The mTOR Pathway in Lung Cancer and Implications for Therapy and Biomarker Analysis

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Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that functions as a key regulatory protein in normal cell growth, survival, metabolism, development, and angiogenic pathways. Deregulation of these processes is a required hallmark of cancer, and dysregulation of mTOR signaling frequently occurs in a wide variety of malignancies, including lung cancer. Targeting of mTOR is thus an attractive strategy in the development of therapeutic agents against lung cancer. In this review, the mTOR-signaling pathway is described, highlighting opportunities for therapeutic intervention and biomarker analysis, and clinical trials in lung cancer including both non–small cell lung cancer and small cell lung cancer.

Key Words: Mammalian target of rapamycin, Non-small cell lung cancer, Small cell lung cancer, Rapamycin, Sirolimus.

Treatment with conventional chemotherapy for advanced non–small cell lung cancer (NSCLC) has reached a therapeutic plateau with a median survival time of 10 to 12 months. Thus, the need for new therapeutic opportunities is great, and the introduction of targeted therapies for subgroups of patients has already demonstrated great promise. One of the most promising targets identified in general is mammalian target of rapamycin (mTOR), which is found to be activated in a substantial number of lung cancer cases as well. This review gives a background to the mTOR pathway and describes the current clinical development of mTOR inhibitors in the treatment of lung cancer, including biomarker analysis.

mTOR SIGNALING PATHWAY

"Target of rapamycin" is the protein target of rapamycin, an antifungal metabolite originating from Easter Island ("Rapa Nui" in the native language), which was later found to have immunosuppressive and antiproliferative properties.[1]

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The mammalian homologue, mTOR, is a 289 kDa protein with serine/threonine kinase activity. The mTOR protein functions in two different complexes, mTORC1 and mTORC2. These mTOR complexes play an integral role in the regulation of many cellular processes including protein synthesis, autophagy, lipid synthesis, mitochondrial metabolism/biogenesis, and cell cycle. Both mTOR complexes are important for maintaining cellular homeostasis and the growth of many types of cancer, but with different mechanisms of action. mTORC1 is involved in regulating cell size, whereas mTORC2 is involved in regulating cell shape through actin cytoskeleton reorganization.

Activation of mTOR is initiated by ligand stimulation of one of several different membrane-bound growth factor receptors, e.g., receptors for epidermal growth factor and insulin-like growth factor-1 and -2 (IGF-1 and -2). This receptor stimulation leads to activation of phosphatidylinositol 3-kinase (PI3-K), which in turn will activate the downstream effector Akt (also known as protein kinase B), a master regulator of cell survival at multiple levels. Akt phosphorylates and suppresses the activity of the downstream tuberous sclerosis complex 1 (hamartin) and 2 (tuberin)(TSC1/2), thereby leading to Rheb-mediated activation of mTOR.[2] This TSC1/2 complex functions as a key player in the regulation of the mTOR pathway by mediating inputs from the PI3-K/phosphatase and tensin homologue (PTEN)/Akt and Ras/Erk1/2 signaling pathways. In cellular stress conditions the TSC1/2 complex is activated by the tumor suppressor serine/threonine kinase 11 (liver kinase B1 [LKB1])–adenosine monophosphate-activated protein kinase (AMPK) pathway, leading to the suppression of the activity of Rheb and switching off mTOR-mediated signals.[3] The two major substrates of mTORC1 are S6K1 and 4E-BP1, which upon phosphorylation initiate eIF4E-mediated cap-dependent transcription and translation of a wide variety of proteins. Genes that are sensitive to eIF4E-mediated translation cover a range of cellular functions, including cell-cycle control (cyclin D1), angiogenesis (vascular endothelial growth factor), and apoptosis (survivin and Mcl-1). mTORC2 has other substrates than mTORC1, and is known to phosphorylate and activate Akt and Protein Kinase Cα (PKCα)[4] (Fig. 1).

DEREGULATION OF mTOR PATHWAY IN LUNG CANCER

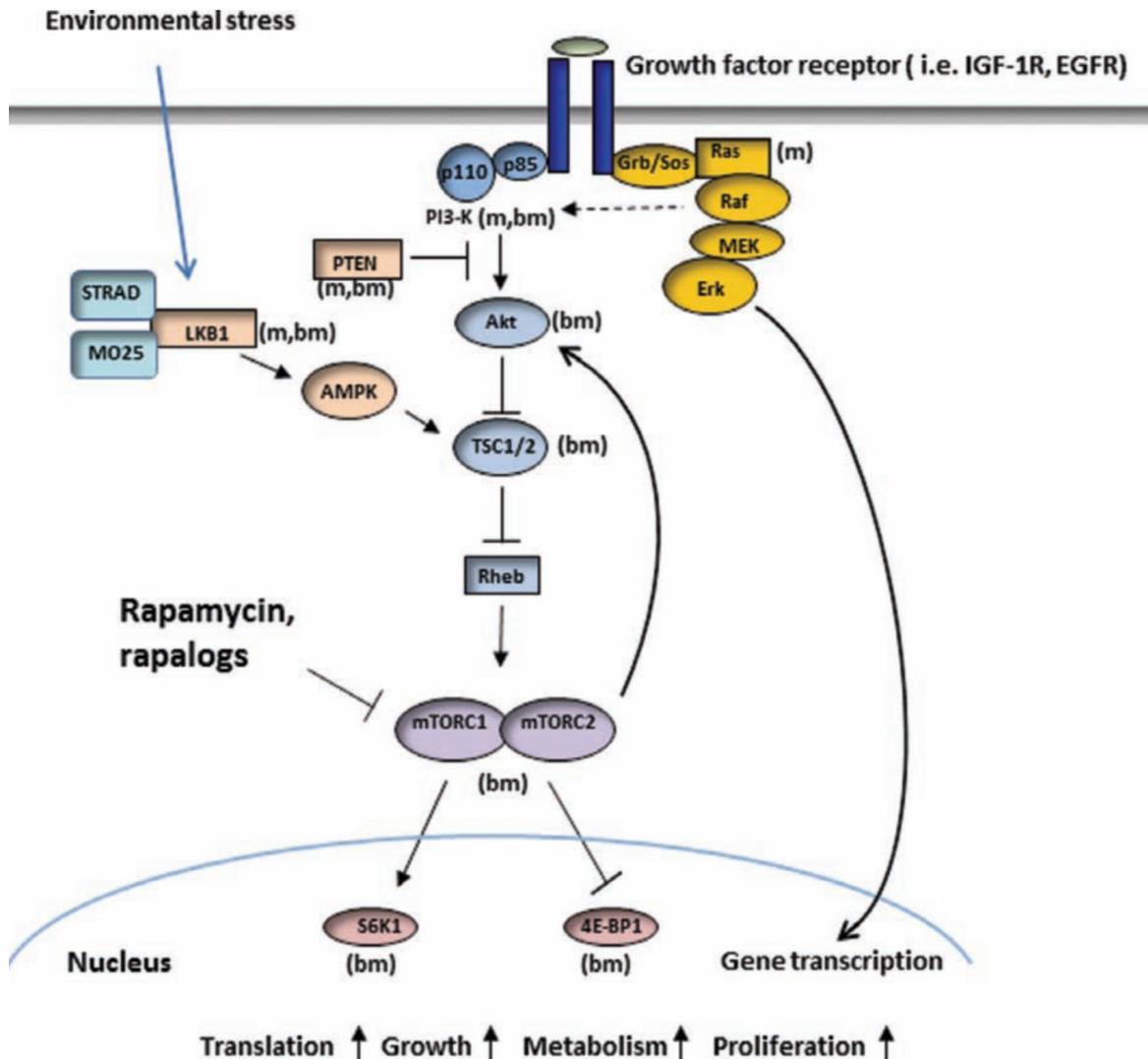Abberant activation of the Akt/mTOR pathway is commonly observed in lung cancer.[1] Deregulated PI3-K/Akt/mTOR activity is known to contribute to lung cancer development and maintenance, and the most downstream effector, eIF-4E, is (1) capable of transforming cells, (2) overexpressed in both squamous lung carcinoma and adenocarcinoma, and (3) a

FIGURE 1. mTOR-signaling network. Activated growth factor receptors trigger activation of the PI3K/Akt pathways and the Ras/MEK/Erk pathway. Activated Akt leads to increased mTOR activity through signaling by means of the TSC1/2 complex. mTOR then phosphorylates S6K1 and 4E-BP1, resulting in increased gene transcription, cell growth, and cell proliferation. PI3-K, phosphatidylinositol 3-kinase; AMPK, adenosine mono-phosphate-activated protein kinase; LKB1, liver kinase B1; PTEN, phosphatase and tensin homologue; STRAD, Ste20-like adaptor protein; TSC, tuberous sclerosis complex; EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin; m, signaling proteins frequently mutated in lung cancer; bm, potential as biomarker; Ras, ras2 Kirsten rat sarcoma viral oncogene homolog; MEK, MAPK/ERK kinase; Erk, extracellular signal-regulated kinase.

poor prognostic factor.[1,5,6] Several lines of evidence support a role for the mTOR pathway in lung carcinogenesis by means of its coupling with eIF-4E, which in numerous studies has been shown to function as an oncogene.[7] eIF-4E expression in bronchial adenocarcinomas (but not squamous cell carcinomas) was found to be substantially higher than in the normal lung,[8] and in an another study of adenocarcinomas eIF-4E expression was found to be elevated and specifically associated with histological grade and invasiveness of the tumor.[9] Several negative regulators of mTOR signaling, including PTEN and LKB1, act as tumor suppressors and have been found to be frequently mutated in lung cancer, indicating a role of the mTOR pathway in lung carcinogenesis.[2,3] Furthermore, in a transgenic mice model

overexpression of IGF-1, a major activator of the Akt/mTOR pathway, significantly increased the incidence of premalignant adenomatous hyperplastic lesions.[10] Further evidence for a role of the mTOR pathway in lung tumorigenesis comes from studies of the activation status of Akt, the upstream regulator of mTOR, in bronchial epithelial lesions, showing an increased activity compared with normal and hyperplastic bronchial epithelia. Also, there was a similar frequency of activated Akt in low-stage tumors (stage I/II) and high-stage tumors (stage III/IV), indicating that activated Akt/mTOR pathway is an early event in tumor progression. Activation of Akt correlated well with phosphorylation of downstream mTOR.[11] Another study used the K-rasLA1 mice model in which somatic activation of the

v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*K-ras*) oncogene induces morphologic changes in alveolar epithelial cells that recapitulate those of precursors of human lung adenocarcinoma. It was observed that the levels of phospho-S6, a downstream mediator of mTOR, increased with malignant progression (normal alveolar epithelial cells to adenocarcinoma) with high levels of phospho-S6 in atypical alveolar hyperplasia, an early neoplastic change. Treatment with the rapamycin analogue CCI-779, causing mTOR inhibition, reduced the size and number of early epithelial neoplastic lesions.[12] Furthermore, activation of the mTOR pathway has been implicated in tobacco-related lung carcinogenesis. It was demonstrated that nicotine stimulated the Akt/mTOR pathway in normal human bronchial epithelial cells, leading to induced survivin expression and increased survival potential of the cells. Small interfering RNA-mediated down-regulation of survivin expression suppressed the tumorigenic potential of premalignant and malignant bronchial epithelial cells exposed to the tobacco components.[13] In another study on factors influencing human bronchial epithelial cell growth it was found that fibronectin stimulates growth through activation of NF-κB, which, in turn, is activated through PI3-K/Akt and integrin-dependent signals. Blocking both NF-κB and PI3-K signals abolished the stimulatory effect of fibronectin on cell growth, suggesting that these signaling pathways are involved in the effect.[14]

Data suggest that mTOR activation is associated with both KRAS and epidermal growth factor receptor (EGFR) mutation and may be a mechanism of resistance to treatment with EGFR inhibitors.[15] There are also data demonstrating that mTOR activation correlates with lymph node metastasis. In a report by Dobashi et al.,[1] mTOR was activated in as much as 90% of adenocarcinomas, 40% of squamous cell carcinomas, and 60% of large cell carcinomas. In small cell lung cancer (SCLC), expression data are more scarce, but in a recent report, the Akt/mTOR pathway was found to be widely expressed and activated in tumor specimens from SCLC patients. However, it did not correlate to survival.[16] There are several studies on SCLC cell lines demonstrating that the PI3K/Akt/mTOR pathway is activated, including different mechanisms of activation, such as PTEN mutation and PI3-K overexpression.[17]

The activation of Akt through PI3-K is subject to negative regulation by the product of the PTEN gene on chromosome 10,[3] a tumor suppressor which acts by removing a phosphate group from PI3-K lipid product phosphatidylinositol 3,4,5-trisphosphate. Loss of PTEN in NSCLC is a frequent finding and has been associated with a worse prognosis and resistance to EGFR tyrosine kinase inhibitors (TKI).[4] Another negative regulator of mTOR is LKB1, also known as serine/threonine kinase 11. LKB1 activates AMP-activated protein kinase through direct phosphorylation, which in turn phosphorylates TSC2, the guanosine triphosphate (GTP)-activating protein of Rheb, resulting in Rheb inactivation and mTOR inhibition. LKB1 loss-of-function somatic mutations are frequently observed in NSCLCs (20%–30%), and recent studies have identified LKB1 as an important factor in lung cancer, especially in the metastatic process.[5]

Epithelial-mesenchymal transition (EMT) leads to increased motility and invasive behavior in tumors and has been identified as a mechanism of resistance to EGFR TKIs.[18] Transforming growth factor-β has emerged as a major inducer of EMT through activation of downstream-signaling pathways, including Smad and non–Smad signaling pathways. Among the non–Smad pathways, the PI3K-Akt-mTOR axis plays a major role in transforming growth factor-β–induced EMT.[19] The exact role of mTOR in EMT transition in lung cancer has not yet been studied in detail but is expected to be similar as in other cancer types.

There are indications that the mTOR signaling pathway plays a central role in some characteristic clinical features of lung cancer. Lung cancer exhibits a particularly aggressive metastatic phenotype, and it was demonstrated that exposure of NSCLC cells to hypoxia or epidermal growth factor resulted in a significant up-regulation of CXCR4 expression and chemotactic behavior by means of activation of hypoxia inducible factor-1α (HIF-1α). The PI3-K inhibitors wortmannin and LY294002 and the mTOR inhibitor rapamycin inhibited activation of HIF-1α and consequently up-regulation of CXCR4 expression.[20] Bone metastasis is a common phenomenon in lung cancer, contributing to significant morbidity and worse prognosis. Investigating cell signals that may contribute to increased osteoclast differentiation and consequently bone resorption, phospholipase D (PLD)-induced Akt/mTOR activation was identified as one of the key signals. PLD inhibition completely decreased Akt and mTOR phosphorylation, whereas a PI3-K inhibitor only partially decreased mTOR phosphorylation, suggesting that mTOR activation by PLD is through both PI3-K/Akt-dependent and -independent manner.[21] In another study, NSCLC tumors and cell lines were investigated for EphA2 mutations and a particular mutation, G391R, was found to activate the mTOR pathway, leading to increased cell survival and an invasive phenotype.[22]

TARGETING THE mTOR PATHWAY

Sirolimus

The apparent importance of the mTOR signaling network in lung cancer makes its signaling components attractive targets for therapy. The antiproliferative and antitumor effects of sirolimus (rapamycin), a naturally occurring antibiotic produced by the bacterium *Streptomyces hygroscopicus*, have been evaluated in numerous in vitro and in vivo models. However, there is very limited clinical data on sirolimus for the treatment of lung cancer, but clinical trials are ongoing (Table 1). On the basis of the already established sirolimus, several derivatives have been developed and investigated in preclinical and clinical studies including CCI-779 (Cell Cycle Inhibitor-779, temsirolimus, TORISEL; Pfizer, New York, NY), RAD001 (everolimus, Afinitor; Novartis Pharma AG, Basel, Switzerland), and AP23573 (Ariad Pharmaceuticals, Cambridge, MA). For ongoing clinical trials with mTOR inhibitors, see Table 1.

CCI-779 (Temsirolimus)

CCI-779 is a water-soluble ester of sirolimus that is approved for the treatment of advanced renal cell carcinoma. The N0323 trial was a two-stage single-arm phase-II trial that evaluated the response and toxicity rates of CCI-779 administered as a frontline single-agent treatment for stage IIIB (pleural effusion) or stage IV NSCLC. Fifty-five patients were accrued and the median progression-free survival (PFS) and

TABLE 1. Clinical Trials With mTOR Inhibitors in Lung Cancer[a]

Trial	Stage	Patients (*n*)	Intervention	Main Endpoint
Sirolimus				
Sarkaria et al.,[45] phase I	NSCLC III	7	Radiation 60 Gy/weekly cisplatin/sirolimus	Safety, tolerability
NCT00993499 phase I (ongoing)	NSCLC IIIB/IV	42	Afatinib (BIBW 2992) /sirolimus	Maximum tolerated dose
NCT00923273 phase I/II (ongoing)	Refractory/relapsed NSCLC	82	Pemetrexed/sirolimus	Maximum tolerated dose, safety
Temsirolimus (CCI-779)				
Hidalgo et al.,[46] phase I	Advanced cancer including lung cancer	63	CCI-779 (temsirolimus)	Maximum tolerated dose, safety
Buckner et al.,[47] phase I	Advanced cancer including lung cancer	24	CCI-779 (temsirolimus)	Maximum tolerated dose
Molina et al.[23] phase II	NSCLC IIIB/IV	55	CCI-779 (temsirolimus)	Response rate, toxicity
Pandya et al.,[24] phase II	Extensive disease SCLC	87	CCI-779 (temsirolimus)	PFS, toxicity
Bryce et al.,[48] phase I	Advanced solid tumors including lung cancer	48	CCI-779 (temsirolimus)/EKB-569 (EGFR inhibitor)	Maximum tolerated dose
NCT00921310 phase I/II (ongoing)	Refractory/relapsed NSCLC	43	Pemetrexed/temsirolimus	Maximum tolerated dose, response rate
Everolimus (RAD001)				
Tarhini et al.,[25] phase II	Relapsed SCLC	40	Everolimus (RAD001)	Disease control rate at 6 weeks
Ramalingam et al.,[49] phase I	Advanced NSCLC	24	Docetaxel/everolimus	Recommended phase 2 doses
Vansteenkiste et al.,[50] phase I	NSCLC IIIB/IV	24	Pemetrexed/everolimus	Cycle 1 dose-limiting toxicity
Soria et al.[26] phase II	NSCLC IIIB/IV	85	Everolimus	Overall response rate
Milton et al.,[51] phase I	NSCLC IIIB/IV	10	RAD001 (everolimus)	Maximum tolerated dose, safety
Price et al.,[29] phase II	NSCLC IIIB/IV	62	Gefitinib/everolimus (RAD001)	Response rate
NCT00401778 phase I (ongoing)	NSCLC I-IIIA	60	RAD001 (everolimus)	Clinical response as measured by PET scan
NCT01317615 phase II (ongoing)	NSCLC IV LC-NEC	85	RAD001(everolimus)/carboplatin/paclitaxel	Efficacy, safety
NCT01167530 phase I (ongoing)	NSCLC unresectable stage III/IV	34	RAD001 (everolimus)/radiotherapy	
NCT01427946 phase I/II (ongoing)	NSCLC III/IV KRAS mutant	45	Retaspimycin HCl (IPI-504)/everolimus	Safety/efficacy
NCT01063478 phase I (ongoing)	NSCLC II/III/Mx	24	RAD001 (everolimus)/radiotherapy/chemotherapy	Safety
NCT01079481 phase I (ongoing)	SCLC relapsed or refractory	15	Everolimus/paclitaxel	Maximum tolerated dose

[a]For ongoing clinical trials, please refer to www.clinicaltrials.gov. Accessed December 20, 2011.
NSCLC, non–small cell lung cancer; EGFR, epidermal growth factor receptor; SCLC, small cell lung cancer; PFS, progression-free survival; PET, positron emission tomography.

overall survival (OS) were 2.3 and 6.6 months, respectively. An interesting clinical benefit was observed with four patients (8%) having confirmed partial responses and 15 patients (30%) with stable disease. The most common grade 3/4 toxicity included dyspnea (12%), fatigue (10%), hyperglycemia (8%), hypoxia (8%), nausea (8%), and rash/desquamation (6%). Although the study did not meet the predefined success criteria, single-agent CCI-779 showed activity similar to other signal transduction inhibitors, and had good tolerability.[23] In a randomized phase II trial by the Eastern Cooperative Oncology Group (E1500), 87 patients with extensive SCLC having received four to six cycles of cisplatin or carboplatin plus etoposide or irinotecan and with either stable or responding disease, were randomized between two different dose levels of temsirolimus. The study concluded that temsirolimus, given at 25 or 250 mg weekly, did not increase the PFS in this patient population. There

were 86 patients with reported toxicities, 36 (42%) of whom had grade 3 toxicities, the most common of which were thrombocytopenia, hypophosphatemia, and fatigue. An additional 12 (14%) had grade 4 toxicities, the most common of which was neutropenia. No patients experienced lethal toxicities.[24]

RAD001 (Everolimus)

RAD001 (everolimus) is an oral sirolimus analogue that is currently approved for the treatment of advanced renal cell carcinoma. Recently, a phase-II study evaluated everolimus in previously treated patients with relapsed SCLC. A total of 40 patients were treated, and one patient (3%) showed a partial response, eight (23%) had stable disease; the primary endpoint disease control rate at 6 weeks was 26%, whereas median survival was 6.7 months, and median time to progression

was 1.3 months. Baseline S6 kinase protein expression was significantly higher in patients with disease control versus patients with progression ($p = 0.0093$). Some grade 3 toxicities were reported, including bone-marrow depression (thrombocytopenia [$n = 2$] and neutropenia [$n = 2$]), infection ($n = 2$), pneumonitis ($n = 1$), fatigue ($n = 1$), elevated levels of transaminases ($n = 1$), diarrhea ($n = 2$), and acute renal failure ($n = 1$). In conclusion, everolimus was well tolerated but had limited single-agent antitumor activity in unselected previously treated patients with relapsed SCLC.[25] Another phase-II study of RAD001 assessed the efficacy of RAD001 monotherapy, where advanced NSCLC patients with two or fewer prior chemotherapy regimens, one platinum based (stratum 1) or both chemotherapy and epidermal growth factor receptor TKIs (stratum 2), received RAD001 10 mg/day until progression or unacceptable toxicity. Primary objective was overall response rate. Eighty-five patients were included, and overall response rate was 4.7%, overall disease control rate 47.1%, and median PFSs 2.6 (stratum 1) and 2.7 months (stratum 2). Common events that were graded 3 or more were fatigue, dyspnea, stomatitis, anemia, and thrombocytopenia. Pneumonitis, probably or possibly related, mainly grade 1/2, occurred in 25% of patients. The study concluded that RAD001 10 mg/day was well tolerated, showing modest clinical activity in pretreated NSCLC.[26] Pneumonitis is a known side effect of inhibitors of mTOR and has been described for sirolimus, temsirolimus, and everolimus. A recent retrospective study of patients with advanced NSCLC suggests that a mostly low-grade pneumonitis with a possible or probable relationship to everolimus was relatively frequent, occurring in 25% of the evaluated patients, suggesting a need for monitoring of pulmonary adverse events in future studies with everolimus.[27]

Combination Treatment With mTOR and EGFR Inhibitors

Increasing knowledge about mechanisms of resistance to EGFR-TKIs supports a significant role for the Akt/mTOR pathway in resistance development,[28] and has led to the initiation of clinical trials evaluating the combination of EGFR-TKIs and mTOR inhibitors. A phase-II trial assessed the efficacy of the combination of gefitinib and everolimus in patients with advanced NSCLC. Sixty-two patients were enrolled, and partial responses were seen in eight of 62 patients (response rate, 13%). Three partial responders had an EGFR mutation. Interestingly, both patients with a KRAS (G12F) mutation responded. The median time to progression was 4 months and the median overall survival was 12 months for all patients. By subgroups, the median overall survival was 27 months for patients with no prior chemotherapy and 11 months for patients previously treated with chemotherapy. Although the trial did not meet the pre-specified response threshold to pursue further study of the combination of gefitinib and everolimus, further investigation of mTOR inhibitors in patients with NSCLC with KRAS G12F-mutated tumors is warranted.[29]

Combination Treatment With mTOR and IGF-1R Inhibitors

Thus far, limited anticancer therapeutic success with IGF-1R targeted therapy has been reported; also, there is limited knowledge about resistance mechanisms to IGF-1R directed therapy.[30–33] One study on cixutumumab (IMC-A12), an anti-IGF-1R monoclonal antibody (mAb), demonstrated induced activation of Akt and mTOR, resulting in de novo synthesis of EGFR, Akt1, and survivin proteins and activation of the EGFR pathway in cixutumumab-resistant head-neck carcinoma and NSCLC cells.[34] Targeting mTOR and EGFR pathways by treatment with rapamycin and cetuximab (an anti-EGFR mAb), respectively, prevented cixutumumab-induced expression of EGFR, Akt, and survivin and induced synergistic antitumor effects in vitro and in vivo. An ongoing phase-II study (NCT01016015) is evaluating the combination of temsirolimus and cixutumumab in treatment of patients with locally advanced, metastatic, or recurrent soft-tissue sarcoma or bone sarcoma. Also, for mTOR inhibitors a connection between mTOR and IGF-1R signaling has been found. In rhabdomyosarcoma cell lines and xenografts, rapamycin mediated Akt activation through an IGF-1R–dependent mechanism, reportedly involving abrogation of a mTOR-mediated negative feedback signal through S6K and Grb10.[35,36] Combined mTOR and IGF-1R inhibition with rapamycin and the anti-IGF-1R mAb h7C10, respectively, resulted in additive inhibition of cell growth and survival.[37] Thus, combining an mTOR inhibitor and an IGF-1R antibody/inhibitor may be an appropriate strategy to enhance mTOR-targeted anticancer therapy.

Other mTOR Inhibitors

Ridaforolimus (AP23573) is a novel non-prodrug sirolimus analogue that selectively targets mTOR and has shown potent antitumor activity both in cell lines and in xenograft tumor models. It is currently under clinical evaluation, and in phase-I studies has shown good tolerability with dose-limiting stomatitis, in both single and combination treatments. Ridaforolimus has demonstrated clinical benefit in advanced soft-tissue sarcoma and in hematological malignancies, and several phase-I studies in various tumor types are ongoing.[38] Clinical data for treatment in lung cancer are yet to be reported.

mTORC1 is sensitive whereas mTORC2 is resistant to rapamycin and other rapalogs. Second-generation inhibitors of mTOR, referred as mTOR kinase domain inhibitors, are being developed that target rapamycin-resistant pathways by inhibiting the adenosine-5'-triphosphate binding site of both mTORC1 and mTORC2. Two such novel mTOR inhibitors, CC-115, and CC-223 (Celgene), are currently under clinical investigation in phase-I trials in patients with advanced solid tumors, non-Hodgkin's lymphoma or multiple myeloma to assess safety and tolerability (NCT01353625 and NCT01177397, respectively). CC-115, being a dual DNA-protein kinase and mTOR inhibitor, differs from other mTOR inhibitors whereas CC-223 is a dual mTOR inhibitor targeting both mTORC1 and mTORC2. They both exist as oral formulations.

Other Inhibitors of the mTOR Pathway

Alternative ways of inhibiting the mTOR pathway include PI3-K inhibition. The first-generation PI3-K inhibitors, Wortmannin and LY2994002, proved unsuitable for clinical use despite demonstrating preclinical antitumor activity.[39] Novel PI3-K inhibitors, including BKM120 (an oral pan-class I PI3-K inhibitor), XL 147 (an adenosine-5'-triphosphate-competitive reversible PI3-K inhibitor), and GDC-0941 (a dual PI3K/mTOR inhibitor) have been developed and early clinical trials in lung cancer and other solid tumors are ongoing, but data regarding lung cancer are still pending. Akt inhibition is another strategy to block signaling in the mTOR pathway and is being explored in early clinical studies in lung cancer, including MK2206 in advanced NSCLC patients who previously progressed on erlotinib treatment (www.clinicaltrials.gov).

BIOMARKERS

Promising yet limited success has been achieved in clinical applications of mTOR inhibitors. An important strategy in the evaluation of mTOR inhibitors is the identification of patients who are most likely to benefit by using a biomarker approach. Several biomarkers have been developed to monitor the effects of mTOR inhibitors, including measurements by Western blotting or immunohistochemistry of S6K and 4E-BP1 phosphorylation (see Fig. 1 for potential biomarkers). In one study, activation of S6K and/or 4E-BP1 was a determinant of cisplatin resistance in NSCLC.[40] Biomarker data are limited in lung cancer but some experience can be derived from studies of other tumor types. On investigating sensitivity to ridaforolimus in sarcoma and endometrial cancer cells, it was found that the proportion of cells in the G(0)–G(1) phase of the cell cycle before treatment correlated significantly with ridaforolimus sensitivity, also including a higher expression of several G(1) phase cell-cycle proteins such as p21 and p27.[41] Another study using a mammary carcinoma OncoMouse model demonstrated that the antitumor efficacy of temsirolimus was associated with significantly suppressed tumor vascular density and macrophage burden, two microenvironmental factors of importance for tumor progression.[42] An association with decreased levels of phospho-S6 proteins was also seen. There is a need for efficient biomarkers not only to predict who will benefit from chemotherapy but also to avoid the development of unnecessary toxicity. In one study in advanced NSCLC patients using single nucleotide polymorphism analysis, genetic variations within the PI3K/PTEN/AKT/mTOR signaling pathway associated with increased risk of toxicity from platinum-based chemotherapy.[43] However, existing biomarkers have been found to be only partially predictive and additional biomarkers are warranted. For example, S6K activity showed reduction both in "sensitive" and "insensitive" cells in a study on everolimus.[34] S6K may also be a prognostic marker for clinical outcome independent of type of treatment, according to findings in both breast cancer and renal cancer patients.

Besides the classical mTOR/S6K/4E-BP1 pathway, alternative biomarkers for response to mTOR inhibitors have been explored in different tumor types. In a study on patients with persistent/recurrent epithelial ovarian cancer/primary peritoneal cancer, an association between positive pretreatment circulating tumor cells and lack of response to temsirolimus was indicated.[44] Because of the inherent complexity in the regulation and function of mTOR, a combined use of different classes of biomarkers may be needed to accurately predict responses to mTOR inhibitors. Moreover, the molecular mechanisms involved in mTOR activation, e.g., abrogation of PTEN function, may be tumor-specific. The mTOR inhibitors will most likely show activity in selected patient populations with specific molecular alterations. Therefore, the importance of simultaneous development of predictive biomarkers is crucial in future clinical trials.

In conclusion, the mTOR pathway plays a critical role in mediating proliferative and survival signals in cancer cells and has been shown to be an important factor in lung carcinogenesis and tumor progression,[7] and in resistance to chemotherapy and EGFR inhibitors.[15] Several mTOR-targeted agents are under clinical development, both in NSCLC and SCLC, given as single treatment or in combination with chemotherapeutic agents or EGFR-TKIs. So far, promising clinical data have been observed, including partial responses and manageable toxicity, stimulating further clinical development. To further enhance the clinical benefit of mTOR inhibitors, there is a need to incorporate biomarker analyses in future clinical trials, improving our ability to identify the optimal patients and dosing and combination strategies with other molecular-targeted therapies.

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