competing for ATP binding in mTOR pathway

Researchers have created numerous ATP-competitive mTOR inhibitors that specifically target mTORC1 and mTORC2 more fully suppress mTOR. These inhibitors may be more effective against tumours reliant on the mTOR signaling system. In contrast to rapamycin analogues, ATP-competitive mTOR inhibitors reduce cell proliferation and trigger apoptosis. A panmTOR inhibitor called MLN0128 (sapanisertib) has been investigated in mouse model animals against solid tumors such as bone and soft tissue malignancy and breast cancer. It shows anti-tumour solid effects in vitro and in vivo [51]. In colorectal cancer with PIK3CA mutations, MLN0128 could overcome tumour resistance to Everolimus and reduce tumour size by about 20% [52]. MLN0128 induced tumour shrinkage in patients with xenografted pancreatic neuroendocrine tumors, even in Everolimus-resistant tumors [53]. Another ATP-competitive mTOR inhibitor, PP242 (Tokinib), has good antitumor activity against malignant tumors such as gastric and colon cancer [54]. In platinum-resistant tumour cells that rely on the Akt-mTOR signaling pathway, we found that PP242 re-sensitizes platinum-resistant ovarian tumour cells to carboplatin in vitro and in vivo [55]. In addition, AZD2014 (Vistusertib) and its analogues are highly effective as ATP-competitive mTORC1/2 inhibitors in the treatment of estrogen receptor-positive breast cancer and also inhibit rapalogs and paclitaxel-resistant breast cancer [56].

Targeting PI3K/mTOR pathway with inhibitors

PI3K and mTOR inhibitors may be more effective against cancer than mTOR inhibitors alone. Some substances can inhibit both PI3K and mTOR due to the similarities between the two proteins. Several PI3K isomers are inhibited by NVPBEZ235 (Dactolisib) and mTOR, which can cross the blood-brain barrier, exhibits a strong anti-cancer effect and are used to treat glioma and overcome glioma resistance to muzolimine. In addition, for a type of gastric cancer showing up-regulation of PI3K/mTOR activity, NVPBEZ235 can inhibit its resistance to paclitaxel [57]. SAR245409 (Voxtalisib) is a PI3K inhibitor that significantly inhibits tumor growth in various human tumor xenotransplantation models. SAR245409 and the MEK (mitogen-activated protein kinase) inhibitor Pimasertib can synergistically inhibit the growth of some endometrial tumor cells [58]. GSK2126458 (Omipalisib) is an oral inhibitor of PI3K and mTOR, which can effectively reduce the viability of human rhabdomyosarcoma cells and inhibit the growth of rhabdomyosarcoma in vivo [59].

The various methods used by mTOR inhibitors to target the subtleties of the mTOR signaling pathway define this class of drugs. Different kinds can be identified depending on their action locations, such as allosteric, ATP-competitive, and dual-binding site inhibitors. Due to their ability to interfere with vital cellular processes thought to be involved in carcinogenesis, these inhibitors show considerable potential for treating cancer. While ATP-competitive inhibitors like Torin1 directly target the kinase domain to disrupt mTOR complexes, allosteric inhibitors like rapamycin interfere with mTORC1 signaling via binding to allosteric sites. Dual-binding site inhibitors, such as AZD8055, use a mixture of binding techniques to obstruct mTOR activities completely. These inhibitors' modes of action provide numerous methods to alter the cellular pathways linked to cancer, laying the groundwork for sophisticated treatment approaches.

Unraveling mechanisms and therapeutic approaches for tumor resistance to mTOR inhibitors

Drug resistance is one of the significant problems in the effective treatment of tumors. Due to the heterogeneity of the tumors, some tumors do not even respond to a given drug. Clonal selection, adaptive evolution and resistance to cell death are common mechanisms of drug resistance in tumors. Due to the complexity of the signaling network, tumour cells may be able to adapt to one or more inhibitors by targeting a given signaling pathway to influence the activation of other signaling pathways. Therefore, the in-depth study of the underlying mechanism of tumour resistance to mTOR inhibitors is significant.

Drug efflux mediated by ABC transporters

One of the ways that most cancers develop medication resistance is by upregulation of the ATP-binding cassette (ABC) transporter. ABC transporters may function as drug efflux pumps, lowering the concentration of medicines in cells and impacting the treatment of conditions like tumors. Glycoproteins and breast cancer-resistant proteins are substrates for the rapamycin and NVP-BEZ235 mTOR inhibitors [60]. Breast cancer resistance protein was shown to be overproduced in luminal breast cancer cell lines that were resistant to Everolimus [61], and overexpression of ABCG2 resulted in tumor cells that were very resistant to the PI3K inhibitor PF-4989216. Competitive substrates or ABCG2 inhibitors can stop this process. Since different mTOR inhibitors have varying affinities for ABC transporters, blocking the function of ABC transporters or lowering their affinities for ABC transporters may increase mTOR inhibitors' effectiveness in treating tumors.

Stem cells in tumorigenesis

Tore stem cells (TSC) are a subgroup of tumor masses highly resistant to therapy. mTOR is one of the mediators of transforming growth factor-β signaling pathways that enhances tumour drug resistance. Transforming growth factor-β can induce epithelial-mesenchymal transformation, thereby promoting the generation of tumor stem cells [62]. Previous studies have shown that some mTOR inhibitors have inhibitory effects on tumor stem cells, such as PI3K/mTOR inhibitor VS-5584, which can reduce CSC levels in mouse xenotransplantation

Table 1 mTOR inhibitors for tumour treatment

mTOR inhibitor	Category	Tumour type
Sirolimus	Rapalog	Renal cell carcinoma
Everolimus	Rapalog	Gastric cancer, non-small cell lung cancer
Sapanisertib	ATP-competitive	Bone and soft tissue sarcoma, breast cancer
Tokinib	ATP-competitive	Stomach and colon cancer
Vistusertib	ATP-competitive	ER-positive breast cancer
Dactolisib	Targeting PI3K and mTOR	Glioma, gastric cancer
Voxtalisib	Targeting PI3K and mTOR	Endometrioma
Omipalisib	Targeting PI3K and mTOR	Rhabdomyosarcoma

models with multiple human tumors [63]. The sensitivity of TP53 (tumor suppressor p53) mutation and BCL2 (B-cell lymphoma 2) phosphorylation to mTOR inhibitors in shadow glioblastoma [64, 65]. Moreover, BCL2 phosphorylation in TP53 wild-type glioblastoma stem cells reduced glioblastoma sensitivity to mTORC1/2 inhibitors compared to TP53 mutated glioblastoma stem cells. Therefore, an in-depth study of the genetic background of tumors and tumour stem pathways will be conducive to developing more effective mTOR inhibitors and small molecule compounds to resist tumour resistance.

Mutation of the mTOR gene

Genetic mutations can affect drug sensitivity. A variety of mTOR activating mutations, such as mTOR kinase domain mutations M2327I, S2215Y, L2230V, E2388Q and V2046A, have been found in human tumors and may be resistant to ATP competitive inhibitor MLN0128 [66, 67]. Despite this breakthrough, further research is needed to clarify which unknown tumour-associated mutations in Raptor, Rictor, and RHEB may be associated with resistance to mTOR inhibitors.

Dysregulation of tumor metabolic pathways

Other tumour pathways regulate the sensitivity of mTOR inhibitors. The MEK/ERK pathway may become activated by mTOR inhibition, whereas ERK may become inactive by palbociclib inhibition via CDK4/6. Inhibitors of CDK4/6 and mTOR can thus work together to suppress the growth of tumors [68, 69]. A multifunctional enzyme called transglutaminase2 is involved in signal transmission, apoptosis, and cross-linking polypeptide chains, among other things. Transglutaminase2 inhibition efficiently renders tumour cells with aberrant mTORC1 activity susceptible to rapamycin [70–73]. Furthermore, mitochondrial homeostasis is necessary for cell survival and proliferation. The adaptive mitochondrial response to mTOR inhibition prevents apoptosis and prolongs the viability of tumor cells [74–77]. As a result, medication resistance in tumors may be caused by the aberrant metabolic pathways of tumors and their interaction. More investigation into the intricate mechanism of tumour metabolic pathways will be helpful in effectively using mTOR inhibitors in clinical therapy.

Exploring the interplay of mTORC1 and mTORC2

The cross-regulation of mTORC1 and mTORC2 also affects tumour sensitivity to mTOR inhibitors. MTORC2 is activated by PI3K in response to growth factors, and the activated mTORC2 phosphorylates Akt and up-regulates the activity of mTORC1, which enhances the survival and proliferation ability of tumour cells and thus enables tumour cells to develop specific resistance to drugs [78–80]. Meanwhile, activated mTORC2 inhibits the expression of pro-apoptotic microRNA (miR-9-3p), thereby reducing its negative regulation of pro-survival factor E2F1 (E2F transcription factor 1) to inhibit the apoptosis of tumour cells and promote the survival of tumour cells [81–84]. The activity of mTORC2 is also regulated by the substrate S6K of mTORC1. The downstream substrate S6K of mTORC1 inhibits insulin signal transduction by phosphorylating IRS1 (insulin receptor substrate 1) at different sites and down-regulates the PI3K signaling pathway to inhibit mTORC2 activity. S6K also phosphorylates Rictor and mSin1, respectively, to destabilize mTORC2 [85–87]. Therefore, inhibitors targeting mTORC1 can relieve mTORC1's inhibition of PI3K/mTORC2 signaling, thus enhancing the survival ability of tumour cells [88–92]. Therefore, further research and elucidation of the potential interactive regulatory mechanism between mTORC1 and mTORC2 are conducive to the rational use of mTOR inhibitors and small molecule drugs to overcome tumour resistance.

Conclusions

Protein synthesis in cells is known to need a significant amount of energy. The protein mTOR plays a crucial role in regulating mRNA translation, allowing cells to adapt between energy-rich and energy-poor conditions quickly. Under limited energy conditions, cells can reduce the production of unnecessary proteins due to a regulatory preference. Although the primary role of many components of the translational machinery is to support cellular development and proliferation rather than quiescent states, it is reasonable to prioritize suppressing their translation during adverse conditions to preserve cellular quiescence. On the other hand, it is shown that both mRNA translation and mTOR pathways often undergo overexpression in specific abnormal proliferative conditions, such as malignant tumours. The complex network of translational regulating within the mechanistic target of the rapamycin (mTOR) pathway is centered on pivotal components like 4E-BP, S6K, and LARP1. While these factors effectively address fundamental discoveries in mTOR-mediated translational regulation, unresolved complexities remain. Some messenger RNAs (mRNAs) that mTOR regulates for translation may not possess the usual TOP/PRTE motif. Additionally, 4E-BP, S6K, and LARP1 do not control all TOP mRNAs. This observation suggests that uncharacterized proteins may be involved in this biological pathway. In recent years, there has been a growing recognition of the significance of RNA modifications, such as m6A (N6-methyladenosine), in regulating mRNA metabolism. The reversible m6A modifications in eukaryotic mRNAs are the most common internal modifications. These changes are detected by effector proteins, which play a crucial role in regulating the modified mRNAs' translation process, stability, and cellular distribution.

Interestingly, previous studies have reported that mTORC1 has a role in increasing the production of the principal methyl donor known

as S-adenosylmethionine (SAM). This is achieved by regulating the expression of a specific enzyme called methionine adenosyltransferase two alpha (MAT2A). In addition, it has been observed that mTORC1 upregulates the levels of Wilms' tumor 1-associating protein (WTAP), which serves as a favorable regulatory subunit within the m6A RNA methyltransferase complex. The protein synthesis and cellular growth process are facilitated by mTORC1 signaling, activated by the stimulation of m6A RNA alterations. Identifying and clarifying previously unknown RNA-binding proteins (RBPs), cap-binding proteins, and RNA modification-associated proteins within the mechanistic target of the rapamycin (mTOR) pathway are of utmost significance. Understanding the mTOR signaling pathway is crucial for developing novel therapeutic approaches for cancer and metabolic disorders.

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