Challenges and Emerging Opportunities for Targeting mTOR in Cancer

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ABSTRACT

The mechanistic target of rapamycin (mTOR) plays a key role in normal and malignant cell growth. However, pharmacologic targeting of mTOR in cancer has shown little clinical benefit, in spite of aberrant hyperactivation of mTOR in most solid tumors. Here, we discuss possible reasons for the reduced clinical efficacy of mTOR inhibition and highlight lessons learned from recent combination clinical trials and approved indications of mTOR inhibitors in cancer. We also discuss how the emerging systems level understanding of mTOR signaling in cancer can be exploited for the clinical development of novel multimodal precision targeted therapies and immunotherapies aimed at achieving tumor remission.

Introduction

We have recently gained an unprecedented understanding of mechanistic target of rapamycin (mTOR) signaling and its role at the intersection of growth factor and nutrient sensing, cell metabolism and bioenergetics, proteostasis, and transcriptional and translational control. Indeed, mTOR plays a key role in normal and malignant cell growth. However, the results of efforts to pharmacologically target mTOR in cancer have been disappointing, with few patients showing clinical benefit in spite of mTOR's aberrant hyperactivation in most solid tumors. Possible reasons for this reduced clinical efficacy include (i) limited evidence of selective dependence on mTOR for growth in the cancer types in which these agents were analyzed in unselected patient populations; (ii) a dearth of studies supporting biochemical mTOR inhibition in cancer lesions in the clinical setting; (iii) limited mechanistic biomarkers and genetic alterations of predictive value of a favorable response; (iv) rapid activation of resistance mechanisms, including vertical and horizontal compensatory pathways rendering mTOR inhibitors ineffective; (v) mechanistic limitations of clinical mTOR inhibitors; and (vi) undesirable toxicities, including immune modulating effects. However, lessons learned from approved indications of mTOR inhibitors in cancer, emerging results from recent clinical trials and immune competent preclinical tumor models, and new insights into pathway regulation and pharmacology can guide future clinical development of mTOR inhibiting strategies.

The mTOR Pathway: Regulation, Function, and Hyperactivation in Cancer

mTOR is a 289 kDa serine/threonine kinase that serves as the catalytic subunit of two distinct complexes, mTORC1 and mTORC2. mTORC1 is comprised of mTOR, mLST8, and RAPTOR alongside the accessory inhibitory factors PRAS40 and DEPTOR, which bind mTORC1 via RAPTOR and mTOR, respectively (**Fig. 1**). This complex, which is partially inhibitable by rapamycin and its derivatives, phosphorylates substrates that promote protein, lipid, nucleotide, and ATP production while inhibiting the catabolic breakdown of these species through autophagy. Under normal physiologic conditions, mTORC1 is only activated when energy, growth factors, and macromolecular building blocks are abundant. This is achieved through an elegant mechanism orchestrated by two classes of small G proteins, Rheb and the Rag GTPase family. Briefly, when growth factors and ATP are present, active GTP-bound Rheb is localized to the surface of the lysosome, where it can activate mTORC1. However, mTORC1 only colocalizes with active Rheb when amino acids and other nutrients are also abundant. This colocalization is mediated by Rag protein heterodimers, which become activated in the presence of nutrients, resulting in mTORC1 recruitment to the lysosome surface. Thus, an elegant 'AND gate' only enables mTORC1 activation in cellular environments that can sustain growth (1, 2).

mTORC2, by contrast, is comprised of mTOR, mLST8, and RICTOR. Like mTORC1, mTORC2 is regulated by accessory factors mSIN1 and PROTOR1/2, which bind to RICTOR, and DEPTOR, which directly binds mTOR (**Fig. 1**). In comparison with mTORC1, the roles and regulation of mTORC2 are less well understood. This complex cooperates with PDK1 to activate several classes of PKCs, along with SGK1 and AKT, mediating survival and proliferative signaling. mTORC2 further regulates the organization of the actin cytoskeleton and chemotaxis, and through these effects, it has been shown to drive the migration and metastasis of cancer cells (1, 2).

Because of its central roles as a positive orchestrator of cell growth, proliferation, anabolic metabolism, and survival, it is perhaps unsurprising that mTOR has been documented to be hyperactive in up to 80% of human cancers (1, 2). While the mTOR kinase itself is somewhat rarely mutationally activated in cancer, its regulation by diverse cellular inputs provides abundant upstream sources to drive its hyperactivation. These inputs include receptor tyrosine kinases, G protein–coupled receptors, and the downstream Ras–MAPK and PI3K–AKT signaling pathways, which are collectively hyperactivated by mutational mechanisms in the majority of human cancers, where they activate mTORC1 through repression of TSC1/2, negative regulators of Rheb. Indeed, widespread genetic alterations (mutations, copy-number alterations, and epigenetic silencing) in core components of the PI3K–AKT–mTOR pathway itself occur in over 30% of all tumors (**Fig. 1**; ref. 3). Further, recent evidence suggests that loss of

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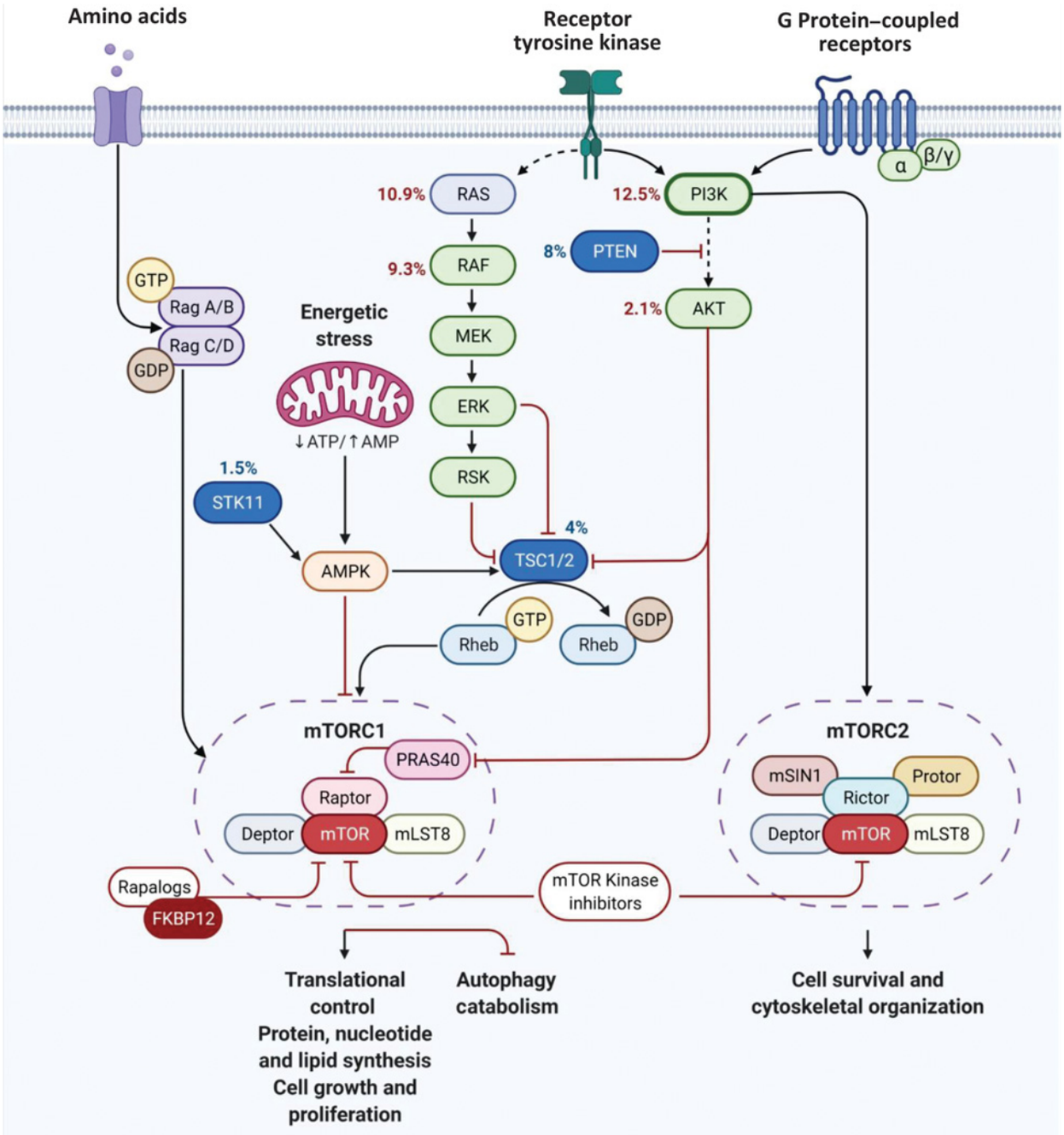

Figure 1.

mTOR signaling pathway in cancer. Schematic depicting major upstream regulatory mechanisms controlling the mTORC1 and mTORC2 complexes, their major functions, and mechanistically distinct classes of pharmacologic inhibitors. Numbers indicate the percentage of tumors harboring mutations in indicated pathway members [data retrieved from The Cancer Genomic Atlas (TCGA) Pan-Cancer Atlas Studies via cbioportal.org; mutation frequencies above 1% are shown]. Adapted from "mTOR Signaling Pathway" by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

nutrient sensing capacity upstream of the Rag GTPases can render mTOR insensitive to the low nutrient levels found in many tumors. For example, mutations in each of the core components of the GATOR1 complex (DEPDC5, NPRL2, and NPRL3), which are required for linking nutrient sensing to the Rag GTPases, lead to their loss of function in glioblastoma. Similarly, mutations in RagC and FLCN, an upstream regulator of the Rag GTPases, block metabolic checkpoints restricting mTORC1 activity in follicular lymphoma and Birt-Hogg-Dubé syndrome, respectively (2). mTOR's pervasive activation in cancer, and evidence suggesting its key role in disease

progression, explain why it has been the subject of intense interest as a cancer therapeutic target.

Pharmacologic Targeting of the mTOR Pathway in Cancer: Successes and Failures

Efforts to target mTOR signaling in cancer have largely relied upon two classes of drugs: the rapamycin derivatives, which partially inhibit mTORC1, and the ATP-competitive mTOR inhibitors, which fully inhibit both mTORC1 and mTORC2.

Early studies revealed that rapamycin is active in lymphangioleiomyomatosis, which involves the overgrowth of abnormal smooth muscle-like cells caused primarily by mutations in *TSC1* and *TSC2* genes, and in Kaposi's sarcoma, a virally induced endothelial-derived malignancy often arising in renal transplant patients. Rapamycin derivatives ("rapalogs"), most notably the orally available agent everolimus, are now approved for the treatment of a range of malignancies, including renal cell carcinomas, $HR^+/HER2^-$ breast cancers, and pancreatic neuroendocrine tumors, as well as tumors associated with tuberous sclerosis complex, including adult renal angiomyolipoma and subependymal giant cell astrocytoma. However, particularly when used as a single agent, this drug has been associated with only modest survival increases and single digit response rates (4, 5).

Successful targeted cancer therapies often show activity only in tumors harboring biomarkers indicative of dependency on the drug's target. In contrast, in most clinical studies of rapalog therapy, patients have been enrolled on the basis of disease tissue of origin and stage, often in the absence of molecular evidence of mutational mTOR pathway activation and/or clear clinical and experimental evidence of mTOR pathway dependence. When the mutational status of common upstream activators of mTOR such as *PIK3CA*, *PTEN*, and *KRAS* has been considered, clinical responses have also been infrequent. Thus, we currently lack mechanistic biomarkers and genetic alterations of predictive value of a favorable response (4). However, "exceptional responses" to rapalogs have been observed, including in patients whose tumors harbored mTOR activating genomic alterations. For example, several patients with perivascular epithelioid cell neoplasm, a rare sarcoma with mutations in *TSC1*/*TSC2*, displayed exceptionally deep and sustained responses to rapamycin derivatives, as did a patient with metastatic bladder cancer with an inactivating *TSC1* mutation. Similarly, patients with bladder cancer, renal cell carninoma, and anaplastic thyroid cancer harboring activating *MTOR* mutations or inactivating *TSC1*/*TSC2* mutations have also displayed exceptional responses. These results suggest that patients with alterations in *MTOR* or *TSC1*/*2*, or those with certain other alterations in the mTOR signaling pathway, may be strong candidates for mTOR-targeted therapy. This hypothesis is bolstered by preclinical studies, which have shown that tumor cell lines with *DEPDC5*, *NPRL2*, *NPRL3*, *RHEB*, and activating *MTOR* mutations can respond well to rapamycin derivatives (5). However, the story is complicated, as many patients harboring these alterations nevertheless fail to respond, as demonstrated by a recent histology-agnostic Phase II study, which disappointingly revealed only two objective responses in 29 patients with solid tumors harboring *TSC1* or *TSC2* mutations (6).

A significant advance in the field arrived in 2009, when the first highly selective, ATP competitive mTOR kinase inhibitors were described (1). Interestingly, while the improved activity of these agents in model systems was originally assumed by many to derive from their ability to inhibit both mTORC1 and mTORC2, studies suggested that the major driver of this differential activity actually owes to the ability of these agents to fully inhibit cap-dependent translation downstream of mTORC1 (7). Unfortunately, while ATP-competitive mTOR inhibitors have been valuable research tools, their clinical activities have largely been disappointing. For example, in a Phase II study of women with advanced-stage, HR^+, $HER2^-$ breast cancer treated with fulvestrant, with or without the ATP competitive inhibitor vistusertib (AZD2014) or everolimus, the median PFS with vistusertib plus fulvestrant (7.6–8.0 months) was slightly better than fulvestrant alone (5.4 months) but worse than everolimus plus fulvestrant (12.3 months). Other ATP competitive mTOR inhibitors have fared similarly: There were no reported responses to AZD8055 in a Phase I trial in solid tumors and lymphomas, and responses in a similar Phase I trial with sapanisertib (a TORC1/TORC2 inhibitor, also known as INK128) were infrequent. Despite these disappointing single agent results and the fact that, as yet, ATP competitive mTOR inhibitors have failed to offer clinical advantages relative to rapalogs, ongoing trials are nevertheless assessing the response of certain tumors, including those harboring *RICTOR* amplification or *TSC1*/*2* mutations, to these agents (4).

We lack a satisfying answer to the question: Why have mTOR inhibitors so often failed to deliver impressive clinical responses? However, clues exist. Cap-dependent translation initiation can occur independently of mTOR, particularly under hypoxic conditions in the tumor microenvironment. Thus, rapalogs, because of this incomplete ability to inhibit cap-dependent translation, and their propensity to drive feedback-mediated AKT survival signaling, may simply be incapable of inhibiting the full range of mTOR effectors needed for a robust antitumor response in many settings (4). In addition, mTOR inhibitors can promote the activation of multiple vertical and horizontal compensatory pathways, such as enhanced receptor tyrosine kinase signaling and ERK MAPK superactivation, thereby rendering mTOR inhibitors ineffective. mTOR inhibitors may also exert undesirable immune modulating effects, and ATP competitive inhibitors in particular may be largely dose-limited by toxicity (4).

New, Potentially Improved Strategies to Target mTOR in Cancer

Lessons learned from the experience with mTOR inhibitors in the clinic and novel network-based systems biology approaches can guide the development of new therapeutic strategies targeting mTOR in cancer. First, new generations of mechanistically distinct mTOR pathway inhibitors, as embodied by recently described inhibitors of class I glucose transporters and Rheb as well as the Rapa-Link compound series, may have the ability to completely block the disease-driving functions of mTORC1 while sparing toxicities secondary to mTORC2 inhibition (8). Second, recent studies have described approaches that may enable the selective inhibition of the sources of mTORC1 hyperactivation in tumor cells, leading to tumor-selective pathway inhibition rather than global mTOR blockade. For example, HER3 sustains persistent PI3K–AKT–mTOR signaling in head and neck cancers that do not harbor *PIK3CA* mutations, thus HER3 blockade inhibits mTOR in cancer cells without affecting mTOR function in immune cells (9). A particularly elegant example of this concept occurs in pancreatic cancer, where macropinocytosis supplies protein-derived amino acids to activate mTORC1 via the lysosomal amino acid transporter SLC38A9, the inhibition of which selectively blocks mTORC1 signaling and resultant pancreatic ductal

adenocarcinoma tumor growth (10). Third, we are now in a position to harness the power of systems biology approaches to identify novel synthetic lethalities resulting from mTOR blockade, as well as to develop multimodal precision strategies cotargeting mTOR and its compensatory resistance mechanisms. Finally, rapalogs, mTOR kinase inhibitors, and new targeting agents may be significantly improved through a better understanding of the relationship between tumor lineage/genetics and mTOR dependence. Ultimately, the emerging systems level understanding of mTOR signaling in cancer can now be exploited for the development of novel drugs and combination therapies aimed at achieving cancer remission as well as preventing cancer recurrence in at-risk patients.

Authors' Disclosures

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