



REVIEW ARTICLE OPEN

Multifaceted role of mTOR (mammalian target of rapamycin) signaling pathway in human health and disease

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The mammalian target of rapamycin (mTOR) is a protein kinase that controls cellular metabolism, catabolism, immune responses, autophagy, survival, proliferation, and migration, to maintain cellular homeostasis. The mTOR signaling cascade consists of two distinct multi-subunit complexes named mTOR complex 1/2 (mTORC1/2). mTOR catalyzes the phosphorylation of several critical proteins like AKT, protein kinase C, insulin growth factor receptor (IGF-1R), 4E binding protein 1 (4E-BP1), ribosomal protein S6 kinase (S6K), transcription factor EB (TFEB), sterol-responsive element-binding proteins (SREBPs), Lipin-1, and Unc-51-like autophagy-activating kinases. mTOR signaling plays a central role in regulating translation, lipid synthesis, nucleotide synthesis, biogenesis of lysosomes, nutrient sensing, and growth factor signaling. The emerging pieces of evidence have revealed that the constitutive activation of the mTOR pathway due to mutations/amplification/deletion in either mTOR and its complexes (mTORC1 and mTORC2) or upstream targets is responsible for aging, neurological diseases, and human malignancies. Here, we provide the detailed structure of mTOR, its complexes, and the comprehensive role of upstream regulators, as well as downstream effectors of mTOR signaling cascades in the metabolism, biogenesis of biomolecules, immune responses, and autophagy. Additionally, we summarize the potential of long noncoding RNAs (lncRNAs) as an important modulator of mTOR signaling. Importantly, we have highlighted the potential of mTOR signaling in aging, neurological disorders, human cancers, cancer stem cells, and drug resistance. Here, we discuss the developments for the therapeutic targeting of mTOR signaling with improved anticancer efficacy for the benefit of cancer patients in clinics.

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INTRODUCTION

The mTOR belongs to the class of evolutionarily conserved threonine and serine kinases which recognize and incorporate a variety of extracellular and intracellular signals to maintain cellular homeostasis and metabolism.^{1–4} The name mTOR was obtained from rapamycin isolated from a soil bacterium in 1970 on Rapa Nui.^{1–4} Further, the structural elucidation of the rapamycin revealed 14–16 membered lactone rings and reduced saccharide substituents. Interestingly, the physiological characterizations have uncovered immunosuppressive properties, curtailed organ rejection, kidney transplantation, and inhibition of T-cell mitogenesis.^{2,5} Mechanistically, the mTOR has dual kinase activity and can phosphorylate serine/threonine or tyrosine residue. The mTOR has been considered a part of the phosphoinositide 3-kinase (PI3K) family due to the presence of the catalytic domain within the mTOR structure which has a similarity with lipid kinases like PI3K. mTOR has been reported to be crucial for many biological processes, including cell growth, cell survival, immunity, autophagy, and metabolism.^{1,2}

This has been reported that mTOR can generate two different functional complexes named mTORC1 and mTORC2.⁶ The

mTORC1 was discovered as a complex of several proteins that consist of mTOR, Raptor (regulatory associated protein of mTOR), GβL (G protein β subunit-like protein)/mLST8 (mammalian lethal with SEC13 protein 8), DEPTOR (DEP-domain-containing mTOR-interacting protein), and PRAS40 (the 40 kDa proline-rich Akt substrate).^{7,8} The mTORC2 is composed of mTOR, GβL/mLST8, Rictor (rapamycin-insensitive companion of mTOR), Protor/PRR5 (Proline-rich protein 5), DEPTOR, and mSIN1 (mammalian stress-activated protein kinase-interacting protein 1).^{7–14} The mTORC1 amalgamate signals from a variety of growth factors, and nutrients to enhance cellular proliferation especially when there is adequate energy and/or catabolism when the body is hungry.^{5,15} The mTORC1 is well known for its function in cell growth and metabolism, whereas the mTORC2 regulates proliferation and survival.¹ Several groups reported that mTOR is crucial for several signaling cascades like AKT, PI3K, TSC1/TSC2 (tuberous sclerosis complex subunit 1 and 2), Rheb, LKBL/AMP-activated protein kinase (AMPK), VAM6/Rag GTPases.¹⁶ The mTOR signaling was demonstrated to enhance gene transcription and translation to control cellular growth, autophagy, and apoptosis.^{2,5,17}

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Dysregulation of mTOR has been found to be strongly linked with several diseases like aging, arthritis, insulin resistance, osteoporosis, cancers, and neurological disorders.¹⁸ Cancer development is a complex, and multifactorial process, including genetic aberration, epigenetic modifications, dysregulated expression of hormones, tumor suppressors, and conversion of proto-oncogenes to oncogenes.^{19–21} The frequent alteration of mTOR was noticed to play an important role during tumorigenesis, distant metastasis, and drug resistance in human malignancies, such as lung, breast, liver, renal, pancreatic, and prostate.^{22–25} The stimulation of the mTOR cascade has been displayed to increase tumor growth through the regulation of glycolysis, angiogenesis, growth factor receptor pathway, lipid metabolism, and autophagy.^{5,9} Therefore, mTOR represents an important and promising target for therapeutic intervention against human malignancies.^{26,27} In this current review, we have discussed the structure of mTOR complexes along with their molecular functions, upstream regulators, as well as downstream effectors of mTOR signaling, the association of mTOR signaling to modulate cellular metabolism and autophagy. Also, how the dysregulated mTOR signaling is associated with aging, neurological disorders, and cancers (Fig. 1). Importantly, we have highlighted the opportunities and challenges for pharmacological targeting of mTOR signaling for therapeutic intervention and management of human malignancies.

STRUCTURAL ARCHITECTS OF MTOR AND ITS COMPLEXES

Mechanobiology of mTOR unfolds it as a complex protein kinase intricated with multielement complexes via its communication network with other proteins. The cryo-electron microscopic structure of the mTOR has revealed a hollow rhomboid architecture with dimensions of $\sim 280 \times 210 \times 130$ (Å³).²⁸ This has been noticed that the C-terminus of mTOR was comprised of the FAT domain (FKBP12 rapamycin-associated protein, ataxia telangiectasia, and transformation or transactivation domain associated protein), FRB (FKBP12 rapamycin binding) domain which is responsible for the interaction of FKBP protein bound to rapamycin with mTOR, kinase domain that is an important site for phosphorylation to control the activity of mTOR, and FATC (FAT carboxyterminal domain). Also, the N-terminal part of the mTOR consists of 20 HEAT repeats. The HEAT repeats were found to be essential for interaction with Raptor and Rictor.^{22,29}

The structural architect of mTORC1 defines the complex and symmetric organization of Raptor, PRAS40, DEPTOR, and mLST8 (GβL) as its major components along with centrally located mTOR protein.³⁰ Interlocking interactions allying the two mTOR and two Raptor subunits configure dimeric interfaces. Distal foot-like perturbances of mLST8 (GβL) subunit interpose mTOR inside complex 1. PRAS40 circumscribes itself to the adjoining adjacency of Raptor subunits i.e., the middle section of the central core of complex 1.^{31,32} Raptor is critical for the assembly, proper localization, and stability of the mTORC1. Raptor was reported to be important for the recruitment of the substrate on mTORC1. PRAS40 has been shown to inhibit the activation of mTORC1 unless it is phosphorylated through growth factor receptor signaling by growth factors/other stimuli. PRAS40 has an essential role in human cancers and metabolic disorders. The mLST8 was found to be associated with the kinase domain of the mTORC1 and can help in the stabilization of kinase activity. DEPTOR acts as an inhibitory subunit in the mTORC1. The crystal structure and functional analysis revealed that rapamycin-FKBP12 can efficiently bind with the FRB domain of mTOR to obstruct the substrates from active sites.^{1,29,31–33} The mTORC1 has been found to control cellular growth by increasing the biogenesis of ribosomes, mRNA translation, and autophagy.³⁴

The mTORC2 complex is a hollow rhombohedral fold with dimensions of $220 \text{ Å} \times 200 \text{ Å} \times 130 \text{ Å}$.²⁸ The complex embraces binary symmetry, and each promoter incorporates one copy of

mTOR, GβL/mLST8, mSin1, and Rictor. mTOR-mLST8 (GβL) heterodimer embraces overall architecture alike to complex 1 with a root mean square deviation of 6.7 Å for 3550 α-carbon atoms.^{30,35} The two monomers of mTOR pack against each other to form a central scaffold yielding a binding surface for the other three components. Two copies of mLST8 (GβL), mSin1, or Rictor bind symmetrically to the mTOR dimer.^{31,32} Rictor is important for the assembly, substrate recognition, and stability of the mTORC2. This has been observed that mSIN1 acts as a scaffold protein that helps in the mTORC2 interaction with serum and glucocorticoid-activated kinase 1 (SGK1) and negatively controls the kinase activity of mTORC2.¹² Protor-1, a Rictor-binding protein was found to regulate mTORC2-dependent phosphorylation of SGK1.³⁶ The mTORC2 has been displayed to be associated with the cytoskeleton, cell proliferation, cell survival, and migration.

Harwood et al. have identified another rapamycin-insensitive complex known as mTORC3. The E26 transformation-specific transcription factor ETV7 was found to interact with mTOR in the cytoplasmic compartment. The mTORC3 displayed bimodal mTORC1/2 activity that was independent of the components of mTORC1/2. This was noticed that mTORC3 is robustly activated in several cancers. The loss of mTORC3 expression in cancer cells displayed marked sensitivity to rapamycin. Interestingly, this study also demonstrated that mTORC3 induced tumorigenesis in a murine model of rhabdomyosarcoma. Interestingly, the transgenic ETV7 expression further enhanced tumor onset and penetrance.³⁷ The detailed structure of the mTOR and its complexes, along with their function, has been described in Fig. 2.

UPSTREAM REGULATORS OF MTOR SIGNALING

The mTOR signaling cascades control the cellular growth and mitotic divisions by generating prominent metabolic energy from glucose, lipid, protein, and nucleotides while inhibiting catabolic processes like autophagy.³⁸ Hence, the mTORC1 plays a central role in maintaining the equilibrium between anabolism and catabolism, especially in response to environmental stress. mTOR signaling augments energy depots and consumption. mTORC1 can enhance cellular growth by integrating stimuli from growth factors, DNA damage, oxygen, nutrients, amino acids, and energy, whereas augmented mTORC1 prompts insulin resistance by halting insulin receptor signaling and fat accumulation (Fig. 3).^{1,38} This has been observed that dysregulated oncogenes, enhanced anabolism, angiogenesis, and suppression of autophagy underlie the tumorigenic behavior of mTORC1. mTORC2 is activated by growth factors which in turn activates AKT and AGC family leading to increased cellular proliferation and survival.^{1,38} Growth factors are known to regulate several signaling cascades that intersect on TSC, including receptor tyrosine kinases (RTK), IGF-1, Wnt, TNFα, inflammatory cytokines, and Ras signaling cascade. These growth factors stimulate the phosphorylation of TSC2 through AKT. The phosphorylation was associated with the inhibition of TSC through its dissociation from the lysosomal membrane. The RTK-mediated Ras signaling was found to activate the mTORC1 pathway through MAPK/ERK and its effector p90^{RSK} leading phosphorylation of TSC2. Moreover, the other growth factors like Wnt, and TNFα were noticed with the activation of mTORC1 through repression of TSC1.³⁹ Importantly, the environmental/extracellular and intracellular stresses are sensed by the mTORC1 and modulate cellular growth and survival under hypoxia, lower levels of ATP, and DNA damage conditions.⁴⁰ Under glucose deprivation conditions, there is a marked reduction in cellular energy levels that stimulates energy stress-sensing metabolic regulator AMPK. The stimulation of AMPK was reported to repress the mTORC1 either through direct phosphorylation of Raptor or indirectly by phosphorylating TSC2.⁴¹ Furthermore, low glucose levels were found to suppress mTORC1 signaling by inhibiting the Rag GTPases activity, especially in cells lacking

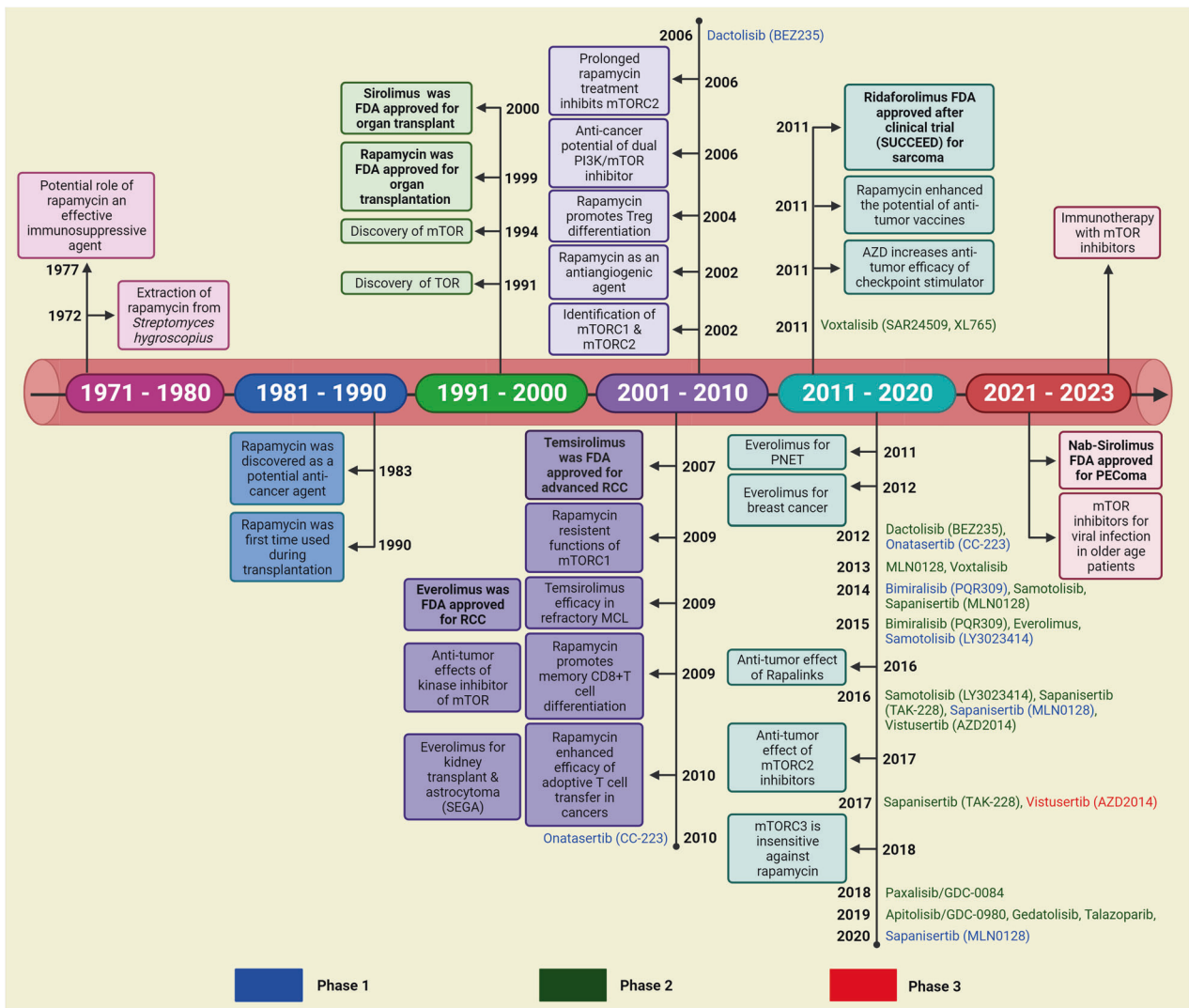


Fig. 1 History of research on the discovery and development of mTOR signaling. The figure describes the journey of mTOR signaling from its origin to the most advanced scientific discoveries including the identification, isolation, development of inhibitors, and their application as therapeutics in human health and diseases. Created with BioRender.com. FDA Food and Drug Administration, TOR target of rapamycin, mTOR mammalian target of rapamycin, mTORC1 mTOR complex 1, mTORC2 mTOR complex 2, MCL mental cell lymphoma, Nab-sirolimus nanoparticle albumin-bound sirolimus, PEComa perivascular epithelioid cell tumor, PNET pancreatic neuroendocrine tumor, RCC renal cell carcinoma, SEGA subependymal giant-cell astrocytoma

AMPK. Recently, Dai et al. have reported that AMPK-dependent phosphorylation of WDR24 can modulate the glucose-dependent activation of mTORC1.⁴² These results indicated that mTORC1 can efficiently sense glucose or energy stress through multiple molecular mechanisms.^{43,44} Hypoxia or oxygen deprivation stress was reported to produce an inhibitory effect on the mTORC1 through activation of AMPK, and induction of REDD1 that resulted in the activation of TSC. Also, the induction of the DNA damage response signaling was found to repress mTORC1 via induction of p53 target genes like *AMPKβ*, *PTEN*, and *TSC2* leading to increased activation of TSC activity. Amino acids are not only required as the building blocks of proteins but also a great source of carbon and energy for a variety of metabolic signaling cascades.⁴⁵ The activation of the mTORC1 pathway is coupled with diet-mediated changes in the concentration of amino acids. Interestingly, the mechanism of sensing the amino acids through mTORC1 with the help of Rag GTPases as essential components of mTORC1 signaling was one of the groundbreaking discoveries in the field of mTOR signaling.⁴⁶ Rags were discovered as heterodimers of RagA/RagB with RagC/RagD. These are mostly

bounded by the membrane of the lysosome through their close association or interaction with a well-characterized pentameric complex composed of p14, HBXIP, p18, MP1, and c7ORF59.^{47,48} The amino acid stimulation converts the Rags to an active nucleotide-bound state which allows Rags to bind with Raptor and recruit mTORC1 on the surface of the lysosome, where Rheb is localized. The mTORC1 has been found to sense cytosolic as well as intra-lysosomal amino acids through different molecular mechanisms.^{49,50} SLC38A9, lysosomal amino acid transporter was displayed to interact with the Rag-v-ATPase complex which is responsible for arginine transport and activation of mTORC1.^{50,51} The cytosolic arginine and leucine signal to mTORC1 through GATOR1 and GATOR2 complexes. The GATOR1 is comprised of Npr12/3, DEPDC5 and acts as a GAP for RagA/B to inhibit the mTORC1 pathway. The KICSTOR complex comprised of Kaptin, c12orf66, ITFG2, and SZT2 and bound to GATOR1 on the surface of the lysosome to modulate the mTORC1 pathway for nutrient/amino acids sensing.⁵² On the other hand, GATOR2 was discovered as a pentameric complex of WDR24, Seh1L, Mios, WDR59, and Sec13. GATOR2 interacts with GATOR1 on the surface

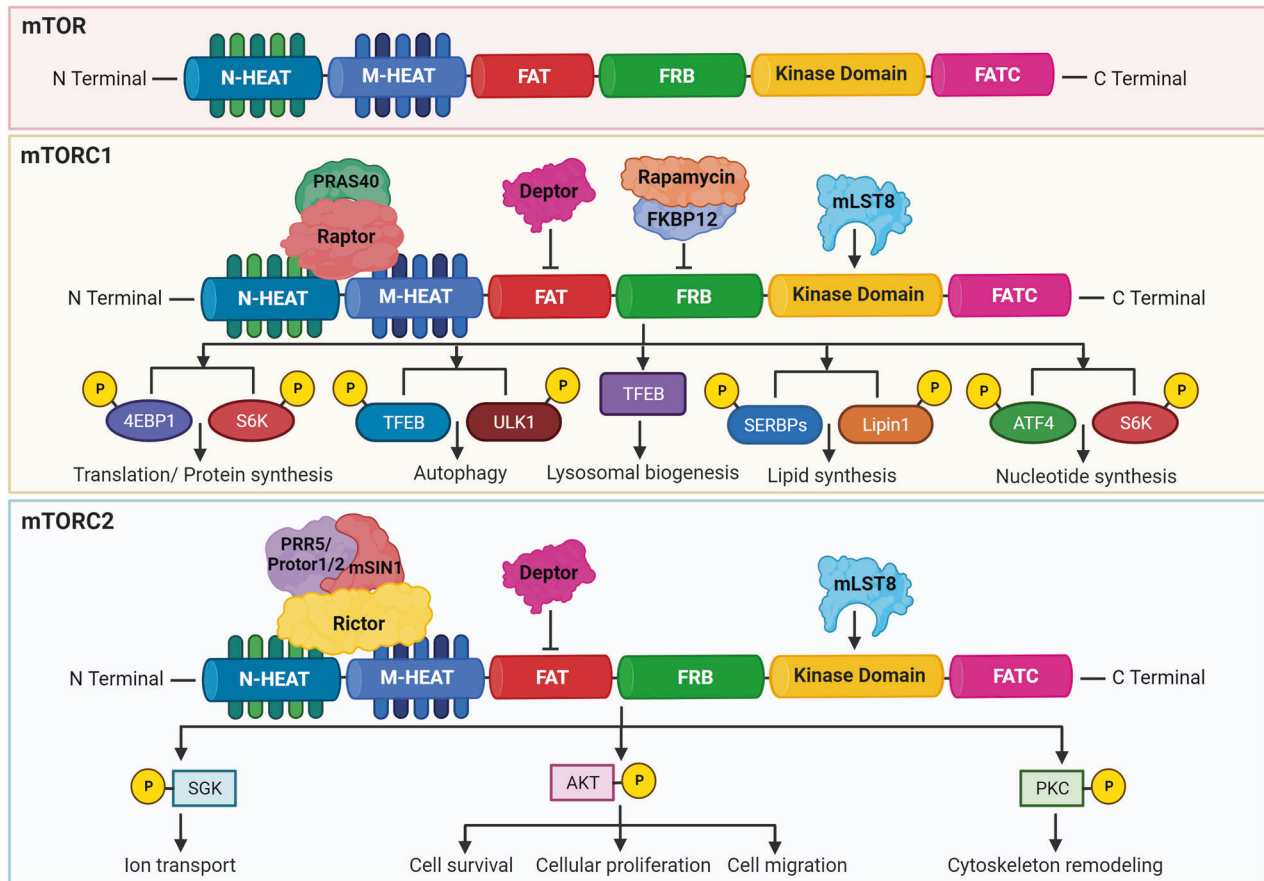


Fig. 2 The domain structures of mTORC1 and mTORC2, their downstream signaling targets and functional role. N-terminal domain of mTOR possesses tandem HEAT repeats and C-terminal domains composed of FATC, kinase, FRB, and FAT. The mTOR signaling pathway is majorly constituted of two distinctive mTOR complexes named mTORC1 and mTORC2. The mTORC1 is a complex of DEPTOR, Raptor, PRAS40, mLST8, mTOR, and phosphorylate downstream targets to regulate protein synthesis or mRNA translation, lipid synthesis, nucleotide synthesis, lysosomal biogenesis, and autophagy. The mTORC2 is a complex of mTOR, DEPTOR, mSIN1, Rictor, Protor, and mLST8 to regulate cell survival, proliferation, migration, and cytoskeleton remodeling. Created with BioRender.com

of the lysosome as a positive regulator of the mTORC1 pathway.⁵³ Sestrin2 was discovered as a GATOR2 interacting protein partner that senses cytosolic amino acid. This led to the inhibition of mTORC1 signaling under the deprivation of the amino acid.⁵⁴ Structural and biochemical analyses revealed that Sestrin2 is a direct sensor of leucine and upstream of mTORC1. Further, Sestrin2 was shown to be transcriptionally induced after prolonged amino acid starvation through ATF-4. These results indicated that Sestrin2 can function as an acute leucine sensor and indirect mediator of prolonged starvation of amino acid.⁵⁵ Arginine activated mTORC1 via the GATOR1/2-Rag pathway and binding the CASTOR1. CASTOR1 interacts and suppress GATOR2 in the absence of arginine and dissociates upon arginine binding resulting in the mTORC1 activation.^{56,57} These findings verified that CASTOR1 has the arginine sensing ability for the mTORC1 pathway. Additionally, other molecular mechanisms that control amino acids mediated mTORC1 signaling were reported and recruitment of Folliculin-FNIP2 complex on the lysosome cooperates as a GAP for RagC/D in the presence of amino acids.⁵⁸ The glutamine is utilized as a source of nitrogen by highly dividing cells to stimulate mTORC1 independent of the Rag GTPases via Arf family GTPases.⁵⁹ The long noncoding RNA-SPAR was found to cooperate with the v-ATPase-Ragulator complex. This cooperation caused a hamper in the process of the mTORC1 recruitment to lysosomes.⁶⁰ Recently, Yan and colleagues have performed genome-wide CRISPR-Cas9 screening and identified interleukin enhancer binding factor 3 (ILF3) as a critical regulator for the

sensing of the amino acids in a mTORC1-dependent manner. ILF3 was found to tether with GATOR complexes on the lysosomes. Further, the addition of the sequences that specifically target lysosome to the GATOR2 component WDR24 was found to bypass the ILF3 requirement and modulated the amino acid-mediated mTORC1 pathway.⁶¹ Another study by Jiang and colleagues demonstrated that WDR24 or Ring domains were critical for GATOR2 to disseminate amino acids availability to mTORC1 during embryonic development.⁶² Further, studies have confirmed the potential of mTORC2 as an effector of IGF and PI3K signaling. The mSin1 of the mTORC2 was found to have a phosphoinositide-binding domain which proved to be important for the insulin-regulated activity of the mTORC2. In the insufficiency of insulin, the PH domain of the mSin1 was noticed to hamper the mTORC2 catalytic activity. This autoinhibition was rescued upon binding to PI3K-generated PIP₃⁶³ and mSin1 was phosphorylated by AKT, indicating the existence of a positive-feedback loop that partially activates Akt and promoted the activation of mTORC2.⁶⁴ The S6K1 was found to inhibit the mTORC2 signaling through the degradation of IRS1, insulin receptor substrate-1.⁶⁵ The negative feedback loop among insulin-dependent PI3K pathway and mTORC1 is another mechanism for the mTOR2 regulation (Fig. 3). The mTORC1 was found to phosphorylate Grb10 (negative regulator of IGF-1R signaling) that is upstream to AKT and mTORC2.^{66,67} During the past several decades, the emerging pieces of evidence have provided valuable information about the regulation of the mTOR signaling that leads to the development of

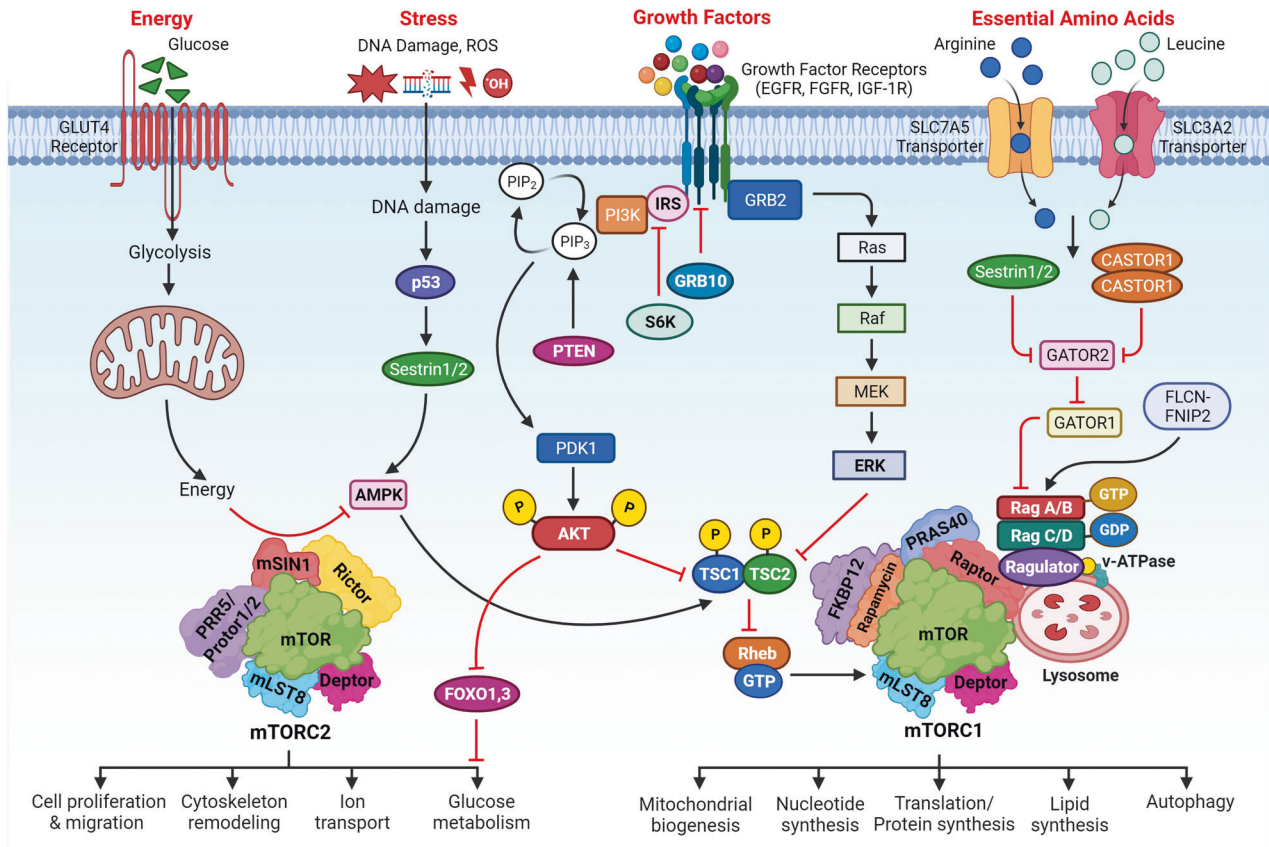


Fig. 3 The major upstream regulators of mTORC1 and mTORC2. Growth factors, amino acids like arginine and leucine, energy from glucose or other sources, cell stresses including DNA damage, and ROS stimulate mTORC1 to modulate various biological processes like mitochondrial biogenesis, nucleotide synthesis, mRNA translation (protein synthesis), lipid synthesis, and autophagy. The growth factors are the main regulators of the mTORC2 to control cell proliferation, migration, cytoskeleton remodeling, ion transport, and glucose metabolism. Created with BioRender.com

several clinical drugs. However, the complete knowledge of the integration of a variety of signals through TSC to regulate mTORC1/2 activity remains an open research question in the field.

Role of the mTOR signaling cascade in glucose metabolism
The energy requirement of the cell is regulated by mTORC1 by AMPK, the sensor of intracellular energy levels. Augmented glucose metabolism promotes mitochondrial activity by prompting AMP levels that disturb the ATP: AMP ratio resulting in the activation of the AMPK leading to phosphorylation of TSC2 which enhances the GAP activity of TSC2 for the Rheb to repress the mTORC1 activity.⁶⁸ Moreover, AMPK can directly phosphorylate Raptor and reduce mTORC1 activity under energy-deprived conditions.⁴¹ Also, mTORC1 can favor cellular proliferation through a prominent shift in glucose metabolism from oxidative phosphorylation to glycolysis. This metabolic reprogramming is known as the Warburg effect and is characterized by an increase in the uptake of glucose and the production of lactate, even in the presence of oxygen (aerobic glycolysis).⁶⁹ mTORC1 enhanced the Warburg effect by increasing the gene expression and catalytic activity of key enzymes required during glycolysis, such as pyruvate kinase muscle isozyme 2, hexokinase 2, and lactate dehydrogenase A, among others. This results in increased flux through the glycolytic pathway, which provides the building blocks and energy required for cell growth and division.^{69–71} Hypoxic stress or anaerobic condition has been found to promote the reduction of pyruvate to lactate by NADH through lactate dehydrogenase that may augment the lactic acid concentration leading to lactic acidosis. Lactic acidosis favors oncogenesis by modulating the tumor microenvironment. Hypoxia-inducible

factor 1 alpha (HIF-1 α) was reported as a well-known regulator to increase the expression of the glycolytic enzymes, and glucose transporters.^{70,71} Glucose transporters thus facilitate the transport of glucose into cells while glycolytic enzymes catalyze the breakdown of glucose into energy.^{69–72} Moreover, mTORC1 has been found to enhance the translation of the HIF-1 α which in turn activates the expression of key enzymes involved in glycolysis like phospho-fructokinase. The mTORC1-mediated SREBP stimulation caused increased flux by the pentose phosphate pathway leading to the generation of NADPH from the glucose and other intermediate metabolites for proliferation and growth.⁵⁶

Role of the mTOR signaling cascade in lipid metabolism
mTOR plays a significant role in regulating lipid biosynthesis required for cell growth and division by maintaining the cellular membrane. The mTORC1 has been shown to enhance the synthesis of lipids through the regulation of SREBP (sterol regulatory element-binding protein) that modulates the expression of the genes associated with cholesterol and fatty acid biosynthesis.⁵⁶ Generally, during lower levels of sterol, the SREBP gets activated. The mTORC1 signaling cascade was reported to stimulate in two ways (1) SREBP activation via the S6K1 mechanism, and (2) through phosphorylation of the Lipin-1 that can repress SREBP activity in the absence of mTORC1.^{56,73} In the first mechanism, mTORC1 activates SREBP through the downstream effector S6K1, which phosphorylates and activates SREBP cleavage-activating protein (SCAP). This leads to the translocation of the active form of SREBP to the nucleus and the upregulation of genes involved in lipid synthesis. In the second mechanism, mTORC1 phosphorylates and inactivates Lipin-1, which is a negative regulator of SREBP activity.⁷³ In the absence of

mTORC1 signaling, Lipin-1 represses SREBP activity by promoting the formation of a repressor complex that inhibits SREBP-mediated transcription. However, under conditions of mTORC1 activation, Lipin-1 is phosphorylated and inactivated, leading to the derepression of SREBP activity and the upregulation of lipid biosynthesis.⁷³ Overall, the regulation of SREBP by mTORC1 provides an important mechanism by which mTORC1 can promote lipid synthesis and support cellular growth and proliferation (Figs. 2 and 3).

Role of the mTOR signaling cascade in nucleotide biogenesis

This has been confirmed that mTORC1 can promote the biosynthesis of nucleotides, especially in the proliferative cells to support the replication of the DNA and biogenesis of ribosomes. Moreover, mTORC1 was displayed to enhance the expression of MTHFD2 (methylene tetrahydrofolate dehydrogenase 2) in an ATF-4-dependent manner that can provide carbon units for the synthesis of purine. Ben-Sahra and colleagues have shown that S6K1 phosphorylates and stimulates carbamoyl phosphate synthetase (CAD) that helps in the pyrimidine synthesis pathway.⁷⁴ Robitaille and colleagues have performed quantitative phosphoproteomics and identified that mTOR regulates the phosphorylation of approximately 335 proteins.⁷⁵ This study showed that mTORC1 can phosphorylate CAD at serine 1859 via S6K and activated de novo synthesis of pyrimidines leading to the cell cycle through the S phase (Figs. 2 and 3). Therefore, mTORC1 regulates the production of nucleotides to adjust the RNA and DNA synthesis required for ribosome biogenesis.⁷⁵

Role of mTOR signaling in protein biogenesis

mTOR signaling cascade is well known for protein synthesis through the phosphorylation of eIF4E binding protein (4EBP), and S6K1. mTORC1 can phosphorylate S6K1 at Thr389 residue which leads to its phosphorylation and activation via PDK1 (3-phosphoinositide-dependent protein kinase 1). S6K1 can lead to the phosphorylation and activation of a variety of substrates that promote mRNA translation initiation, particularly the eIF4B which is crucial for the 5' cap binding eIF4F complex.⁷⁶ Dorrello and colleagues have revealed that programmed cell death protein 4 (PDCD4) repressed the translation initiation factor eIF4A. During mitogen response, the phosphorylation of PDCD4 at Ser67 by the S6K1 leading to its degradation through the ubiquitin ligase SCF (β -TRCP) enables the synthesis of protein synthesis and cellular proliferation.⁷⁷ Exon junction complex has been displayed to regulate mRNA synthesis.⁷⁸ The SKAR-dependent recruitment of S6K1 to the newly generated mRNPs acts as a bridge between mTOR signaling and translation. The mTOR kinase has been recognized as one of the master regulators of translation to meet the demand for cancer cells. The global ribosome profiling was performed to unravel the mechanism of translation that regulates gene expression through mTOR in cancers. This study showed the enrichment of the specific genes associated with cellular growth, invasion, and metabolism. These genes were downstream targets of mTOR signaling in prostate cancer.⁷⁹ Moreover, the potent and ATP-competitive mTOR inhibitor repressed mRNA translation and suppressed cellular proliferation.⁸⁰ Another study has revealed that mRNAs that are controlled by mTORC1 are 5' terminal oligopyrimidine (TOP) motifs. Moreover, the 4EBPs suppressed the initiation of translation by hampering the interaction between eIF4E and eIF4G1. This diminished the ability of eIF4E to interact with TOP and TOP-like mRNAs which explains why mTOR inhibition selectively repressed their translation.⁸¹ The mTORC1 phosphorylates its substrate 4EBP that triggers its dissociation from eIF4E allowing 5' cap-dependent mRNA translation (Figs. 2 and 3).^{82,83}

MTOR SIGNALING IN HUMAN CANCERS

The deregulation of mTOR signaling has been noticed in human malignancies. The emerging data has suggested that the mTOR

signal is frequently altered in approximately 30% of cancers.^{23,84} The activation of the mTOR pathway is dependent in three different ways (1) the activating mutations in the *mTOR*, and *mTORC1/2* or mutations in upstream genes lead to hyperactivation of the mTOR signaling (2) overexpression/amplification of the components of mTORC1 and mTORC2 (3) loss of function of negative regulators in the mTOR signaling cascade.⁸⁵ The gain of the function mutations in the kinase domain of mTOR can directly activate the mTOR pathway. The genome sequencing of human tumors has reported approximately 33 mutations in the *mTOR* gene. These mutations were associated with the activation of the mTOR pathway in colorectal cancer, endometroid carcinoma, stomach cancer, lung carcinoma, renal cell carcinoma (RCC), and melanoma.⁸⁶ This study displayed that *mTOR* mutations were clustered in six distinct regions in the c-terminal part of mTOR in human tumors. These mutations did not affect the mTOR complex assembly but suppressed the binding of DEPTOR. Interestingly, the cell lines with *mTOR* mutations displayed marked sensitivity to mTOR inhibitors in both in vitro and murine models.⁸⁶ Moreover, mutations in the components of *mTOR* complexes have been observed in several cancers. For instance, RICTOR was reported to be highly amplified in patients with lung and breast carcinoma. The *RICTOR* amplification in squamous cell lung carcinoma was linked with a bad prognosis and short survival.⁸⁷ Further, this study showed the sensitivity of mTORC1/2 inhibitors against RICTOR-amplified lung cancer cells. Interestingly, the patient was treated with mTORC1/2 inhibitors and displayed stabilization of the tumor for at least 18 months.⁸⁷ Joly et al. also confirmed that RICTOR was robustly expressed in the HER2-amplified breast carcinoma specimens which in turn enhanced phosphorylation of AKT at S473 residue.⁸⁸ A case study by Shamieh and colleagues reported that amplification in the *RICTOR* gene was associated with metastasis and drug resistance in TNBC.⁸⁹ Also, upregulation of RICTOR was associated with increased mTORC2 activity which promoted the cellular motility and proliferation of the glioma cell.⁹⁰ In addition, the hyperactivation of mTOR signaling can be the result of mutations in the upstream genes, including oncogenes and tumor suppressor genes.^{34,91} Gao et al. have reported the overexpression of Rheb1 in acute myeloid leukemia (AML) that was associated with worse median survival. Depletion of Rheb1 in the murine MLL-AF9 model displayed increased survival through suppression of mTOR signaling.⁹² Ghosh et al. reported the mutations in FAT domain of the *mTOR* gene in RCC. This study also identified Rheb mutations in patients with RCC leading to an increase in mTORC1 activity.⁹³ The mutations, amplification, and overexpression of *PIK3CA*, *KRAS*, *AKT*, *IGFR*, and *EGFR* are more common in cancer, which are upstream molecular targets for mTOR complexes resulting in the activation of the mTOR signaling cascade in human malignancies. *PIK3CA* mutations are frequently observed in a variety of cancers, including breast, colorectal, and ovarian cancer, and result in the activation of the PI3K/AKT/mTOR cascade. Similarly, mutations in *KRAS*, a key mediator of cell growth and differentiation, can lead to the marked activation of the mTOR signaling and contribute to the process of carcinogenesis. Also, the inactivation of the p53, PTEN, STK11, and TSC1/2 was noticed to enhance the activation of the mTOR signaling cascade in human tumors.^{34,94} The loss of p53 function can contribute to oncogenesis through multiple mechanisms, including promoting cell proliferation and survival, reducing apoptosis, and increasing genomic instability.⁹⁵ The emerging data from various sources have shown that p53 can negatively regulate mTOR signaling by inducing the expression of the mTOR inhibitor, REDD1, and by inhibiting the expression of S6K1, a downstream target of mTOR. In the absence of functional p53, these negative regulatory mechanisms are disrupted, leading to increased activation of the mTOR pathway and promoting tumor growth and survival. Additionally, p53 inactivation can lead to increased expression of growth factors and cytokines, such as IL-6, that can activate the mTOR pathway through other mechanisms. Alterations of PTEN were reported in breast, multiple

myeloma, and endometrial cancers, and treatment with mTOR inhibitors displayed strong antitumor activity in these cancers.^{96–98} Inactivation of TSC1/2 has been observed in tuberous sclerosis and can initiate tumorigenesis. The TSC1/2 mutations have been noticed in many cancers like pancreatic neuroendocrine, urothelial, bladder, and renal.^{99–101} Another side of mTOR signaling is to control the cellular growth and metabolism of the cancer cells through enhanced ribosome biogenesis. Recently, the mTOR complex has been shown as a nutrient sensor in cancer metabolism which includes glucose, lipid, amino acids, nucleotides, growth factors, etc.

Long noncoding RNAs (lncRNAs) as a regulator of mTOR signaling in cancers

lncRNAs are >200 nucleotides long RNAs with a close structure of the mRNA that includes 3'-polyadenylated tails, 5'-caps, transcription start site, and splicing resulting in a final gene product (transcript) but do not code for protein.^{102–105} The lncRNAs perform their function by acting as a signal molecule, decoy, guide, and scaffold.^{106–109} During the last decade, a variety of studies have revealed that dysregulated expression of lncRNAs can modulate mTOR signaling and vice versa.^{110,111} The lncRNAs can modulate the mTOR activity in several ways, including (1) direct binding to components of the mTOR complexes and (2) regulating upstream or downstream targets of mTOR. DLEU1 and HAGLROS lncRNAs were reported as direct targets of the mTOR complex through RNA immunoprecipitation.¹¹² DLEU1 was overexpressed in endometrial carcinoma than in healthy endometrial.¹¹² The overexpression of DLEU1 showed a significant increase in proliferation, clonogenicity, and migration while suppressing apoptosis through the mTOR pathway. Overexpression of DLEU1 resulted in the phosphorylation of mTOR and subsequent activation of downstream targets like PI3K, AKT, and pS70K. This study suggested that DLEU1 enhanced endometrial carcinogenesis through its binding with mTOR protein and activation of the PI3K/AKT/mTOR axis.¹¹² Chen and colleagues have noticed that overexpression of HAGLROS was associated with worse outcomes in patients with gastric cancer.¹¹³ Silencing of HAGLROS suppressed the expression of the mTOR leading to increased expression of ATG9A and ATG9B. Moreover, HAGLROS was found to control mTOR signaling through the sponging of microRNA-100-5p (miR-100-5p) to activate mTOR and its interaction with mTORC1 components to stimulate the mTORC1 pathway.¹¹³ However, these interactions need to be confirmed through other assays based on RNA/protein crosslinking methods. Several lncRNAs are found to regulate upstream and downstream molecules of the mTOR complex to modulate the mTOR pathway. For example, the NBR2 lncRNA was found to function through the LKB1-AMPK by maintaining the NBR2-AMPK feedback-forward loop. Further, the RNA pulldown experiments confirmed the interaction of NBR2 with the AMPK- α subunit. This interaction was markedly enhanced under glucose starvation conditions. NBR2 regulates cell growth, autophagy, and apoptosis in response to energy-related stresses through mTOR signaling.¹¹⁴ MALAT1 lncRNA was found to act as oncogenic lncRNA in hepatocellular carcinoma (HCC) via splicing factor SRSF1/mTOR/S6K1 axis.¹¹⁵ Overexpression of LINC00152 was reported to increase HCC tumorigenesis regulating the EpCAM expression through mTOR signaling cascade.¹¹⁶ Another study displayed that H19 lncRNA was downregulated in human pituitary adenomas. Forced expression of H19 suppressed the cellular proliferation and tumor growth of pituitary cancer cells. Mechanistically, H19 interacts with 4E-BP1 which hampered the 4E-BP1 interaction with Raptor.¹¹⁷ This study displayed the potential role of the H19-mTOR-4E-BP1 axis in pituitary tumors.^{117,118} LINC00963 overexpression was associated with poor prognosis, cell proliferation, invasion, and metastasis in non-small cell lung carcinoma (NSCLC). RNA precipitation and mass spectrometry analysis confirmed that LINC00963 interacts with PGK1 which in turn caused activation of

AKT/mTOR signaling cascade in NSCLC.¹¹⁹ Several groups have displayed that altered lncRNA expression can change mTOR activity or vice versa. The upregulation of the GAS5 lncRNA inhibited the tumorigenesis of gastric carcinoma through the miRNA-106a-5p/AKT/mTOR axis in both in vitro and nude mice xenograft models.¹²⁰ Restoration of the GAS5 expression enhanced the sensitivity of the cisplatin in glioma through mTOR-mediated autophagy.¹²¹ Depletion of CASC9 suppressed the tumor growth of OSCC xenograft by autophagy-dependent apoptosis via AKT/mTOR pathway.¹²² Silencing of TUG1 lncRNA caused apoptosis of HCC cells through mTOR signaling. Moreover, this study used both the activators and inhibitors of the mTOR/S6K pathway and confirmed that TUG1 controls HCC growth via the mTOR/S6K axis.¹²³ CRNDE is one of the highly overexpressed lncRNA in patients with glioma and glioma cell lines. Overexpression of CRNDE promoted the growth, clonogenicity, invasion, and migration of glioma cells through increased expression of P70S6K. Mechanistically, the acetylation of histones at the promoter region can lead to the upregulation of CRNDE.¹²⁴ HULC has been found to regulate many cellular processes that are overexpressed in human malignancies. Depletion of HULC decreased angiogenesis, proliferation, and invasion by inhibiting the phosphorylation of ERK/AKT/mTOR and downstream target eIF4E.¹²⁵ The UCA1 lncRNA was found to support the increased glycolysis due to the activation of hexokinase 2 via the mTOR-STAT3/miRNA143 axis in bladder cancers.¹²⁶ ZNNT1 lncRNA is localized on chromosome-8 and has only a single exon. ZNNT1 was identified as a downstream target of the mTOR pathway. ZNNT1 expression was induced upon treatment with rapamycin in uveal melanoma.¹²⁷ The overexpression of the ZNNT1 was reported to induce autophagy that regulates tumorigenesis by regulating the expression of ATG12 in uveal melanoma.¹²⁷ We have discussed the role of several other lncRNAs involved in the mTOR signaling in Fig. 4. However, the molecular mechanism(s) behind the deregulation of the lncRNA/mTOR axis need to be investigated in greater detail.

Role of the mTOR signaling in cancer stem cells

CSCs have been characterized as a unique sub-population that has the ability of self-renewable, metastasis, and drug resistance leading to relapse.^{102,128} During the last decades, CSCs have been observed in a variety of human cancers, including ovarian, pancreatic, breast, liver, and lung.^{129,130} Several groups have displayed the involvement of the Wnt/ β -catenin, Hedgehog, STAT3, TGF- β , PI3K/AKT/mTOR signaling cascade in the CSCs. Recently, mTOR signaling has been reported to be critical for CSC self-renewal, maintenance, and tumorigenicity. Zhou et al. have shown the potential role of mTOR signaling in breast cancer stem cells that increased the clonogenic ability and tumor formation in vitro and xenograft models, respectively.¹³¹ Treatment of HCC with branched-chain amino acids resulted in the activation of mTORC1. Further, the overexpression and silencing approach displayed that mTORC1 activation or silencing of mTORC2 inhibited the CSC population and tumorigenicity by suppressing the expression of EpCAM in HCC.¹³² The activation of the PI3K/AKT pathway was noticed in glioblastoma multiforme (GBM) neurospheres. Interestingly, the combination of alpelisib with pharmacologic mTOR inhibition led to a dramatic and significant decrease in the growth of glioma stem cells.¹³³ In another study, the silencing of mTOR or the treatment of rapamycin in A172 cells resulted in the repression of NSC/progenitor markers in GSCs leading to decreased sphere formation. NVP-BEZ235 (PI3K/mTOR inhibitor) treatment demonstrated a significant decrease in the growth of GSCs derived from patient samples in xenograft models.¹³⁴ Importantly, the Yamanaka stem cell factors were used to generate CSCs for understanding the molecular basis of CSCs in human breast cancer cells. This study observed that transcriptional suppression of mTOR repressors plays an important role during

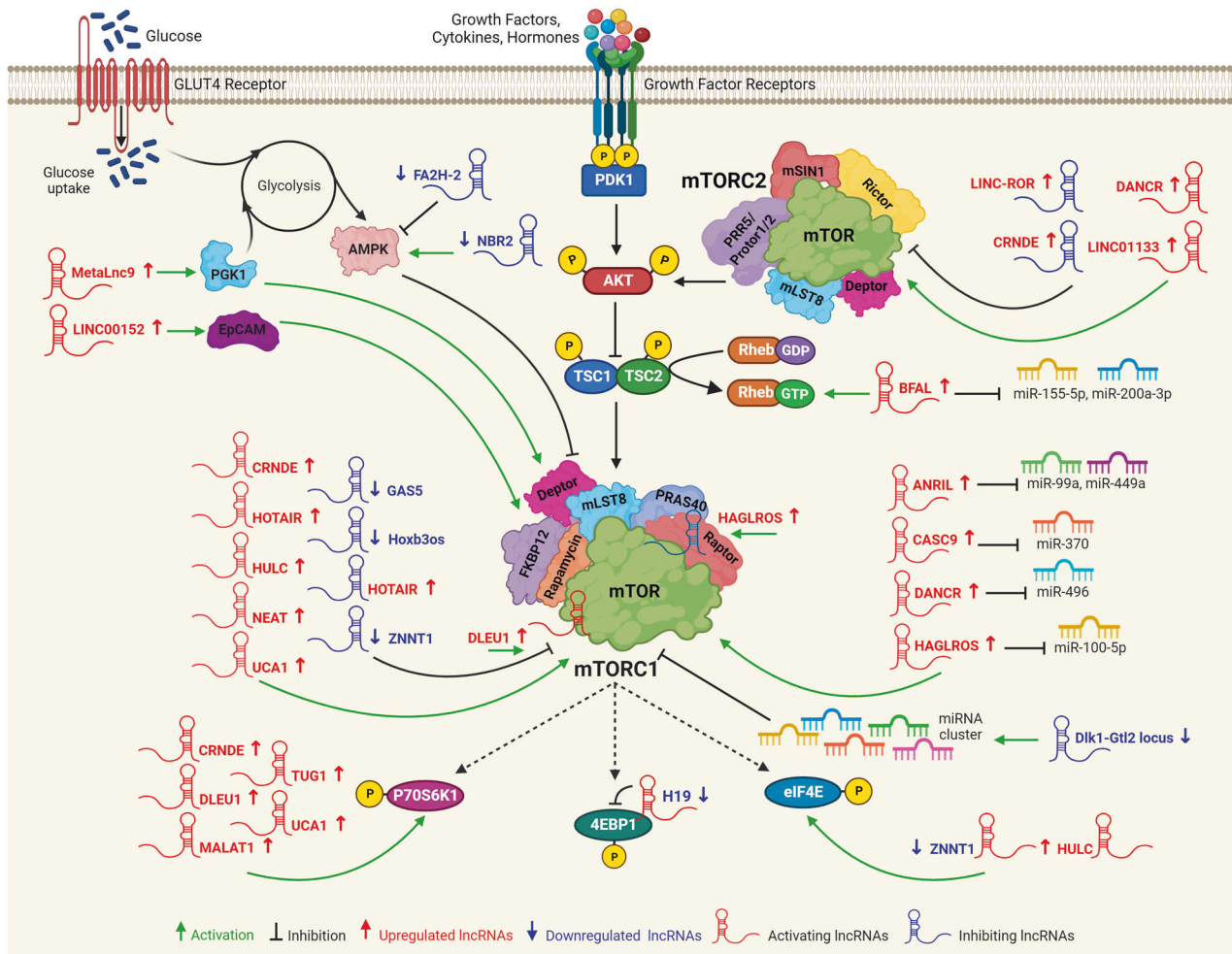


Fig. 4 The association of long noncoding RNAs in mTOR signaling. The lncRNAs act as a sponge for miRNA that controls the expression of the upstream or downstream protein coding gene involved in the mTOR signaling cascade. DLEU1 and HAGLROS can directly bind with mTOR whereas FA2H-2 and NBR2 lncRNA regulate mTOR signaling through AMPK. LINC-ROR, CRNDE, DANCER, and LINC01133, regulate mTORC2-mediated signaling. HULC, ZNNT1 activate eIF4E, H19 inhibit 4E-BP1, and CRNDE, DLEU1, TUG1, UCA1, and MALAT1 activate P70S6K1 to modulate mTOR signaling. Created with BioRender.com

the attainment of the CSCs-like characteristics.¹³⁵ This has been reported that PTEN/PI3K pathway is essential for sphere formation and maintenance of CSCs in prostate carcinoma. The NVP-BEZ235 (PI3K/mTOR inhibitor) was effective in suppressing the CSCs and growth of prostate cancer.¹³⁶ The hyperactivation of the PI3K/Akt/mTOR pathway was linked with the upregulation of CXCR4 in A549 gefitinib-resistant (A549-GR) lung cancer cells. This study showed that the population of CXCR4⁺ cells is quite high in the A549-GR cell and has a high capability of self-renewal in vitro and tumorigenicity in the murine model. The CXCR4-mediated STAT3 signaling was also active in A549-GR cells, indicating its importance during the stemness in lung cancer cells.¹³⁷ A study found that phosphorylated IGF-1R was markedly high in breast cancer stem cells (BCSCs) which resulted in mammosphere and tumorigenicity. Interestingly, rapamycin (mTOR inhibitor) displayed a significant reduction in BCSCs in vitro and xenograft model.¹³⁸ Hoshii et al. have generated conditional knockouts of *Raptor*, a component of mTORC1. Depletion of *Raptor* was associated with the inhibition of leukemia in a murine AML model through apoptosis of differentiated leukemia cells. Further, the transplantation of *Raptor*-deficient AML cells demonstrated that mTORC1 is critical for the initiation of leukemia, suggesting that loss of mTORC1 supports the self-renewable ability of leukemic stem cells (LSCs).¹³⁹ Ghosh et al. have reported that

depletion of S6K1 enhanced the survival of mice transplanted with MLL-AF9⁺ LSCs through AKT and 4E-BP1 phosphorylation. The S6K1 can work through many targets of the mTOR signaling to increase the renewal and progression of LSCs. The inhibitors against PI3K/mTOR pathway sensitize chronic myeloid leukemia stem cells with tyrosine kinases like nilotinib as well as restore the response of progenitors against nilotinib even in the presence of stem cell factor.¹⁴⁰ On the other hand, pharmacological inhibition of mTOR was reported with increased expression of CD133 in gastric cancer both in time and dose-dependent fashion.¹⁴¹ Yang and colleagues have revealed that suppression of mTOR signaling markedly blocked the conversion of CD133⁺ to CD133⁻ in liver cancer. In xenograft models, the treatment of rapamycin enriched the population of CD133⁺ cells and promoted tumorigenesis of HCC cells.¹⁴² Altogether, the data from several groups suggested the dual role of mTOR signaling in cancer stem cells in human malignancies. This might be because of different cell types. Therefore, careful thoughts are needed to use mTOR inhibitors against different cancer types.

Resistance to mTOR inhibitors in human cancers

Drug resistance is one of the most serious problems during the treatment of human cancers in clinics. Several reasons for drug resistance include (1) tumor heterogeneity, (2) clonal selection, (3)

evolution of new clones, (4) intrinsic resistance to cell death, (5) complexity and crosstalk among signaling pathways, and adaptation to other survival pathways. The mTOR inhibition revealed promising anticancer efficacy in preclinical models. On the contrary, resistance against mTOR inhibitors has been noticed in several tumors. Efflux of the chemotherapeutic drugs by ABC transporters is one of the essential molecular mechanisms of drug resistance and poor outcomes of treatment. The overexpression of ABC transporters has been noticed in a variety of cancer cell lines treated with mTOR inhibitors. The mTOR inhibitors including AZD8055 and rapamycin were confirmed as a substrate of ABCB1.¹⁴³ NVP-BEZ235 and AZD8055 were found to be transported through ABCG2.¹⁴³ The *Abcb1* and *Abcg2* knockout mice showed enhanced penetration of rapamycin, AZD8055, and NVP-BEZ235 in the brain compared to wild-type mice.¹⁴³ The overexpression of the ABCB1 was associated with resistance to everolimus in luminal breast cancer cells.¹⁴⁴ NVP-BEZ235 was used in combination with sunitinib against metastatic castration-resistant prostate (mCRPC) cancer and resulted in a synergistic antitumor effect.¹⁴⁵ The resistance against PF-4989216 PI3K/mTOR inhibitor was observed in lung carcinoma through ABCG2 upregulation. The resistance against PF-4989216 was reversed by inhibition ABCG2.¹⁴⁶ This was reported that LY3023414 acts as a substrate for both ABCG2 and ABCB1 transporters. The overexpression of ABCG2 and ABCB1 was shown to suppress the intracellular uptake of LY3023414 leading to resistance in cancer cells.¹⁴⁷ To understand the mechanism of mTOR inhibitors resistance in human cancers, a resistance screen was performed in MCF-7 breast cancer cells and discovered somatic mutations A2034V and F2108L in the FRB-FKBP12 domain of the mTOR to acquire resistance against rapamycin. Also, the M2327I somatic mutation in the kinase domain of the mTOR was observed in the case of an ATP-competitive inhibitor AZD8055.¹⁴⁸ Importantly, the clinical relevance of somatic mutations has been supported when the F2108L mutation was conferred in the patient who relapsed after the treatment of everolimus in anaplastic thyroid carcinoma.¹⁴⁹ The mutant tumor cells showed sensitivity to mTOR kinase inhibition. Somatic mutations in the kinase domain including M2327I have been noticed in the drug-naïve patients.⁸⁶

ROLE OF THE MTOR SIGNALING IN AUTOPHAGY

Autophagy is one of the important processes which are critical for cellular digestion to remove damaged organelles and macromolecules.^{150,151} Apart from this, autophagy is critical in maintaining cellular equilibrium by providing energy and building blocks under stress conditions.^{150,151} Autophagy was reported when the electron microscope revealed the structure of vesicles has amorphous materials and cytoplasmic organelles in the kidneys of newborn murine.^{151–153} Later, studies have demonstrated that the deprivation of amino acid can robustly enhance the process of autophagy perfused livers of rats and mammalian cells.^{151,152} Also, several groups have reported that amino acids are one of the important regulators of the mTORC1 cascade. Under nutrient and growth factor-deprived conditions, mTORC1 activity was reported to be suppressed, indicating that there is an inverse relation between autophagy and activation of mTORC1.^{154,155} The induction of autophagy through inhibition of mTORC1 has been well studied in yeast and drosophila models.⁶ Interestingly, the molecular basis of mTORC1 to regulate autophagy in mammalian cells is quite recent and emerging. mTOR controls autophagy through the regulation of a protein complex composed of UNC-5-like autophagy-activating kinase 1 (ULK1), autophagy-related gene 13 (ATG13), and focal adhesion kinase family-interacting protein of 200 kDa (FIP200). Studies have revealed that mTORC1 can inhibit the ULK complex through the phosphorylation of ATG13 and ULK1/2 (Fig. 5). Silencing of mTORC1 was found to be associated with enhanced activity of

ULK1/2 kinase leading to phosphorylation ATG13 and FIP200, the important components of ULK1/2 kinase complex.¹⁵⁶ The mTORC1 was found to phosphorylate ULK1 at Ser-758 which in turn blocks the interaction with AMPK halting ULK1 activation.¹⁵⁷ Moreover, mTORC1 was reported to decrease the stability of the ULK1 through phosphorylation of autophagy/beclin 1 regulator 1 (AMBRA1).^{158,159} The mTORC1 and AMPK control the activity of the VPS34 complex which is needed for the generation of the autophagosome.¹⁵⁹ The ATG14L-associated VPS34 complex has been noticed to play a crucial role in the regulation of autophagy.¹⁶⁰ Under nutrient stress conditions, AMPK stimulates the autophagy VPS34 complex through phosphorylation of Beclin 1 while suppressing the non-autophagy VPS34 complex via Thr163/Ser165 phosphorylation in VPS34. On the other hand, mTORC1 leads to the phosphorylation of ATG14L in the VPS34 complex to suppress lipid kinase activity of VPS34, suggesting another mechanism for autophagy inhibition through mTORC1.¹⁶¹ Moreover, the precise role of autophagy in human malignancies is still not clear because activation or suppression of autophagy was found to be tumorigenic or anti-tumorigenic. Emerging pieces of evidence have shown that activated autophagy can suppress the process of cancer progression, especially in precancerous lesions. However, several studies indicated that autophagy acts to promote tumor survival and growth in advanced cancers.^{162,163} Therefore, the inhibition of autophagy can be employed as a therapeutic approach. Under stress conditions, autophagy supports the growth and survival of tumors, especially in poorly vascularized tumors. Dysregulated autophagy has emerged as an adaptive mechanism for the initiation and progression of human cancers through the accumulation of DNA damage, macromolecules, organelles like mitochondria, oxidative stress, chromatin instability.^{164,165} In addition, stress-induced autophagy has been noticed with enhanced stemness and drug resistance in human cancers.^{164,166}

THE IMPORTANCE OF MTOR SIGNALING IN THE AGING PROCESSES

Several studies have observed that mTOR signaling is involved in the key processes associated with aging in a variety of living organisms, such as worms, yeast, flies, and mammals. Initial studies on the *Caenorhabditis elegans* (*C. elegans*) have noticed that suppressed expression of the mTOR homolog known as *ceTOR* or *let-363* as well as *Raptor* known as *daf-15* was associated with an increased life span of almost more than double.¹⁶⁷ The mutations in the *CeTOR* and *raptor* were reported with dauer-like larval arrest and suggested that *CeTOR* is important for the regulation of dauer diapause. The *let-363* and *daf-15* mutants displayed a marked shift in the metabolism that resulted in fat accumulation and an extended adult life span.¹⁶⁸ Later on, other genetic screening studies also noticed that reduced mTOR signaling enhanced the life span of the drosophila, yeast, and murine models.^{169–171} Altogether, these studies have proved that extended life span was highly dependent on nutritional condition (glucose, fat, and protein metabolism) and a close association with the mTOR pathway. Interestingly, rapamycin, a pharmacological mTOR inhibitor has been proven to increase the life span in different model organisms.^{172–174} This is well-established that mTORC1 has a major role in nutrients and insulin sensing. Therefore, the benefits of a calorie-restricted diet on life span are because of the reduced mTORC1 signaling. This was confirmed when a calorie-restricted diet did not extend the life span upon inhibition of mTOR signaling in *C. elegans*, *Drosophila*, and yeast.^{170,175} There are several thoughts regarding the role of mTOR signaling in aging processes in mammalian systems. The repression of the mRNA translation during mTORC1 inhibition was correlated with slower aging by reducing oxidative and proteotoxic stress. This observation was the consistent loss of S6K1 that

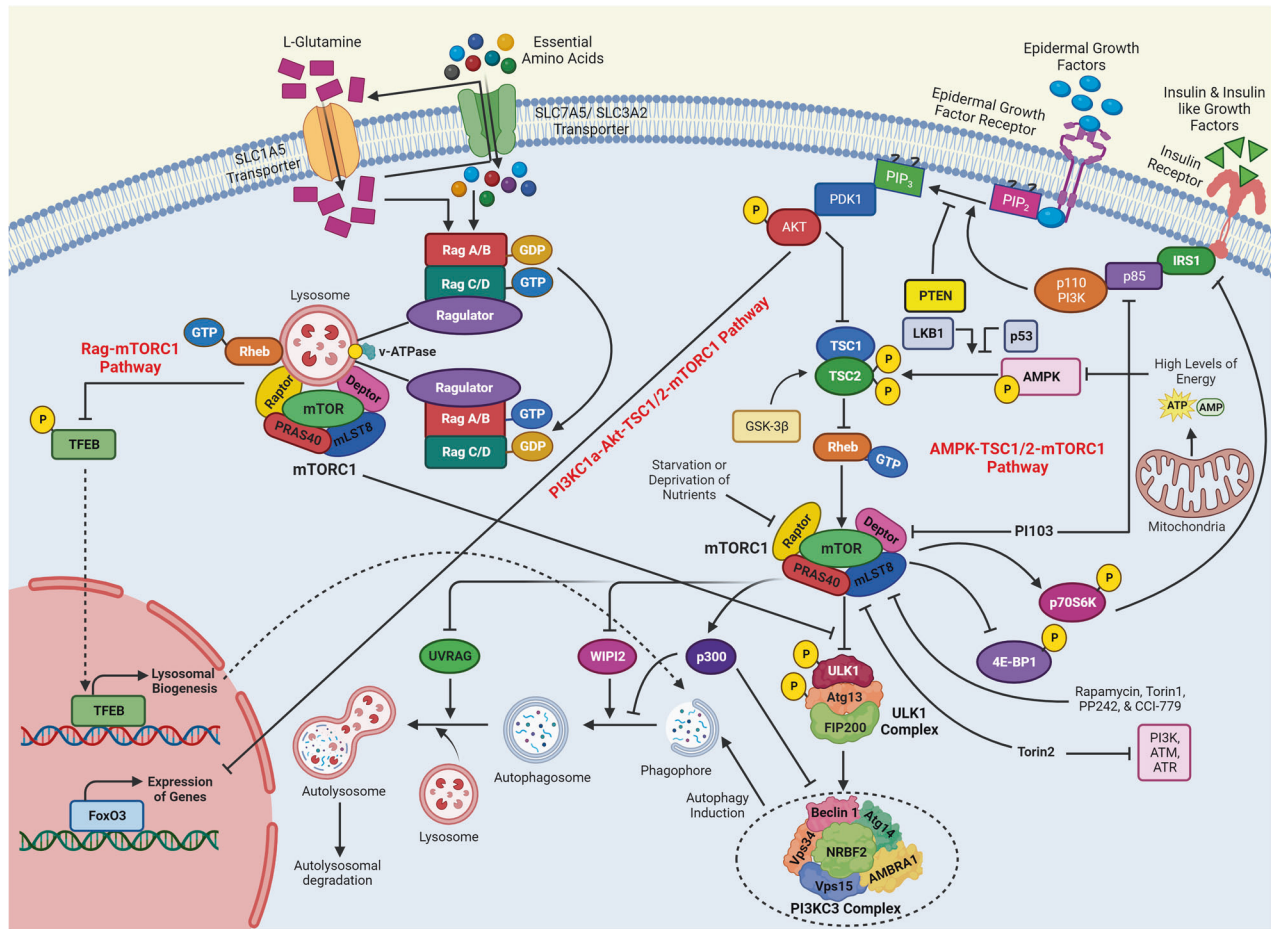


Fig. 5 Key events involved in mTOR-mediated autophagy. The mTORC1 suppressed the ULK1 complex activity via phosphorylation of ULK1/ATG13. The mTORC1 can help in the translocation of TFEB in the nuclear compartment to regulate the process of autophagy. The mTORC1 has an important role in the induction of autophagy, nucleation, phagosome elongation, autolysosome formation, and finally degradation through lysosomes. Created with BioRender.com

extends the life span, and resistance to age-related diseases like compromised immunity, motor dysfunction, and loss of insulin sensitivity in mammals.¹⁷⁶ Also, the loss of S6K1-induced gene expression was similar to a calorie-restricted diet or pharmacological activation of AMPK.¹⁷⁶ RNA polymerase III (POL III) was reported to play a critical role in nutrient signaling, and anabolic activities to accelerate aging by mTORC1.¹⁷⁷ There is another possibility that the depletion of mTORC1 could modulate aging via autophagy. Moreover, this was thought that the attenuation of adult stem cells could be central in maintaining aging processes. Rapamycin treatment was found to increase the self-renewal ability of hematopoietic stem cells and the life span of old age mice.^{178,179} Other studies revealed that mTORC1 and SLC7A5 regulate the self-renewal of intestinal stem cell self-renewal and Paneth cell function in maintaining intestinal niche and physiology.^{180,181} The depletion of foxo was found to restore stem cell aging in germline stem cells of drosophila.¹⁸² Interestingly, the phase IIa clinical trials were conducted on 264 volunteers with an age of ≥ 65 years of age at 12 clinical sites to evaluate the safety, and efficacy of mTOR inhibitors to boost immune responses.^{183,184} The low dosages of everolimus markedly decreased the rate of infections and enhanced the vaccination response against influenza with increased antiviral immunity.¹⁸⁴ Based on these data, alternative rapamycin dosage regimens were proposed for better longevity with minimal side effects.¹⁸⁵ Altogether, mTOR inhibition can increase life expectancy and help delay the onset of age-associated diseases. However, the duration of the treatment

should be decided carefully as it can produce severe side effects like immunosuppression and glucose intolerance.

MTOR SIGNALING IN NEUROLOGICAL PROCESSES, BRAIN DEVELOPMENT, AND NEUROLOGICAL DISEASES

The emerging research studies displayed that mTOR signaling is one of the important regulators of neurological processes like neural stem cells, neural development, synaptic plasticity, circuit formation, learning, and memory.¹⁸⁶ Ablation of Rictor or Raptor in neurons was found to decrease the neuron size and early death.¹⁸⁷ The depletion of Rictor or Raptor was shown to have a differential impact on the differentiation of the oligodendrocyte and myelination of the central nervous system. These data revealed the importance of mTORC1 and mTORC2 during brain development.¹⁸⁷ On the contrary, the hyperactivation of the mTORC1 pathway was observed in the brain and well-documented in human patients with TSC. These patients have been shown with several neurological disorders such as autism, intellectual disability, epilepsy, anxiety, sleep disturbances, and brain tumors.^{188,189} TSC has been characterized as an autosomal disorder caused by the loss of either TSC1 or TSC2. Moreover, the hyperactivation of mTORC1 because of the *Tsc1* or *Tsc2* loss in the neural cells in the murine models displayed severe epileptic seizures. The rapamycin treatment was effective in reducing epileptic seizures in these mice.¹⁹⁰ The mutations in components of the KICSTOR and GATOR1 complexes were associated with

epilepsy in humans. Recently, the retrospective analysis of TSC patients treated with everolimus or sirolimus under the age of 2 years has reported promising benefits on epilepsy.¹⁹¹ This study for the basis for the testing of mTOR inhibition in a large cohort of patients with TSC. The mTORC1 activation in tissue stems promoted mRNA translation near synapses which required neuronal circuit formation. Further, inhibition of mTOR signaling was found to block the ketamine-induced synaptogenesis and behavioral responses.¹⁹² The mTORC1-mediated autophagy was found to be strongly correlated with the pathogenesis of neurodegenerative disorders like Alzheimer's disease (AD), and Parkinson's disease (PD). The rapamycin treatment was found to inhibit the progression of AD and increase the life span in the diseased model of AD.¹⁹³ Rapamycin is used in clinical settings and has shown promising effectiveness against AD and emerged as a potential therapeutics.¹⁹⁴ Dactolisib treatment was shown to protect the AD in a transgenic murine Alzheimer model.¹⁹⁵ In the future, next-generation mTOR inhibitors can be designed and tested in AD and PD mice models for better drugs.

ROLE OF MTOR SIGNALING IN MAINTAINING THE IMMUNE RESPONSE

Recent reports have uncovered a significant regulatory function of the mTOR pathway not only in cancer but also in the differentiation, activation, and functional characteristics of immune cells.¹⁹⁶ Tumors can evade the immune system by dampening its ability to detect and eliminate cancer cells.¹⁹⁷ Recent research has focused on tumor immunotherapy as a promising approach to tackle this challenge. Multiple studies indicate that the mTOR pathway, which is frequently overactive in tumors, plays a role in controlling the development and effectiveness of immune cells.⁵ Moreover, mTOR signaling plays a vital role in enabling T cells to perceive and merge immune signals originating from dendritic cells (DCs).¹⁹⁸ These signals have been found to involve cytokines, co-stimulatory molecules, and antigenic signals as well as environmental cues derived nutrients, growth, and immunoregulatory factors.¹⁹⁸ T cells rely on mTOR signaling to sense and integrate this comprehensive array of immune and environmental inputs. Studies have identified that TSC1 acts as most crucial for maintaining the naive T-cell quiescence and survival to maintain immune homeostasis.^{199,200} The T cells that were deficient for PTEN were reported to upregulate mTORC1 activity to maintain their quiescence before tumorigenesis indicating the importance of mTORC1 on T-cell homeostasis.²⁰¹ Other studies have displayed that phosphorylation of liver kinase B1 or STK11 phosphorylates AMPK under energy deprivation. Further, deletion of *Lkb1* in T cells caused massive T-cell apoptosis while compromising thymic selection, altered metabolism, and proliferation of T cells.^{202,203} The stimulation of T- and B-cell receptors as well as other cytokine receptors, such as the IL-2 receptor, causes mTOR to become active in the adaptive immune system. In cytokine-stimulated T cells, mTOR regulates the transition from the G1 to the S phase of the cell cycle.^{204,205} In addition, once T cells are activated by IL-12, mTOR may drive the development of Th1 cells by stimulating the production of IFN- γ .²⁰⁶ Further, studies have reported that the antigen recognition by naive T cells leads to the mTOR activation, which guides the differentiation of CD4⁺ T cells into the T-helper cell effector lineages. Also, mTOR was shown to regulate the effector fate of CD8⁺ T cells during tumor immunity and infections.²⁰⁷ Interestingly, several studies have primarily focused on mTOR inhibitor rapamycin's ability to hinder T-cell proliferation and IL-2 production and induce anergy (a cellular state when the lymphocytes fail to respond upon stimulation), even in adequately stimulated T cells.^{196,198,205} Upon T-cell receptor (TCR) stimulation, both mTORC1 and mTORC2 are activated. The mTORC1 was found to influence the effector responses of T cell (CD8⁺) whereas

mTORC2 activity was associated with metabolic reprogramming to generate memory T cell (CD8⁺).²⁰⁸ The extent of mTOR activation is directly linked to the duration of the interaction between T cells and DCs, as well as the amount of the corresponding antigen (Fig. 6). In addition, co-stimulatory signals exert a significant influence on mTOR activity. CD28-mediated co-stimulation, a well-known activating signal for the PI3K-AKT pathway, enhances the mTOR activity induced by TCR stimulation. This synergistic effect facilitates effective T-cell activation by upregulating mTOR function. The role of mTOR signaling in B-cell development, differentiation, and function is less studied than in T-cell development. The mTOR hypomorph mouse model was generated by *neo*-insertion which partially disrupts *mTOR* transcription. This murine model displayed a partial block of large pre-B to small pre-B stages of B-cell development and compromised proliferation in response to B-cell mitogenic signals. B-cell receptor (BCR) and CD40 signaling were more highly compromised than TLR signaling.^{209,210} This resulted in the alteration in the splenic populations, production of antibodies, and migration towards chemokines.²⁰⁹ The deficiency of SIN1 resulted in the increased expression of IL-7 receptor (*il7r*), *rag1*, and *rag2* which is responsible for increased V(D)J recombinase activity and survival of the pro-B-cell. This study showed that Akt2 mediates the Sin1-mTORC2-dependent inhibition of *il7r* and *rag* gene expression which in turn control the phosphorylation of FoxO1 during B-cell development. Interestingly, mTOR inhibition using rapamycin enhanced *rag* expression and V(D)J recombination in B cells. This study indicated Sin1/mTORC2-Akt2/FoxO1 axis is critical in B cells.²¹¹ *Rictor*^{fl/fl}*Mx1*-Cre mice displayed an increase in the population of pro-B, pre-B, and immature B cells with a significant decrease in mature B cells.²¹² The deficiency of *Rictor* in B cell resulted in the upregulation of *IL-7R*, *RAG1*, and decreased phosphorylation of Foxo1.²¹² In the absence of T-cell antigens, the mTOR signaling can regulate antigen titration which regulates B-cell activation. Also, the activation of mTOR signaling by BCR stimulation and repression of the mTOR pathway by Fc receptor signaling exhibited the crucial role of mTOR in regulating B-cell functions.²¹³ Raptor was specifically deleted in the murine B cell by crossing *Raptor*^{fl/fl} mice with *Mb1*-Cre transgenic mice. *Raptor*^{fl/fl}*Mb1*-Cre model demonstrated the block in the early pre-B-cell stage with the loss of immature and mature peripheral B cells. *Raptor*-deficient pre-B cells were found to have reduced survival, proliferation, oxidative, and glycolytic metabolic capacity.²¹⁴ Interestingly, the treatment of these mice with Rapamycin recapitulated the early block during B-cell development, and loss of immature B cells with the accumulation of pre-B cells.²¹⁴ Recently, transcriptomic analysis of the B cells displayed that follicular B cells upregulate a network of unfolded protein response genes before the secretion of antibodies. The transcription of unfolded protein response genes needs Raptor and mTORC1 kinase adapter. This study suggested that B cells exploit mTORC1 for subsequent plasma cell function and prior antibody production without Xbp1 activity.²¹⁵

Growth factors, TLR ligands, cytokines, and other extracellular signals can all activate the mTORC1-mTORC2 network in innate immune cells.²¹⁶ Studies have demonstrated that mTOR signaling also plays a crucial role in the differentiation and function of dendritic cells (DCs) and natural killer (NK) cells.²¹⁷ DCs possess strong antigen presentation abilities, and NK cells are important immune cells involved in tumor surveillance.^{218–220} Previous studies have reported that both mTORC1 and mTORC2 exert distinct effects on NK cell function.²²¹ While mTORC2 negatively regulates NK cell function by inhibiting the STAT5/SLC7A5 axis, mTORC1 positively regulates mTORC2 activity, thereby promoting the CD122-mediated interleukin-15 signaling pathway.²²² The cytokine interleukin-15 (IL-15) induces mTOR activity in NK cells in both humans and mice. Additionally, recent studies have linked DCs to the mTOR signaling pathway. Inhibition of mTOR in DCs has been shown to improve

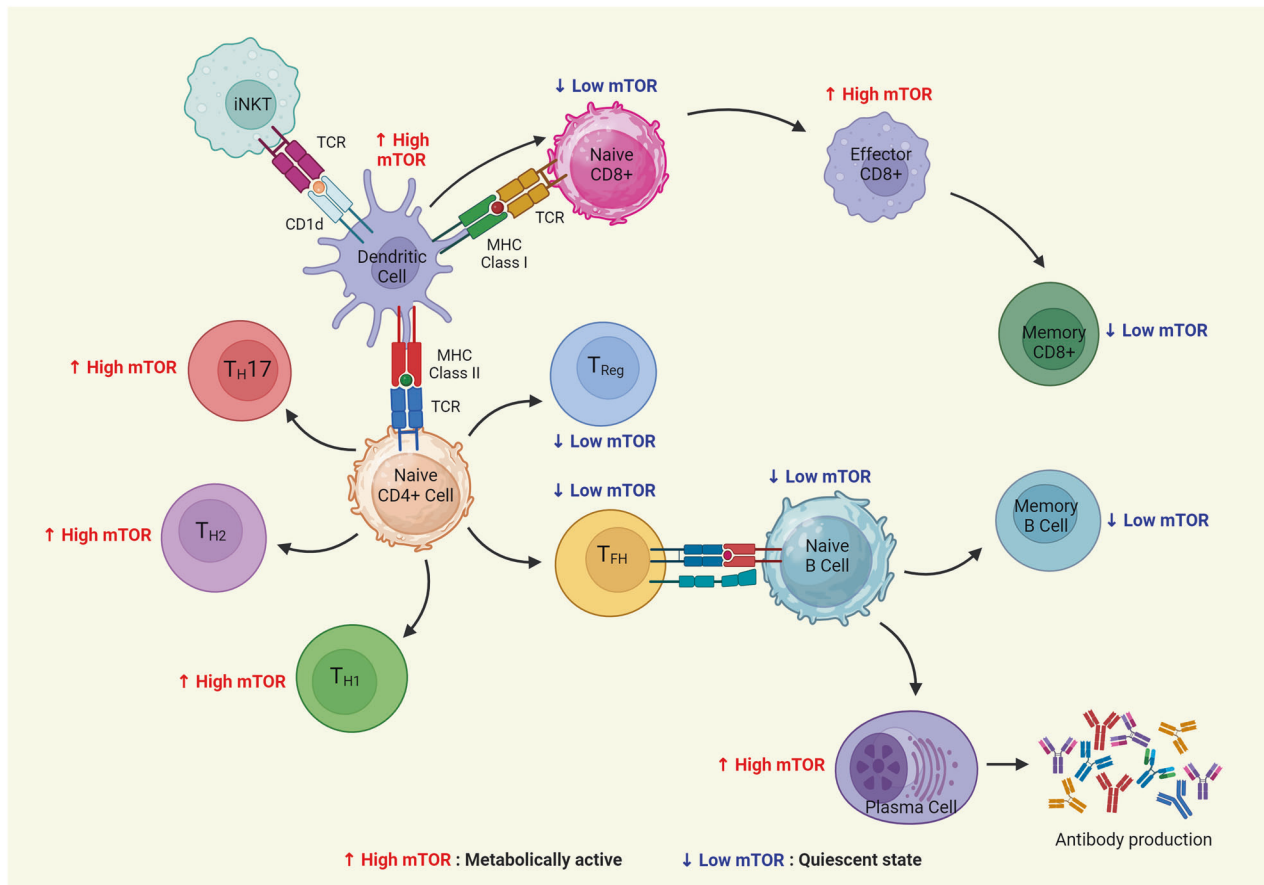


Fig. 6 The mTOR signaling is crucial for modulating the key immune responses. The activated dendritic cells can present the antigen through T-cell receptors that initiate activation, proliferation, and differentiation of invariant natural killer T cells, CD4⁺, and CD8⁺ T cells through stimulation of mTOR. The higher levels of mTOR determine the metabolic active state while lower levels of mTOR define the quiescent state of the T- and B cells. The absence of mTOR signaling during differentiation of naive CD4⁺ T cells generates T-regulatory cell and T-follicular helper cell. The high mTOR activity during activation of naive CD4⁺ T cells supports the expression of the crucial transcription factors needed for their differentiation into Th1, Th2, and Th17 cells. T-follicular helper cells activate the germinal center to generate antibodies. Created with BioRender.com

their antigen presentation capabilities and enhance the activation of cytotoxic CD8 + T lymphocytes, resulting in increased antitumor activity.^{223,224} Therefore, mTOR inhibitors hold promise for enhancing the efficacy of tumor immunotherapy and autologous DC-based vaccination by extending the life span of DCs and improving their antigen-processing abilities.

Macrophages, specifically the M1 and M2 subtypes, play critical roles in tumor development and progression.^{225,226} M1 macrophages can kill tumor cells, while M2 macrophages promote tumor growth, invasion, and metastasis.²²⁷ Dysregulation of the mTOR pathway has been implicated in the polarization and function of macrophages. For instance, decreased expression of miR-30c, which inhibits mTOR activity, leads to the inhibition of M1 macrophage differentiation and function, ultimately promoting tumor growth and metastasis.⁵ These findings highlight the intricate relationship between the mTOR pathway and macrophage polarization, shedding light on potential therapeutic strategies to modulate immune responses in the tumor micro-environment.¹⁹⁶ Studies have shown that enhanced PI3K and mTOR signaling in mouse macrophages leads to increased M2 macrophage markers and activation of the STAT6 pathway, which promotes M2 polarization.²²⁸ Conversely, inhibiting mTORC1 in human macrophages enhances M1 polarization. However, the role of mTORC1 and mTORC2 can be complex, as the deletion of Tsc1, a component of mTORC1, can promote both M1 and M2 polarization depending on different pathways.²²⁹ AKT, another

protein in the pathway, also has isoform-specific effects on macrophage polarization. The PI3K–AKT–mTOR pathway is involved in sensing these cues and affecting macrophage polarization, although the mechanisms are not fully understood. Taken together, mTOR inhibition has enormous potential for vaccine development to boost immunity against human malignancies and pathogens.

OPPORTUNITIES AND CHALLENGES OF MTOR SIGNALING IN CANCER THERAPEUTIC TARGETING

Rapamycin is a natural macrocyclic lactone that is obtained from the bacterium *Streptomyces hygroscopicus*.^{230,231} After the discovery of rapamycin, researchers continued to explore the related targets and signaling involved at the molecular and cellular levels.^{230,232} The FKBP12 and FKBP51 expression was found to be the rate-limiting factor that decides the rapamycin drug response in cell lines and tissues.²³³ This led to the discovery and development of various moieties including synthetic/semisynthetic which were active against mTOR-induced oncogenesis.^{232,234,235} Sirolimus is another approved biochemical functional form of rapamycin that displayed target-specific inhibition.²³⁶ Sirolimus has been found to impair the molecular interaction between mTOR and Raptor by targeting mTORC1.^{234,237,238} Sirolimus is an oral drug that has high protein binding, an elimination half-life of 57–63 h, high drug distribution

to the circulation, and is metabolized by the hepatic enzymes like CYP3A4, CYP3A5, and excretion through p-glycoprotein.²³⁹ Sirolimus was approved by FDA for organ transplantation as an immunosuppressive agent in the year 2009. Interestingly, therapeutic drug monitoring was found to be a critical aspect during sirolimus treatment.²⁴⁰ Sirolimus received FDA approval in the year 2015 for lymphangioleiomyomatosis based on the efficacy reported in clinical trials (ClinicalTrials.gov number, NCT00457808, and NCT00414648).^{241,242} Nab-sirolimus (albumin-bound nanoparticles-based sirolimus formulation) displayed a strong antitumor effect in cases with perivascular epithelioid cell tumors (PEComa). Nab-sirolimus was finally approved by FDA after the success of the AMPECT phase-II trials against metastatic or unresectable PEComa in 2021.²⁴³ Recently, Nab-sirolimus is going through a clinical trial in solid tumors with genetic mutations in *TSC1* and *TSC2*.²⁴⁴ The poor bioavailability of the drug led researchers to focus mainly on improving its pharmacokinetics and stability without disturbing its pharmacodynamic profile.²⁴⁵ Two sides are obligatory to interact with FKBP12 and mTOR providing few options for structural modifications. Synthetic analogs or derivatives thus designed, synthesized, and biologically evaluated by replacing the hydrogen of -OH (C-40) with different moieties led to the development of several newer analogs.²⁴⁶ These analogs have been named Rapalogs with improved pharmacokinetics and stability without disturbing their pharmacodynamic profile. Temsirolimus (a rapalog) was designed by replacing H of the hydroxyl group (C-40) with di-hydroxyl methyl propionic acid ester and was finally authorized by the FDA for the treatment of the patients with metastatic RCC in the year 2007 due to very high bioavailability, specificity, long half-life, good excretion through fecal and urine.^{247–250} Temsirolimus is well-characterized pharmacological derivative of sirolimus. Temsirolimus was reported to interact with FKBP12 that resulted in the formation of a strong trimolecular complex with mTOR. Temsirolimus was found to be equivalent to sirolimus in terms of its inhibitory activity against mTOR kinase both in the cellular and cell-free systems.²⁵¹ Temsirolimus was noticed with remarkable antitumor potential in human preclinical and clinical trials for cancers as a single agent (monotherapy) or in combination with other chemotherapeutic agents in refractory/relapsed acute lymphocytic leukemia, cervix, endometrial and ovarian cancers.^{252–256} Everolimus was designed as an immunosuppressive drug by replacing H of the hydroxyl group (C-40) with a hydroxyethyl group.²⁵⁷ Everolimus is an orally administered drug that possesses high protein binding, and a good half-life, is metabolized mainly through CYP3A enzyme, has good blood-brain penetration, and is eliminated by feces and urine. This has been observed that both temsirolimus and everolimus demonstrated anticancer activity in murine models.⁹ Importantly, these are commonly used chemotherapeutic agents for treating advanced-stage RCC in the clinic. Everolimus is used for patients with advanced breast carcinoma or neuroendocrine pancreatic carcinoma.^{258–260} The combination of exemestane to everolimus markedly enhanced the progression-free survival in HR(+) breast carcinoma and reduced recurrence in breast cancer trials of oral Everolimus-2 (ClinicalTrials.gov; NCT00863655). The combination of everolimus with sunitinib/AZD2014 was not tolerated in the cases with metastatic RCC during clinical trials suggesting that the combination of two parallel signalings should be thought carefully.^{261–263} The everolimus was found to be effective in clinical phase-II trials in patients with advanced thyroid carcinoma with low toxicity and further trials are needed using combination therapy.²⁶⁴ Recently, everolimus was used combined with T-DM1 which resulted in marked antitumor efficacy in HER2-positive breast cancer, suggesting the importance of mTOR-dependent lysosomal processing of T-DM1.²⁶⁵ Everolimus and letrozole were correlated with a promising progression-free survival till 12 weeks of ER⁺ relapsed high-grade ovarian carcinoma in phase-II clinical

trials.²⁶⁶ Everolimus was used in combination with rituximab and reported complete responses in patients with relapsed diffuse large B-cell lymphoma (DLBCL) under NCT00869999 (ClinicalTrials.gov).²⁶⁷ Everolimus treatment has been found to reduce the size of subependymal giant-cell astrocytomas and the frequency of seizures (ClinicalTrials.gov, NCT00411619) in these patients.²⁶⁸ Everolimus has reported promising anticancer efficacy in patients with pancreatic neuroendocrine tumors during clinical trials (RADIANT-3, NCT00510068) and received FDA approval.²⁶⁹ Everolimus was given approval by the FDA in the year 2016 for clinical use in patients with neuroendocrine tumors of the gastrointestinal tract or lung origin.²⁷⁰ Ridaforolimus is the FDA-approved small molecule inhibitor of mTOR(C-40 phosphine oxide substituted formulation of rapamycin) which is available orally or intravenously for the treatment of human soft tissue and bone sarcoma in phase-II clinical trials (ClinicalTrials.gov; NCT00093080) and results showed remarkable efficacy and safety.^{271–273} The results of the phase-III international randomized clinical trial of ridaforolimus (NCT00538239) displayed tumor regression in the patients with metastatic sarcomas compared to the placebo control group.^{272,274} Ridaforolimus displayed potent antitumor activity in advanced endometrial cancer with significant side effects and represent a viable therapeutic agent for PI3K/Akt/mTOR pathway in trastuzumab-resistant and HER2-positive metastatic breast carcinoma and hematological malignancies.^{273–277} The safety and efficacy profiling of the vistusertib/ AZD2014 was evaluated in a variety of preclinical and clinical studies against human cancers.^{278,279} Further, the AZD8055 is another very specific and highly potent dual mTORC1/2 inhibitor with superior anticancer effect in a variety of human malignancies.^{280,281} In the case of leukemia, the AZD8055 treatment displayed enhanced prolonged survival of AML transplanted mice while suppressing the growth of the leukemic cells with managed toxicity profile.^{282–284} These rapalogs were also evaluated for their anticancer activity for the treatment of advanced-stage cancers including liver, gastric, endometrial, lung, and mantle cell lymphoma.^{7,9} Rapalogs have been reported to block mTORC1 substrates selectively and effectively. mTORC1 inhibition and S6K1-dependent pathway inhibition by rapalogs were reported to result in feedback activation of PI3K, Ras/MAPK, mTORC2, RTK's, and AGC kinases.²⁸⁵ Feedback activation of these proteins and pathways was found to compromise the mTORC1 inhibition in disease stabilization, protein biosynthesis, and cell cycle contributing to the development of resistance to rapalogs. O'Reilly et al. found that sirolimus therapy augments AKT phosphorylation at Ser473 residue. The phosphorylation of AKT was also prominent in tumors from patients receiving everolimus as a part of treatment. On the contrary, this has been observed that despite disrupting the S6K-IRS-1-negative feedback loop, sirolimus therapy was potent in preventing carcinogenesis. Also, the genetic variations of the factors associated with the mTOR signaling pathway in cancer cells contribute to de novo resistance to the drugs targeting mTOR.²⁸⁶ A twist in the mTOR story has emerged with the finding that mTORC2 can directly phosphorylate AKT and stimulate a downstream signaling cascade. Unexpectedly, it was noticed that rapamycin can inhibit AKT by disrupting mTORC2 assembly in a few types of cells.²⁸⁷ These discoveries raise many questions regarding the development and application of mTOR inhibitors. To overcome these limitations new strategies were designed and explored. These strategies included combination therapy which includes rapalogs in combination with chemotherapeutic drugs such as paclitaxel or carboplatin and the development of inhibitors that can target both mTOR and PI3K.^{9,288–291} Pharmaceutical companies and academic laboratories have developed numerous such inhibitors by exploiting the structure of the ATP binding pocket of kinases and the small ATP-competitive molecules. Because of sequence similarity among the PI3K and mTOR, several PI3K inhibitors were found to inhibit mTOR

activities. These were called dual PI3K/mTOR inhibitors²⁸⁹ and displayed promising activity to overcome the negative feedback associated with resistance of rapalogs. Because of the diverse functions of different isoforms of PI3K, these dual inhibitors were not well tolerated owing to potential toxicities. These drawbacks fueled the development of inhibitors with higher mTOR specificity than PI3K.²⁹² Researchers then focused on designing such inhibitors that inquest the development of a series of such specific inhibitors. Preclinical and early clinical studies revealed that TOR-KI inhibitors can stop the cell cycle in the G1 phase and suppress the transcription of cyclin D1, which is mTORC1 dependent and activated by AKT.^{9,293} These inhibitors also inhibit the activity of Akt and SGK1, which phosphorylate p27, leading to better inhibition of cyclin-CDK2. By inhibiting the production of HIF-2 α , TOR-KI inhibitors prevent RTK accumulation and growth factor independence, which is favored by HIF-1 and 2 α leading to autonomous growth.^{1,9} Irrespective of the good preclinical and early clinical status of TOR-KI, it was observed by the researchers that the TOR-KI treated cells attenuated mTORC2-mediated phosphorylation of AKT at S473 residue but mTORC1 inhibition can still promote the feedback activation of PDK1 and PI3K derived AKT phosphorylation at T308. This suggested the modest substrate dependency of AKT phosphorylation specifically at S473 residue.²⁹⁴ Although the TOR-KI efficiently inhibited the activity of both TOR complexes but are still quite ineffective because of various feedback loops contributing through upstream signaling pathways as well as the wide range of clinically relevant mutations in mTOR.²⁹⁵ Mutations increase the catalytic activity of mTORC1/2 and thereby reduce the effectiveness of such compounds towards rapalogs, dual inhibitors as well as TOR-KI. To overcome these resistance issues Rodrik-outmezguine and colleagues have generated rapamycin-resistant breast carcinoma cell lines that carry two mutations in the mTOR FRB domain (mTOR A2034V and mTOR F2108L).¹⁴⁸ They also generated the AZD8055-resistant colony that bears mutations in the hyperactive kinase domain. A careful study of the molecular model of mTOR was done revealing the juxtaposition of rapamycin and AZD8055 binding sites. The knowledge of these binding sites has provided the basis for designing new small molecules which finally resulted in a new bivalent mTOR inhibitor.^{7,286} The molecules were designed by linking rapalogs and TOR-KIs using an optimum-length cross-linker. Preclinical studies were performed on murine xenografts of MCF-7 cells bearing these mutations and have reported more sensitivity to rapalink as compared with rapalog and TOR-Kis. This was because of the rapalink(s) capability to inhibit both mTORC1/2, whereas rapamycin and dual inhibitors were not able to block the activities of both complexes effectively even at higher concentrations.¹⁴⁸ Rapalinks are third-generation mTOR inhibitors with advanced structural features of first and second-generation inhibitors that overcome several issues like efficacy, resistance, and feedback activation because rapalink can efficiently bind with the FRB domain of mTOR through binding to FKBP12 and kinase domain of mTOR for its ATP-competitive inhibition simultaneously.²⁹⁶ Sapanisertib (MLN0128 or TAK-228), a selective inhibitor of mTOR reported promising anticancer effects during preclinical analysis. After preclinical evaluation, sapanisertib entered phase-I/II clinical trials for mCRPC, breast carcinoma, lung cancer, endometrial carcinoma, bladder carcinoma, sarcoma, and non-Hodgkin B-cell lymphoma.^{297–304} MLN0128 treatment was found to be very effective in suppressing the tumorigenesis of different subtypes of sarcoma in both in vitro and xenograft models.²⁹⁷ MLN0128 was effective in sensitizing the chemoresistant primary effusion lymphoma.³⁰⁵ Studies have shown that MLN0128 suppresses the growth of HCC tumors and sensitizes them to sorafenib and cabozantinib.^{303,306,307} Interestingly, MLN0128 was effective in sensitizing the everolimus-resistant *PIK3CA* mutant colorectal and pancreatic neuroendocrine tumors.^{308,309} Recently, the DICE trial revealed that TAK-228 along

with weekly paclitaxel chemotherapy resulted in significant improvement and laid the foundation for a phase-III trial in ovarian cancer.³¹⁰ The CC-223 is an oral and potent mTOR kinase inhibitor that was found to suppress the growth and tumorigenesis of HNSCC and HCC through mTORC1/2 in both in vitro and murine model.^{311–314} The preclinical studies displayed that OSI-027 is a selective and potent inhibitor of mTORC1/2 and reported promising antitumor activity in several human cancers.^{315–318} Further, OSI-027 treatment was very effective in enhancing the therapeutic potential of gemcitabine in pancreatic cancer cells and xenograft model.^{319,320} The dual PI3K/mTOR inhibition has emerged as a critical strategy to target mTOR signaling in human malignancies.³²¹ For instance, NVP-BEZ235 (dactolisib) was shown to suppress the PI3K isoforms, mTOR, and ATR which resulted in strong anticancer activity in both solid and blood cancers.^{322–324} Also, NVP-BEZ235 was displayed to cross the blood-brain barrier and reported to sensitize the temozolomide resistance in brain tumors.³²⁵ Strikingly, the combination of dactolisib with immune checkpoint inhibition in primary and metastatic CRPC displayed robust antitumor response by blocking MDSCs.³²⁶ The 17AAG (HSP90 inhibitor) was used in combination with NVP-BEZ235 and resulted in synergistic anticancer activity in melanoma by targeting both MAPK and PI3K/AKT/mTOR signaling pathways.³²⁷ Similarly, paxalisib, apitolisib, voxalisib, PQR309, XH00230381967, omipalisib, and gedatolisib were validated as potent PI3K/mTOR inhibitors with potent antitumor efficacy in many human cancers.^{9,328–330} Paxalisib either as a monotherapy or in combination with ONC201 has entered the phase-II (NCT05009992) clinical trial for the diffuse midline glioma.^{331–334} Apitolisib is an oral and potent inhibitor of mTORC1/2 and class I PI3K. Many preclinical studies have tested the activity of GDC-0980 in solid tumors including lung, brain, endometrial, and gall bladder.^{335,336} Phase-I clinical trials were conducted to evaluate the tolerability, safety, and antitumor efficacy of GDC-0980 in solid tumors.³³⁷ Voxalisib was used at a concentration of 50 mg BID and reported promising anticancer activity in patients with refractory/relapsed follicular lymphoma as well as limited efficacy in DLBCL, chronic myeloid leukemia, and mental cell lymphoma during an open-label, phase 2 trial (ClinicalTrials.gov; NCT01403636) and solid tumors.^{328,338} The combinatorial effect of the voxalisib with low intensity pulsed ultrasound displayed to inhibit GBM tumorigenesis while suppressing the PI3K/AKT/mTOR signaling in CSCs.³³⁹ Another study showed the synergistic effect of the voxalisib when used either with temozolomide plus radiotherapy in glioma.^{340,341} PQR309 was developed as a dual PI3K/mTOR inhibitor and was found to have marked antitumor efficacy in lymphomas as a monotherapy and in combination with venetoclax, lenalidomide, ibrutinib, panobinostat, and rituximab.³²⁹ PQR309 has been reported with promising efficacy against solid and blood cancers.^{279,342,343} LY3023414 (PI3K/mTOR and DNA-PK inhibitor) was reported with anticancer activity alone and in combination with clinically approved chemotherapeutic drugs in several cancers.^{344,345} Du and colleagues have reported that omipalisib acts as a sensitizer for DNA damage-induced apoptosis of HeLa cells through suppression of non-homologous end joining pathway (NHEJ). Further, the combination of omipalisib and doxorubicin-induced γ H2AX in A549 cells. Omipalisib was found to inhibit DNA-PKcs kinase activity in a dose-dependent fashion indicating the ability of omipalisib to directly suppress DNA-PK (a core component in the NHEJ pathway).^{346,347} Interestingly, the gedatolisib was found to be potent to increase the efficacy of the radiotherapy HNSCC and nasopharyngeal carcinoma indicating that gedatolisib may be utilized as a sensitizer to radiotherapy.^{348,349} The phase-I study using gedatolisib in combination with carboplatin and paclitaxel revealed 80% response rate with acceptable tolerability clear cell ovarian carcinoma.³⁵⁰ Recently, phase 1 and 1b studies used the combination of gedatolisib plus cofetuzumab pelidotin and revealed promising clinical anticancer response and tolerable

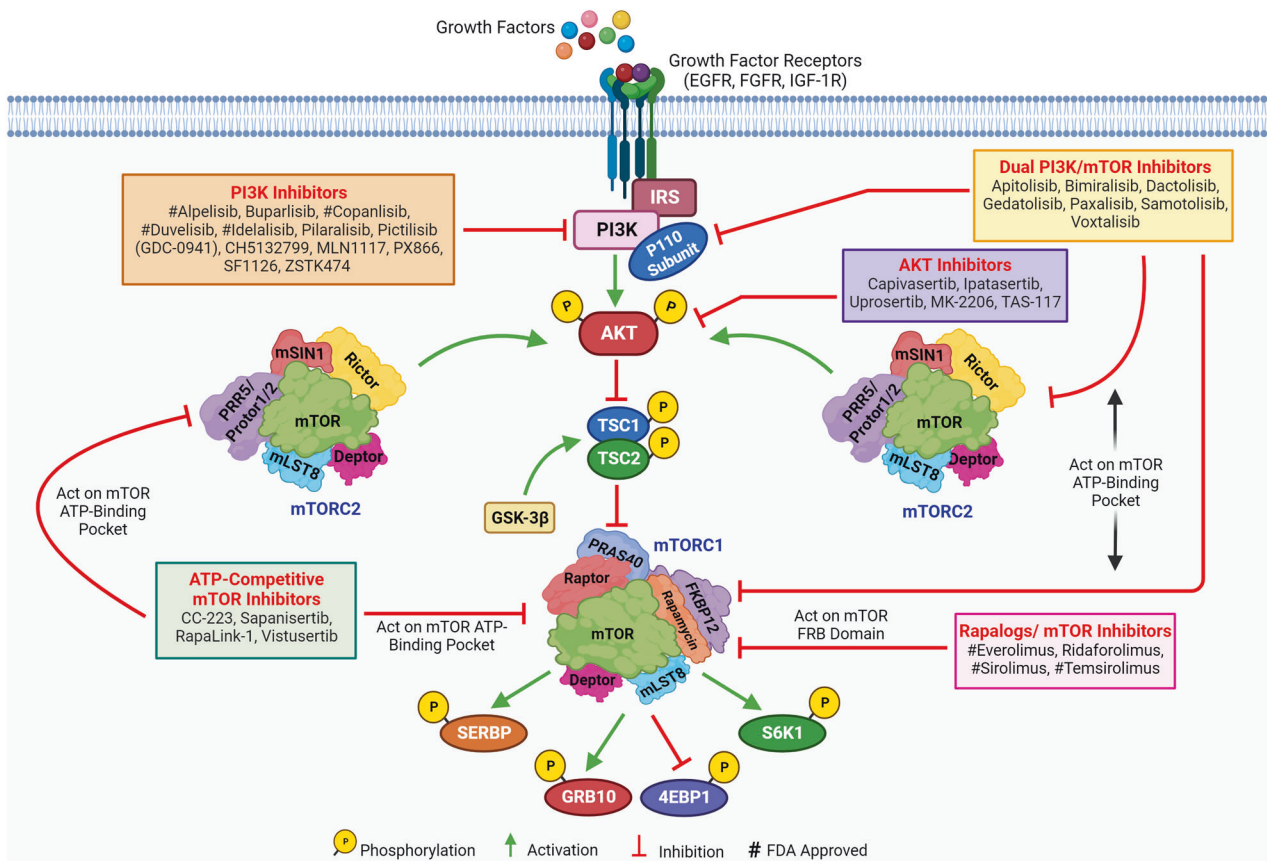


Fig. 7 Pharmacological targeting of the mTOR signaling cascade in human malignancies. The mTOR pathway can be targeted at different levels in human malignancies using Rapalogs or mTOR inhibitors, PI3K small molecule inhibitors, dual mTOR/PI3K inhibitors, compounds targeting AKT, and ATP-competitive inhibitors against mTOR. Created with BioRender.com

toxicities in patients with metastatic TNBC, endometrial and other advanced cancers.^{351–357} Metformin has been shown to activate AMPK through repression of mitochondrial respiratory chain complex I leading to an increase in the AMP/ATP ratio.³⁵⁸ Mechanistically, AMPK negatively regulates the activity of the mTORC1. Wang and colleagues have reported that metformin suppressed mTORC1/2 through the activation of AMPK in myeloma cells. Moreover, metformin suppressed the tumor formation in the mouse model of myeloma through the overexpression of AMPK and downregulation of mTOR.³⁵⁹ In addition, metformin was reported to silence mTORC1 through Rag GTPases/REDD1 axis which is independent of AMPK signaling. Metformin was reported to enhance autophagy through AMPK stimulation and mTORC1 inhibition.³⁵⁹ Metformin treatment displayed strong anticancer efficacy in human malignancies including breast cancer through repression of mTORC1-dependent protein synthesis.³⁶⁰ Recently, the application of metformin in the context to autophagy has been manifested in preclinical models against human cancers. Pharmacological targeting of AKT/PI3K/mTOR axis has been summarized in Fig. 7 and Table 1.

CONCLUSION AND FUTURE PERSPECTIVES

The discovery of mTOR was one of the breakthroughs in understanding the regulation associated with cellular growth, remodeling of the cytoskeleton, cellular metabolism, immune responses, and autophagy in human physiology and cellular homeostasis. The mTOR signaling was noticed to modulate multiple signaling networks to integrate growth factors, amino acids, nutrients, sterols, and nucleotides. The emerging pieces of

evidence have displayed that mTOR activation plays a crucial role in aging, age-related neurological disorders, diabetes, and human malignancies. This has been noticed that inhibition of the mTORC1 signaling prolonged life expectancy while boosting immunity which helps in preventing the onset of age-related, neurological disorders, cancer, and several metabolic diseases in both mammals and humans. Given the fact that mTORC1 is involved in the aging processes, immunity, and other key physiological activities, mTORC1-specific inhibitors can be developed that can produce desirable results in clinical trials. Also, the duration of the mTOR inhibitors should be optimized to prevent side effects like immunosuppression and glucose intolerance. During the last decade, extensive research studies have revealed that the mTOR pathway is highly dysregulated in cancers due to hyperactivation of oncogenic signaling cascades, hotspot mutations/amplifications in the oncogenes, and the deletion or loss of function mutations in tumor suppressor genes. Also, mTOR signaling cascade is well known to influence gene transcription and translation to control cellular proliferation, cytoskeleton, cellular migration, differentiation, tumor metabolism, and immune responses in the tumor microenvironment. Hence, targeting the mTOR pathway provided an opportunity for the discovery of novel therapeutics against human cancers and other diseases. In this direction, several efforts have been taken which led to the discovery of the first-generation mTOR inhibitors called Rapalogs. However, clinical efficacy of the Rapalogs was correlated with a modest anticancer effect in multiple trials on cancer patients. Several mTORC1 inhibitors which include sirolimus, everolimus, temsirolimus, and ridaforolimus were approved through FDA for the management and treatment of several types of human malignancies in clinics. Hence, several groups and pharma companies have focused on

Table 1. mTOR and dual PI3K/mTOR inhibitors in preclinical studies and clinical trials against human malignancies

Name of the drug/inhibitor (s)	Mechanism of action/target (s)	Disease/condition(s)	Status of the clinical phase trial(s) and Identifier(s)	Advantage(s)	Limitation(s)	IC ₅₀ in cancer cell lines	Reference (s)
mTOR Inhibitors							
Sirolimus (Rapamune)	Interact with FKBP12 to form a complex that suppressed mTORC1 activity.	Organ transplants. Lymphangiomyomatosis Relapsed/Refractory Lymphoblastic Leukemia and Lymphoma Relapsed and Refractory Solid Tumors HNSCC	FDA-approved in the years 1999 & 2000 FDA-approved in the year 2015 NCT00414648, Year-2006 NCT01162551, Phase II, Year-2010 NCT01658007, Phase I, Year-2012 NCT01670175, Phase I, Year-2012 NCT01195922, Year-2010	Potent, promising, and well-established drug with good clinical efficacy and safety profile. Enhanced radiosensitivity of HNSCC.	Limited solubility, and bioavailability with a low toxicity profile	Human OS cell line MG63/ ADM = 23.97 nmol/L Murine leukemia cells (P388) = 0.1 nmol T-cell line = 0.05 nmol	234–242
Nab-sirolimus	Bind with FKBP12 to form a complex that suppressed mTORC1 activity.	Advanced unresectable/ metastatic malignant PEComa. Malignant solid tumors harboring inactivating alterations in TSC1 or TSC2.	Nab-sirolimus was FDA-approved through AMPACT trial number NCT02494570 in the year 2021. NCT05103358, Phase II, Year-2022	The ORR of the drug was highly promising, and 2 patients showed a complete response.	Fatigue, stomatitis, rash, nausea, musculoskeletal pain, infection, edema, diarrhea, vomiting, loss of weight and appetite,		243,244
Temsirolimus (Torisel, CCI-779)	FKBP12 subunit of mTORC1	Renal cell carcinoma Relapsed/refractory ALL, recurrent ovarian, endometrial cancer, and mCRPC	FDA was approved in year-2007 NCT01403415, Phase I, Year 2011 NCT01614197, Phase I, Year-2012 NCT01083368, Phase I/II, Year 2009	Promising anticancer efficacy, high bioavailability, life for convenient weekly dosing, good excretion through fecal and urine	Adverse effects, including rash, asthenia, anemia, mucositis, hyperlipidemia, and hyperglycemia	Renal cell carcinoma cells (786-O) = 5 Hep3B = 210 HepG2 = 190 HuH7 = 0.22 PLC = 0.4	247–256
Everolimus (Afinitor, RAD001)	FKBP12 subunit of mTORC1	RCC, neuroendocrine tumors of GI or lung origin, PNET, TSC-associated diseases. SEGA Pediatric ALL	Everolimus received FDA approval in 2009 for RCC, in 2011 for PNET, in 2012 for breast carcinoma, in 2016 for neuroendocrine tumors, 2018 for TSC-associated diseases. NCT00411619, Year-2007 NCT01523977, Phase I, Year-2012	The broad range of clinical applications with enhanced efficacy and prolonged on-target effects	Adverse effects, such as immunosuppression and metabolic disturbances	Renal cell carcinoma cells including 786-O = 1.8–2.6 Breast cancer cell lines include MDA-MB-231 = > 200 MDA-MB-468 = 0.7 BT549 = 1.6	257–270
Ridaforolimus (Deforolimus, AP23573, MK-8669)	FKBP12 subunit of mTORC1	Metastatic soft-tissue and bone sarcomas. Refractory hematologic malignancies, endometrial cancer, breast cancer	Ridaforolimus received FDA approval in the year 2011 for sarcoma during NCT00093080 and NCT00538239. NCT00086125, Phase II, Year-2004 NCT00122343, Phase II, Year-2005 NCT00739830, Phase II, Year-2008 NCT00736970, Phase II, Year-2008 NCT01431547, Phase I, Year-2012	Remarkable potential as an anticancer drug in a variety of human malignancies	Adverse effects include mucositis, myelosuppression, thrombocytopenia, rash, and nausea.	Sarcoma cell lines RH30 = 2–5 nM SYO-I = 23.1 ± 4.6 HS-SY-II = 10.9 ± 2.7	271–277
							278,279

Table 1. continued

Name of the drug/ inhibitor (s)	Mechanism of action/target (s)	Disease/condition(s)	Status of the clinical phase trial(s) and Identifier(s)	Advantage(s)	Limitation(s)	IC ₅₀ in cancer cell lines	Reference (s)
Vistusertib (AZD2014)	mTORC1 & mTORC2	ER-positive breast cancer, symptomatic meningioma, platinum- resistance ovarian carcinoma, and lymphoma	NCT02216786, Phase II, Year-2014 NCT02831257, Phase II, Year-2016 NCT03071874, Phase II, Year-2017 isrcn.org, the Identifier number is ISRCTN16426935 NCT 01316809, Phase I, Year-2011 NCT00973076, Phase I, Year-2009	Potential anticancer agent for inducing apoptosis, cell cycle arrest, and autophagy in human malignancies.	The adverse effects include hyperglycemia, fatigue, lymphopenia, and diarrhea.	IC ₅₀ concentration varies from 30 to 500 nM for the ER- positive breast cancer cell lines.	
AZD8055	mTORC1 & mTORC2	Glioma, advanced solid tumors, leukemia		Improved selectivity and potency	Adverse effects, including hyperglycemia and thrombocytopenia	≤2 nM for BT474 breast cancer, 20–50 nM for RCC cell lines and 400 nM for benign RCC. Glioblastoma cell lines IC ₅₀ range from 100 nM to 500 nM.	280–284
Sapanisertib (MLN0128, TAK- 228, INK128)	mTORC1 & mTORC2	mCRPC, Metastatic thyroid cancer, endometrial, RCC, and advanced solid tumors. Bladder cancer, ER ⁺ breast cancer, & soft-tissue sarcoma	NCT02091531, Phase II, Year-2014 NCT02244463, Phase II, Year-2015 NCT01058707, Phase I, Year-2010 NCT02412722, Phase I, Year-2015 NCT03047213, Phase II, Year-2016 NCT02988986, Phase II, Year-2017 NCT02987959, Phase II, Year-2017	Greater potency with enhanced anticancer efficacy, selectivity, and safety.	Adverse effects, including hyperglycemia and hematologic toxicity	IC ₅₀ value varies from 20 to 100 nM in prostate cancer cells (LNCaP, and DU145); 1–162 nM in breast cancer cell lines; 2–150 nM in different sarcoma cells including RH30, RMS559, A673, TC32, LP5141, SAOS2, ST8814, BC-1 16.20 ± 4.94 nM; BCBL- 137.43 ± 13.13 nM; BC-3, 47.56 ± 7.48, BCP- 19.71 ± 0.76 nM.	297–310
Onatasertib (CC- 223)	mTORC1 & mTORC2	GBM, HCC, NSCLC, Multiple myeloma, DLBCL, HR ⁺ breast cancer, neuroendocrine non-pancreatic origin tumors	NCT01177397, Phase I/II, Year-2010 NCT03591965 and NCT02031419 are active.	ATP-competitive inhibitor, durable response, high selectivity, and efficacy.	The side effects include nausea, vomiting, headache, diarrhea, stomatitis, hyperglycemia, decreased appetite, and asthenia.	0.2–0.35 μM in H929 (B- lymphocyte), 88.17 ± 6.32 nM and 64.32 ± 5.21 nM in the CaOV3 and SKOV3 (ovarian carcinoma cell lines), respectively.	311–314
OSI-027	mTORC1 & mTORC2	Advanced solid tumors, multiple myeloma, lymphoma, and pancreatic cancer	NCT00698243, Phase I, Year-2008	Enhanced the inhibition of the mTOR pathway leading to promising anticancer activity	Adverse effects, including hyperglycemia and neutropenia	22 nM and 500 nM in MM.1 S and JIN3 (multiple myeloma), respectively. 1–30 μM in BxPC3, CFPAC1, PANC1, pancreatic cell lines	315–320
Dual PI3K/mTOR inhibitors Dactolisib (BEZ235)	PI3K & mTOR	PNET, breast cancer	NCT01658436, Phase II, Year-2012 NCT00620594, Phase I/II, Year-2006	Modest clinical efficacy and enhanced efficacy of the immune checkpoint inhibitors	Fatigue, diarrhea, nausea, and mucositis	Ranging from 100 to 2000 nM in breast cancer cell lines (MCF-7, MDAMB231). Ranging from 50 to 300 nM in myeloma cell lines (U266, KMS3, RPMI8226).	322–327
Paxalisib (GDC- 0084)	PI3K & mTOR	GBM	NCT03522298, Phase II, Year-2018 NCT05009992, Phase II, Year-2021 NCT01547546, Phase I, Year-2012	FDA approved for GBM with superior anticancer efficacy.	Hyperglycemia, rashes, mucositis	IC ₅₀ ranged from 300 nM to 1100 nM in glioma cell lines	331–334

Table 1. continued

Name of the drug/ inhibitor (s)	Mechanism of action/target (s)	Disease/condition(s)	Status of the clinical phase trial(s) and Identifier(s)	Advantage(s)	Limitation(s)	IC ₅₀ in cancer cell lines	Reference (s)
Apitolisib (GDC-0980)	PI3K & mTOR	PC	NCT01485861, Phase II, Year-2019 NCT01437566, Phase II, Year 2011 NCT01332604, Phase I, Year 2011		Fatigue, diarrhea, rash, nausea, loss of appetite, Mucosal inflammation, hyperglycemia, vomiting, pruritus, liver dysfunction	The IC ₅₀ value for prostate, breast, and NSCLC < 200. The pancreatic carcinoma and melanoma ranged from 200 to 500 nmol/L. GDC-0980 was reported with <500 nmol/L in 124 out of the 167 cell lines tested.	335–337
Voxtalisis (XL765 or SAR245409)	PI3K & mTOR	Lymphoma, ovarian cancer, GBM	NCT01403636, Phase II, Year 2011 NCT01936363, Phase II, Year-2013 NCT01596270, Phase I, Year-2012	Promising anticancer efficacy	Fatigue, diarrhea, nausea, pyrexia, decreased appetite, and cough		338–341
Bimiralisib (PQR309)	PI3K & mTOR	Refractory lymphoma, primary CNS lymphoma, breast cancer	NCT02249429, Phase II, Year-2015 NCT02669511, Phase II, Year 2016		The side effects of the compound were sweating at night, nausea, crankiness, memory problems	174–324 nmol/L in lymphoma cell lines	279,329,342,343
Samotolisib (LY3023414)	PI3K & mTOR	mCRPC and other advanced cancers	NCT02407054, Phase II, Year-2015 NCT01655225, Phase I, Year 2012	Samotolisib was noticed with high bioavailability and selectively inhibits PI3K as well as mTOR kinase. Highly effective in clinical trials for human cancers.	Samotolisib was associated with nausea, vomiting sensation, loss of appetite, diarrhea, and headache.	The following cholangiocarcinoma cell lines have IC ₅₀ in μM. HuCC1 = 0.52, OZ = 0.45, NCC-BD1 = 0.41, NCC-BD2 = 0.16, NCC-BD3 = 0.32, NCC-CC6-1 = 0.71, NCC-CC4-1 = 0.23	344,345
Gedatolisib (PF-05212384, PKI-587)	PI3K, kinase site of mTOR	TNBC, BRCA1/2-positive, HER2 ⁺ breast and endometrial cancers. Advanced squamous cell Lung, Pancreatic, Head & Neck, and Other Solid Tumors	NCT03911973, Phase II, Year-2019 NCT03698383, Phase II, Year-2018 NCT05501886, Phase III, Year-2022 NCT01420081, Year 2011 NCT01347866, Phase I, Year 2011 NCT00940498, Phase I, Year 2009 NCT03065062, Phase I, Year-2023	Gedatolisib suppresses the tumor burden in patients with advanced solid tumors. Gedatolisib in combination with chemotherapies displayed greater clinical benefit in relapse or refractory cancer patients	Gedatolisib was found to cause neutropenia, abnormal liver function profiles, thrombocytopenia, and anemia.	IC ₅₀ value was 340 nM in SW620 and 7.09 μM in adriamycin resistance SW620 cells. IC ₅₀ value was 0.41 μM, and 3.9 μM in LS180 and LS180/MX cells, respectively. The following cell lines have IC ₅₀ in μmol/L MIAPaCa = 0.022 T47D = 0.002 MCF = 0.036 HT29 = 0.037 HTB44 = 0.43 PC3 = 0.011 A549 = 0.02 H460 = 0.05 DLD1 = 0.31 U87 = 0.012 KB = 0.005	348–357

ALL acute lymphoblastic leukemia, AMPECT advanced malignant PEComa, CNS central nervous system, DLBCL diffuse large B-cell lymphoma, ER + estrogen receptor-positive, FDA Food and Drug Administration, FKBP12 FK506-binding protein-12, GBM glioblastoma, HCC hepatocellular carcinoma, HER2 human epidermal growth factor receptor 2, HNSCC head and neck squamous cell carcinoma, HR + hormone receptor-positive, IC₅₀ half-maximal inhibitory concentration, ISRCTN international standard randomized controlled trial number, mCRPC metastatic castration-resistant prostate cancer, mTOR mammalian target of rapamycin, mTORC1 mTOR complex 1, mTORC2 mTOR complex 2, NCT national clinical trial, NSCLC non-small cell lung carcinoma, ORR overall response rate, OS osteosarcoma, PC prostate cancer, PEComa perivascular epithelioid cell tumor, PI3K phosphoinositide 3-kinase, PNET pancreatic neuroendocrine tumor, RCC renal cell carcinoma, SEGA subependymal giant-cell astrocytoma, TNBC triple-negative breast cancer, TSC1 target tuberous sclerosis 1, TSC2 target tuberous sclerosis 2

the development of new combination therapeutic approaches along with the designing, synthesis, and validation of dual inhibitors (PI3K/mTOR) for effective and relapse-free therapy to overcome resistance. Furthermore, the dual inhibitors targeting PI3K/mTOR axis, inhibition of parallel pathways, and targeted/non-targeted drug combinations were tested in preclinical and clinical studies to figure out the best possibility for mTOR inhibition. The results of these combinations were promising. Currently, the development of third-generation mTOR inhibitors (Rapalink-1) displayed promising results in clinical trials because of their ability to inhibit the mutant kinase activity. During the past decade, the libraries of potential mTOR inhibitors included a variety of scaffolds like urea, quinolines, pyridines, and pyridopyrimidines. Apart from these various small molecules have been designed, manufactured, and assessed as mTOR inhibitors with promising anticancer efficacy. In the future, we hope that the Identification of novel and specific inhibitors of the mTOR pathway or mTOR-associated pathways that can eradicate cancer cells, CSCs, drug resistance, and autophagy either alone or in combination with chemotherapeutic drugs and immunotherapeutic agents could be of great importance for the treatment of human malignancies and research purposes. Recently, Nab-sirolimus (the albumin-bound nanoparticle of sirolimus) has been approved by the FDA for clinical use against human cancer. Therefore, the nano-formulation of the mTOR signaling pathway inhibitors should be a priority for better efficacy and limited toxicity. Moreover, preclinical and clinical studies are required to assess the safety, efficacy, and molecular mechanism of the other chemotherapeutic drugs, and immune therapies in combination with mTOR inhibitors. This could lead to the invention of more effective and less toxic treatment regimens that can improve the relapse-free survival of cancer patients.

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AUTHOR CONTRIBUTIONS

M.G. conceived the idea. S.S., D.K., and M.G. designed the concept of the manuscript. V.P., A.S., M.B., R.K.T., S.A., A.S.R., S.S., D.K., and M.G. wrote the initial draft of the manuscript. A.S., S.S., D.K., and M.G. finalized, revised, and presented the final concept of the manuscript. A.S., S.S., and M.G. created the figures and finalized the tables of the manuscript. All authors have read and approved the article.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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