Review Article

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Modulation of Mammalian Target of Rapamycin Signal Machinery in Human Cancer

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Abstract

Mammalian target of rapamycin (mTOR), a serine / threonine protein kinase, regulates cellular process such as growth, proliferation, motility and survival which are mediated through regulating the transcription and protein synthesis. mTOR is the catalytic subunit of two structurally distinct complexes. mTORC1–a master regulator of cell growth and metabolism and mTORC2 is involved in the cytoskeleton organization. Both these complexes are localized to different sub-cellular compartments, thus affecting their activation and function. Though the mTOR signaling has physiological function in cells, an elevated mTOR signaling has been found in many human cancers. Since deregulation has been observed in the mTOR signaling pathway, overwhelming research on this complex machinery has developed inhibitors to inhibit human cancer. Some of them such as temsirolimus, everolimus, are beginning to use in the treatment of cancer. Natural inhibitors of mTOR are found to be effective in cell cultures but none of them is proved to be effective in clinical use. Hence, it will be noteworthy to discuss the physiological importance as well as pathological impact of this signal machinery. This review discusses the physiological role of mTOR, its regulation, involvement in human cancer and pharmacological inhibitors for modulating mTOR activity in cancer.

Key words: Mammalian Target of Rapamycin; Serine / Threonine Protein Kinase; AMP Dependent Protein Kinase; Protein Kinase B; Phosphatidylinositol 3-Kinase; Temsirolimus; Everolimus.

Introduction

The mammalian target of rapamycin (mTOR) complex (289-kDa) is a serine / threonine kinase of the phosphatidylinositol kinase–related kinase family which was identified in 1991[1]. This complex is one of the important components involved in the phosphatidylinositol 3-kinase (PI3K) / Protein kinase B (Akt) signal pathway and is highly conserved from yeast to mammals [2]. Its major role is the integration of signals originated from growth factors, hormones, nutrients, stress, energy status and altering cellular processes such as cell proliferation, cell motility, cell survival, protein synthesis and transcription [2]. Structurally two distinct complexes, mammalian TOR complex 1 (mTORC1) and mTORC2 are localized in different sub-cellular compartments and found to be associated with mTOR. mTORC1 and mTORC2 exert their actions by regulating other important kinases. Hence, these complexes are central to the mediation of various extrinsic and intrinsic signals. mTORC1 promotes glucose uptake and flux through glycolysis, regulates lipid synthesis, adipocyte differentiation and inhibits autophagy.

Significant advance in the regulation and functions of mTOR in the past decade has revealed the crucial involvement of this signaling pathway in human diseases [3]. Involvement of mTOR in human diseases is depicted in figure 1. Therefore, inhibitors of mTOR are designed and directed against the onset and progression of diseases such as diabetes, cancer, alzheimer's disease and obesity [4]. National Institute on Aging Interventions Testing Program has shown that pharmacologically reduced mTOR

signaling with rapamycin increases median and maximal lifespan in genetically heterogeneous mice [5]. Inhibitors of mTOR in cancer are the major research area that attracted attention during the last decade. This review article discusses the physiological role of mTOR, its regulation, involvement in human cancer and pharmacological inhibitors for modulating mTOR activity in cancer.

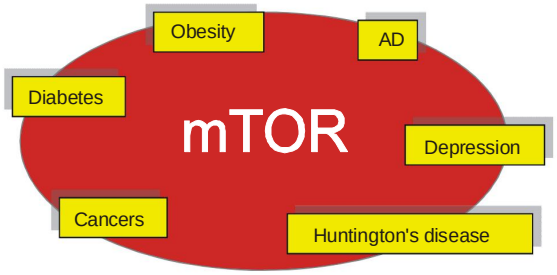

Fig. 1: Involvement of mammalian target of rapamycin (mTOR) in the onset of diseases. AD: Alzheimer's disease

Physiological role and regulation of mTOR signal pathways

mTOR is associated with two distinct multi - protein complexes, mTORC1 and mTOR mTORC2. mTORC1 complex functions as a nutrient / energy / redox sensor and controlling protein synthesis [2]. There are five components of mTORC1complex: (1) mTOR, which is the catalytic subunit; (2) regulatory-associated protein of mTOR (Raptor); (3) mammalian lethal with Sec13 protein 8 (mLST8, also known as GâL [6]; (4) proline-rich AKT substrate 40 kDa (PRAS40); and (5) DEP-domain-containing mTOR-interacting protein (Deptor) [7]. The exact function of most of the mTOR–interacting proteins in mTORC1 complex is still remains elusive. Raptor affects the mTORC1 activity by regulating assembly of the complex and by recruiting substrates for mTOR [8, 9]. The activity of mTORC1 is reduced when PRAS40 and Deptor are recruited to the complex. The activity can also be reduced due to the inhibition of Rheb which is mediated by tuberous sclerosis complex 2

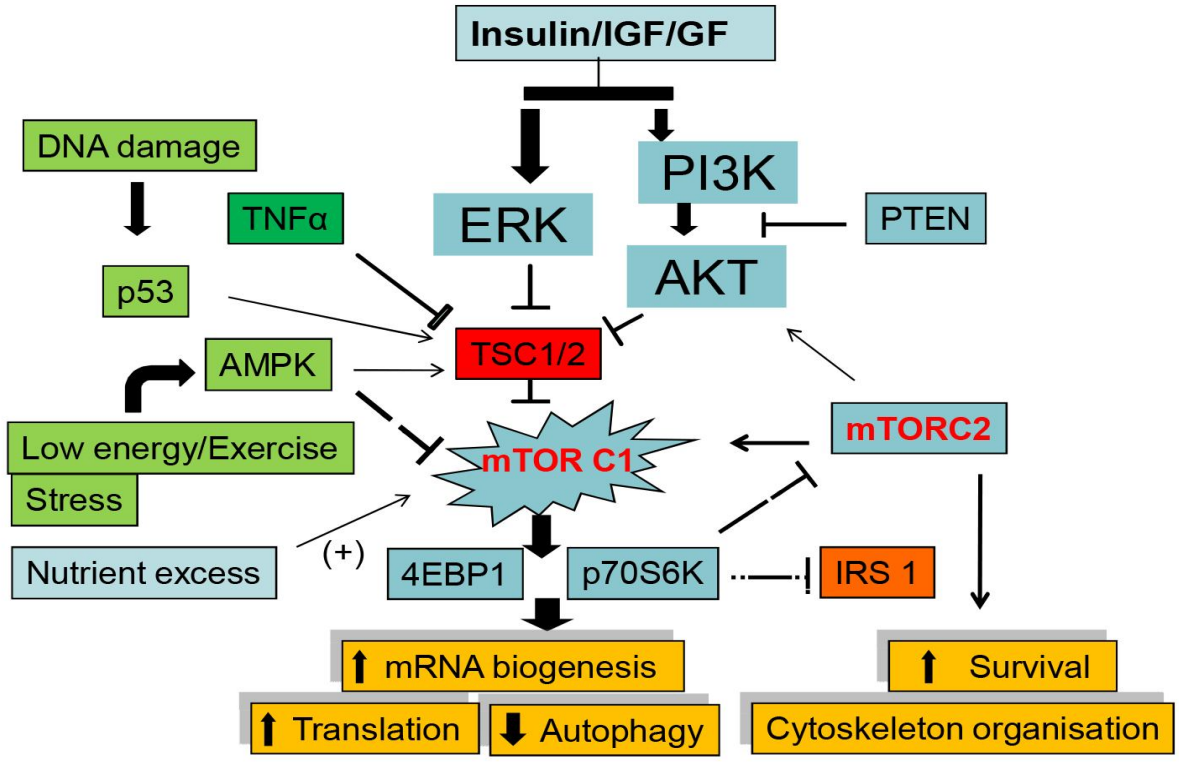

Fig. 2: Role of various factors that contribute the activation of mTOR Complexes. GF, insulin and IGF1 stimulate the PI3K/ Akt pathway which in turn directly inactivate TSC1/TSC2 complex. This leads to the activation mTORC1. The activated mTORC1 phosphorylate S6K1 and 4EBP1, positively regulate the rate limiting step for translation. TNFá phosphorylates and inhibits TSC1 thus activate mTORC1. Activation of complexes finally favors the biological response such as the overall cell growth, survival and proliferation. AMPK inhibit the complex either directly or through TSC 1/2. mTOR C1/C2: Mammalian target of rapamycin mTOR complex 1/2; GF: Growth factor; IGF1: Insulin-like growth factor 1; PI3K: phosphatidylinositol 3-kinase; ERK1/2: extracellular-signal-regulated kinase1/2 (ERK1/2); Akt: Protein kinase B; TSC 1/2: Tuberous sclerosis complex 1/2; RSK 1: Ribosomal S6 kinase; TNFá: Tumor necrosis factor-á; S6K1: p70 ribosomal S6 kinase 1; 4EBP1: Eukaryotic initiation factor 4E-binding proteins; PTEN: Phosphatase and tensin homologue deleted on chromosome ten; AMPK: 5' Adenosine monophosphate-activated protein kinase; IRS-1:Insulin receptor substrate 1.

(TSC2) [8]. Many signals regulate mTORC1 through TSC1/2 (Fig. 2). Growth factors, insulin and insulin-like growth factor 1 (IGF1) stimulate the PI3K and Ras pathways that resulted in the activation of the effector kinases of these pathways such as protein kinase B (Akt/PKB), extracellular-signal-regulated kinase1/2 (ERK1/2), and ribosomal S6 kinase (RSK1). Growth factors are mainly mediated their action though pathways such as ERK 1/2, Wnt and the glycogen synthase kinase 3b pathways [10, 11]. These kinases directly inactivate TSC1/TSC2 complex by phosphorylation and thus activate mTORC1 [12–16]. Akt can activate mTORC1 in a TSC1/2-independent fashion by phosphorylation to dissociation of mTORC1 inhibitor from raptor of PRAS40 [17–20]. Branched chain amino acids, leucine increases intracellular calcium levels, thus activating mTORC1 mediated through calcium/calmodulin-dependent activation and involved in the regulation of energy balance.

The activated mTORC1 positively regulate the rate limiting step for translation by phosphorylating p70 ribosomal S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding proteins (4EBP1). S6K1 in turn phosphorylates number of downstream substrates including the S6 ribosomal protein results in increased mRNA biogenesis and cap-dependent translation and elongation. Among the proteins that are synthesized includes cyclin D1, hypoxia inducing factor 1á, glucose transport protein 1(GLUT-1) and glycolytic enzymes. mTORC1 positively regulates lipid metabolism by activating the sterol regulatory element binding protein 1/2 (SREBP 1/2) [21] and peroxisome proliferator-activated receptor-ã in adipocites [22]. SREBP in turn activate the transcription of genes of lipogenesis pathway such as synthesis of fatty acids, cholesterol etc.

Activation of mTORC1 found to inhibit autophagy which is recognized as an adaptive rescue mechanism for starving cells to conserve energy and also to eradicate the damaged cellular material. Activation of mTOR in presence of the growth factors results in the shuttling of mTOR from cytoplasm to lysosomes and subsequently inhibits the cell autophagy. The Rag GTPases, in the presence of sufficient amino acids–particularly leucine and arginine mediate this lysosomal localization [23–25]. On the lysosomal surface, the Rag GTPases dock mTORC1on Ragulator, a multisubunit complex [26]. mTORC1 directly phosphorylates and suppresses kinases involved in the autophagy [27–29]. Hence, for any upstream signals like originated from growth factors these amino acids are found to be essential for the activation of mTORC1. Therefore, upon amino acid removal during starvation TSC2 is recruited by the Rag GTPases to lysosomes and inhibit the mTOR C1 [30, 31].

Pro-inflammatory cytokines such as tumor necrosis factor-á (TNFá) phosphorylates and inhibits TSC1 which is mediated by IkB kinase b [32]. A cell growth regulator pathway initiated from Wnt signaling also inhibits TSC2 activity results in the activation of mTORC1 [33, 34]. Similarly, low energy and DNA damage can mediate the regulation of mTORC1 through TSC1/2. Furthermore, DNA damage will activate the p53-dependent synthesis of TSC2 and tensin homolog deleted on chromosome 10 (PTEN), causing down regulation of the entire PI3K-mTORC1 axis [35, 36]. Phosphatidic acid activates mTOR signaling at least in part by stabilizing the mTOR complexes [36].

Factors such as stress (ROS), starvation and exercise can inhibit the mTORC1 mediated by the 5' Adenosine monophosphate-activated protein kinase (AMPK). Inoki et al; demonstrated that mTORC1 activity is sensitive to ATP levels [38]. Hence, the factors that lower the ATP level in the cell such as hypoxia or stressors elevate the AMP and inhibit the mTORC1 through AMPK. AMPK will activate TSC2 and furthermore phosphorylate and inhibit Raptor [38, 39]. Starvation and stress can also mediate a transcription dependent, via p53 and transcription independent, by AMPK activation, mechanisms to inhibit the mTORC1 complex.

The other complex of mTOR, mTORC2 comprises six different proteins. Among the proteins Deptor negatively regulates mTORC2 activity [40]. The role of mTORC2 in various cellular processes has not yet been fully elucidated. mTORC2 phosphorylates the serine/threonine protein kinase Akt/PKB at the serine residue S473 thus, affecting metabolism and survival [41]. Phosphorylation of the serine stimulates phosphoinositide dependent protein kinase-1 mediated Akt phosphorylation at a threonine T308 residue, leads to full Akt activation (Fig. 3) [42, 43]. The activated Akt regulates several downstream kinases for the cellular processes such as cell survival, proliferation, growth and apoptosis. Activation of one such kinases, PKC-á regulates cell shape by affecting the actin cytoskeleton [44, 45]. Because of its role in phosphorylating and activating Akt, mTORC2 forms a core component of the PI3K pathway.

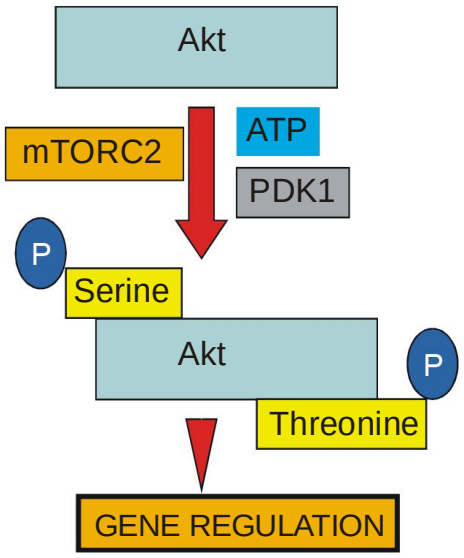

Fig. 3: Role of mTORC2 in the Akt activation. mTORC2 phosphorylates Akt at the serine residue for its full activation. ATP: Adenosine triphosphate; PDK1: 3-phosphoinositide dependent protein kinase-1,

Role of mTOR in tumorigenesis

Since, growth factors can act onto mTORC1 through the multiple signaling pathways that over activate mTORC1 can results in tumorigenesis [46]. Oncogenic protein mediated activation of mTOR signaling induces several processes required for cancer cell growth, survival and proliferation. Activation of cellular oncogene and the subsequent activation of mTOR signaling results in uncontrolled processes such as growth, survival and proliferation required for malignant transformation of cell (Fig. 4). Most of the proteins that are increasingly synthesized in the cells can contribute to abnormal proliferation of cells beyond the physiological demand of the organ involved. Among such proteins cell cycle regulators, growth factors to favor angiogenesis have been demonstrated well. Increase in ribosome biogenesis linked to mTOR activation probably promotes high levels of cell growth and can partially explain the mechanism behind the increased proteins. Estrogen and androgens can allow the entry of amino acid, leucine into the cells those results in the activation of mTOR. Mutations in genes encoding proteins that lie upstream of the mTOR complexes, including *p53*, *Pten*, *Tsc 1/2* and neurofibromatosis type 1 or deregulation of protein synthesis downstream of mTORC1 at the level of 4E-BP1 / eIF4E can also contribute the uncontrolled growth of poorly differentiated cells [47, 48]. Guertin et al; demonstrated the development of prostate cancer in mice induced by the loss of the tumor suppressor PTEN which requires mTORC2 function [48]. Further, activated mTORC2 promotes cell survival [49].

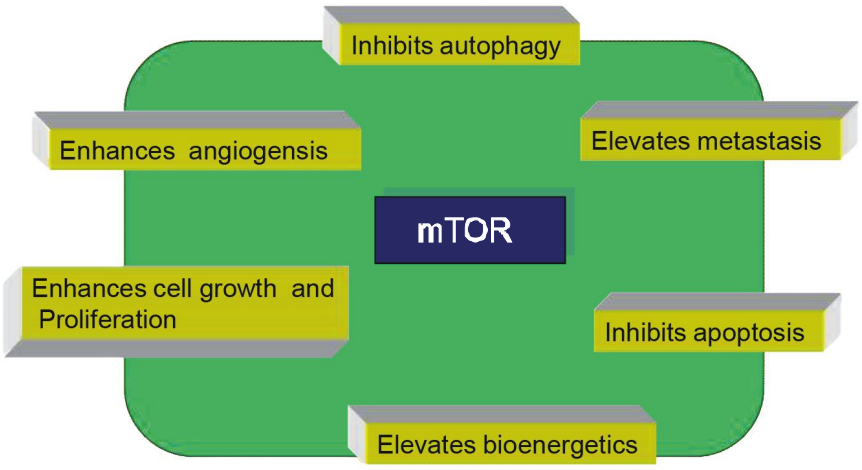

Fig. 4: Role of Mammalian target of rapamycin (mTOR) in tumorigenesis.

Though the activated mTOR-raptor complex 1 (mTORC1) results to enhance protein synthesis in many cell types, activation of mTORC1 strongly represses the PI3K-AKT axis upstream of PI3K. Furthermore, the activation of S6K1 by mTORC1 promotes the phosphorylation of IRS1 and reduces its stability [50]. This auto-regulatory pathway, characterized as the S6K1-dependent negative feedback loop, has been shown to have profound implications for both metabolic diseases and tumorigenesis [51]. Therefore, targeting the mTOR complexes mainly mTORC1 will help to prevent the progression of cell transformation.

Inhibitors of mTOR used in cancer treatment

mTOR complexes are mainly useful for the treatment of cancer and to lesser extent they may also help in identifying predictors of response or resistance. List of inhibitors used in the current clinical trials and therapy are depicted in table 1. Inhibition of mTORC1 and mTORC2 leads to apoptosis; inhibition of mTORC2 alone by PP242 prevents phosphorylation of Ser-473 site on AKT and arrests the cells in G1 phase of the cell cycle [52]. Rapamycin is the first generation of mTOR inhibitors targeted to mTORC1, but they do not bind to mTORC2, which is mostly considered to be rapamycin-insensitive [53]. Rapamycin can enhance apoptosis and also increases sensitivity to cisplatin in vitro [54]. However, targeting to only mTORC1 with rapamycin analog leads to increased signaling through upstream receptor tyrosine kinases and increased Akt activation, which promotes cell survival. Therefore, it has been speculated that rapamycin analog alone has limited clinical activity in cancer due to this mechanism, as well as activation of parallel signaling pathways. This limitation of rapamycin analog has directed the development of alternate methods of targeting the PI3K signaling pathway, with either adenosine triphosphate (ATP)-competitive mTOR inhibitors or by using dual PI3K / mTOR inhibitors. Ongoing breast cancer studies using ATP-competitive mTOR inhibitors and dual PI3K / mTOR inhibitors are in clinical trials. A few of the TOR inhibitors such as temsirolimus and everolimus are beginning to be used in the treatment of cancer [55].

mTOR ATP-competitive inhibitors which target all known functions of mTORC1 as well as mTORC2 can inhibit translation more potently. Although PI3K over activation still occurs, Akt phosphorylation by mTORC2 is impaired. Dual PI3K / mTOR inhibitors block all functions of PI3K, including PDK1- and mTORC2-mediated activation of Akt. However, they might cause increased toxicity. The adverse effect of inhibition is that if phosphatidylinositol 3 kinase (PI3K) and Akt are not activated, leading to decreased glucose uptake and hence enhance the hepatic gluconeogenesis, which in turn cause hyperglycemia or even worsen the hyperglycaemia in diabetes patients. The incidence rates of adverse metabolic effects with either mTORC1 or dual mTORC1 / mTORC2 inhibitors have a wide range from hyperglycemia (22–50%), hypertriglyceridemia (27–71%), and hypercholesterolemia (24–76%) [56]. Non-infectious pneumonitis was reported among advanced renal cell carcinoma patients treated with everolimus [57]. Iacovelli et al; in a meta-analysis reported the 10.4% incidence and risk of pulmonary toxicity in patients treated with mTOR inhibitors for malignancy [58].

Preclinical studies, using hormone receptor–positive cancer cell lines, have demonstrated the activation of PI3K / mTOR pathway after long-term estrogen deprivation [59, 60]. This suggested as one of the important mechanisms of acquired endocrine resistance in hormone replacement therapy. High Akt activity has been shown to contribute to resistance to endocrine therapy as well [61], which can be reversed by rapalogs [62, 63]. Therefore, anti-hormonal treatment for priming of the PI3K pathway might be important in sensitizing cancer cells to PI3K / mTOR inhibitors. Rapalogs were synergistic with anti-estrogens, including tamoxifen and letrozole for blocking both pathways not only enhanced anti-tumor activity but also Akt-induced endocrine therapy resistance is reversed by inhibition of mTOR signaling [62, 63]. Boulay et al. demonstrated that dual inhibition of mTOR and estrogen receptor signaling in vitro induces cell death in models of breast cancer [64]. Treatment with mTOR inhibitors is an effective strategy for overcoming preclinical trastuzumab resistance secondary to PTEN loss [65, 66]. Sirolimus (Rapamune), a rapamycin analog has also been shown to inhibit the growth of cancer cell lines and xenografts from different tumor subtypes [67].

Temsirolimus was the first rapamycin analog approved by the US Food and Drug Administration (FDA) in 2007 for the treatment of advanced renal cell cancer. With each of the three mTOR inhibitors temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573), a safe schedule of treatment has been defined and promising results of anti-tumour activity have been achieved in a variety of solid tumours, thus confirming the preclinical expectations [68]. In a randomized phase 2 study, everolimus in combination with tamoxifen increased overall survival compared with tamoxifen alone in postmenopausal women with aromatase inhibitors resistant metastatic breast cancer [69]. Various natural compounds, including curcumin, resveratrol, epigallocatechin gallate, and caffeine have also been reported to inhibit mTOR when applied to isolated cells in culture [70–73]. However, there is as yet no evidence that these substances inhibit mTOR when taken as dietary supplements.

Table 1: Novel MTOR inhibitors in clinical trial and used in treatment

Inhibitor	Action	Remarks
AZD8055	Novel ATP-competitive mTOR inhibitor with excellent selectivity against PI3K isoforms and ATM/DNA-PK [74].	In Phase 1 trial
Temsirolimus (CCI-779)	Suppresses mTOR activity and inhibits the mTOR-mediated phosphorylation of S6K1 and 4E-BP1[75]	TORISEL® used in advanced RCC
Everolimus (RAD001)	inhibits FKBP12 and blocked phosphorylation 4EBP1 and inactivated the S6K1 [76].	AFINITOR ® used in RCC, pNET, astrocytoma
KU-0063794	ATP-competitive mTOR inhibitors, effectively inhibited both mTORC1 and mTORC2 [77]	
BEZ235 (NVP-BEZ235)	A dual ATP-competitive PI3K and mTOR inhibitor [78]	In Phase 2 trial

ATM: ataxia telangiectasia mutated; DNA-PK: DNA-dependent protein kinase; RCC: renal cell carcinoma; FKBP12; FK506 (an immunosuppressant) binding protein; pNET: Pancreatic neuroendocrine tumors; 4EBP1: eukaryotic initiation factor 4E-binding protein 1; S6K1: ribosomal protein S6 kinase 1.

Conclusion

The mammalian target of rapamycin (mTOR) is a serine / threonine kinase of the phosphatidylinositol kinase-related kinase family. It regulates cell growth process such as cell proliferation, cell motility. The mTOR pathway is dysregulated in human diseases, such as diabetes, obesity, depression, and certain cancers. Activation of (S6K1) by mTORC1 promotes the phosphorylation of serine residue in IRS1 and reduces its stability. The S6K1–dependent negative feedback loop has been shown to have profound implications for both metabolic diseases and tumorigenesis. mTOR inhibitors such as temsirolimus and everolimus are beginning to be used in the treatment of cancer. Inhibitors of mTOR signal pathway are required as adjuvant to the conventional chemotherapeutic agents. In patients with HER2–positive, combinations of PI3K / mTOR inhibitors with anti-HER2 therapies are encouraging. Various combinations of dual PI3K / mTOR inhibitors and other pathway inhibitors, such as MEK or IGF1R, are being studied in clinical trials to either overcome loss of feedback inhibition or PI3K activation. But the results from larger studies are not available yet. Hence, further research is warranted to rule out the potent therapeutic application of mTOR inhibitors.

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