A Selective Small Molecule c-MET Inhibitor, PHA665752, Cooperates with Rapamycin

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ABSTRACT

Purpose: c-MET is believed to be an attractive receptor target for molecular therapeutic inhibition. TPR-MET, a constitutively active oncogenic variant of MET, serves as an excellent model for testing c-MET inhibitors. Here, we characterized a small molecule c-MET inhibitor, PHA665752, and tested its cooperation with the mammalian target of rapamycin inhibitor as potential targeted therapy.

Experimental Design: The effect of PHA665752 treatment was determined on cell growth, motility and migration, apoptosis, and cell-cycle arrest of TPR-MET-transformed cells. Moreover, the effect of PHA665752 on the phosphorylation of MET, as well as its downstream effectors, p-AKT and p-S6K, was also determined. Finally, growth of TPR-MET-transformed cells was tested in the presence of PHA665752 and rapamycin. H441 non–small cell lung cancer (NSCLC) cells (with activated c-Met) were also tested against both PHA665752 and rapamycin.

Results: PHA665752 specifically inhibited cell growth in BaF3.TPR-MET cells (IC50 < 0.06 μmol/L), induced apoptosis and cell cycle arrest. Constitutive cell motility and migration of the BaF3.TPR-MET cells were also inhibited. PHA665752 inhibited specific phosphorylation of TPR-MET as well as phosphorylation of downstream targets of the mammalian target of rapamycin pathway. When combined with PHA665752, rapamycin showed cooperative inhibition to reduce growth of BaF3.TPR-MET- and c-MET-expressing H441 NSCLC cells.

Conclusions: PHA665752 is a potent small molecule–selective c-MET inhibitor and is highly active against TPR-MET-transformed cells both biologically and biochemically. PHA665752 is also active against H441 NSCLC cells. The c-MET inhibitor can cooperate with rapamycin in therapeutic inhibition of NSCLC, and in vivo studies of this combination against c-MET expressing cancers would be merited.

INTRODUCTION

c-MET is a unique receptor tyrosine kinase (RTK) located on chromosome 7p and activated via its natural ligand hepatocyte growth factor. c-MET can also be mutated in a variety of solid tumors (1), such as in the tyrosine kinase domain for hereditary papillary renal cell carcinoma (2, 3) and in the soma and juxtamembrane domains in small cell lung cancer (SCLC; ref. 4). Many of these are activating mutations and potentially targeted by specific c-MET small molecule inhibitors.

The TPR-MET oncogene is a transforming variant of the c-MET RTK and was initially identified after treatment of a human osteogenic sarcoma cell line transformed by the chemical carcinogen N-methyl-N-nitro-N-nitrosoguanidine (5–7). The TPR-MET fusion oncoprotein is the result of a chromosomal translocation, placing the TPR locus on chromosome 1 upstream of a portion of the c-MET gene on chromosome 7 encoding only for the cytoplasmic region (6, 7). Some studies suggest that TPR-MET is detectable in experimental cancer (8–10). Dimerization of the M765,000 TPR-MET oncoprotein through a leucine zipper motif encoded by TPR leads to constitutive activation of the c-MET kinase (11, 12). TPR-MET acts similarly to the activated wild-type c-MET RTK and can activate crucial cellular growth pathways, including the Ras pathway (13–16) and the phosphatidylinositol 3-kinase (PI3K)/AKT pathway (17, 18). Conversely, in contrast to c-MET RTK, TPR-MET is ligand independent, lacks the CBL binding site in the juxtamembrane region in c-MET, and is mainly cytoplasmic (5–7, 19, 20). We have recently shown that the TPR-MET fusion oncoprotein has potent transforming activity and can be an excellent model for testing inhibitors as it has tremendous constitutively elevated c-MET tyrosine kinase activity that can readily be inhibited (21).

When introduced into an interleukin-3 (IL-3)–dependent cell line, BaF3, TPR-MET induces oncogenic phenotypes such as growth factor independence and constitutive tyrosine phosphorylation of multiple cellular proteins, including TPR-MET itself (21). Recently, a novel-specific small molecule inhibitor of TPR-MET and c-MET kinase activity, SU11274, was identified which has in vitro inhibitory activities in TPR-MET cells and in c-MET expressing SCLC cells (i.e., H69 and H345) at low micromolar concentrations (21). SU11274 has cellular IC50 between 2.5 and 5 μmol/L in SCLC.

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To further explore the potential of targeted therapy against the c-MET kinase, we have characterized here a small molecule c-MET inhibitor with relatively improved potency and selectivity, PHA665752 (22). PHA665752 was recently identified and characterized to be active against several solid tumors (22). We have recently reported that non-SCLC (NSCLC) tumors can overexpress c-Met, and the H441 NSCLC cell line has been shown to overexpress and contain an activated c-Met. Here, we found that PHA665752 specifically inhibited cell growth, motility, and migration of cells transformed with TPR-MET. Inhibition of c-MET kinase activity by the small molecule drug induced apoptosis and cell cycle arrest in TPR-MET-transformed BaF3 cells. We also found that the dose-dependent reduction in tyrosine phosphorylation of cellular proteins after PHA665752 treatment correlated with reduced activation of the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway in these cells. Finally, we tested the specific mTOR inhibitor rapamycin alone or in combination with PHA665752 and found that rapamycin can cooperate with the c-MET inhibitor in inhibiting cell viability of BaF3, TPR-MET cells as well as NSCLC H441 cells. Our results suggest that the combination of c-MET inhibitors such as PHA665752 with mTOR inhibitor (rapamycin) may be a feasible approach to improve the therapeutic efficacy in c-MET expressing cancers including NSCLC.

MATERIALS AND METHODS

Cells. The murine pre-B cell line BaF3 was grown in RPMI 1640 containing 10% FCS and 10% WEHI-conditioned medium as a source of murine IL-3. BaF3 cell lines transfected with a BCR-ABL, TEL-ABL, TEL-JAK2, or TEL-PDGFβ cDNA were grown in the absence of growth factors. A TPR-MET expressing BaF3 cell line was generated by transfection of cDNA into BaF3 cells. We also found that non-SCLC (NSCLC) tumors can overexpress c-Met, and the H441 NSCLC cell line has been shown to overexpress and contain an activated c-Met. Here, we found that PHA665752 specifically inhibited cell growth, motility, and migration of cells transformed with TPR-MET.

Inhibition of c-MET kinase activity by the small molecule drug induced apoptosis and cell cycle arrest in TPR-MET-transformed BaF3 cells. We also found that the dose-dependent reduction in tyrosine phosphorylation of cellular proteins after PHA665752 treatment correlated with reduced activation of the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway in these cells. Finally, we tested the specific mTOR inhibitor rapamycin alone or in combination with PHA665752 and found that rapamycin can cooperate with the c-MET inhibitor in inhibiting cell viability of BaF3, TPR-MET cells as well as NSCLC H441 cells. Our results suggest that the combination of c-MET inhibitors such as PHA665752 with mTOR inhibitor (rapamycin) may be a feasible approach to improve the therapeutic efficacy in c-MET expressing cancers including NSCLC.

In vitro Toxicology Assay Kit, Sigma) or trypsin blue exclusion.

Chemicals. PHA665752, (3Z)-5-[(2,6-dichlorobenzyl)sulfonyl]-3-[(3,5-dimethyl-4-[(2R)-2-(pyrrolidin-1-ylmethyl)-pyrrolidin-1-yl]carbonyl]-1H-pyrrol-2-yl)methylene]-1,3-dihydro-2H-indol-2-one (Pfizer, Inc., La Jolla, CA), and rapamycin (Calbiochem, La Jolla, CA) were dissolved in DMSO and used for immunoblotting. In addition, polyclonal antibodies against phospho-MET [Tyr1230/1234/1235], –[Tyr1349] and –[Tyr1366] (recognizing phospho-[Tyr365/365/366], –[Tyr460] and –[Tyr469] respectively in TPR-MET) were obtained from Biosource International for the immunoblotting. The Tyr amino acid numbering of the phospho-MET antibodies is based on the shorter splice variant with 100-bp-long exon 10, instead of the full-length 154 bp.

Apoptosis Assays. The activity of caspase-3 was measured in cell lysates (CaspACE Assay System, Promega, Madison, WI) and Annexin V positive staining was determined by fluorescence-activated cell sorting analysis (Annexin-V-Fluos Staining Kit, Roche Diagnostics) according to the manufacturer’s directions in cells that were either treated with PHA665752 or the solvent DMSO.

Cell Cycle Analysis. Fixed cells were stained with propidium iodide and cell cycle variables analyzed by fluorescence-activated cell sorting analysis.

RESULTS

The Small Molecule c-MET Inhibitor PHA665752 Specifically Regulates Cell Growth in TPR-MET-Transformed BaF3 Cells. PHA665752 was identified as a prototype ATP-competitive small molecule inhibitor of the catalytic kinase activity of the c-MET RTK (22). We initially sought to determine if PHA665752 could inhibit cell growth in TPR-MET-transformed BaF3 cells (Fig. 1A). Treatment of BaF3.TPR-MET cells with PHA665752 was found to inhibit cell growth in a dose-dependent manner with an IC_{50} < 0.06 μmol/L. To further determine if the growth inhibitory effect of PHA665752 on BaF3.TPR-MET cells is maintained over time, cell growth was determined over a 72-hour culture. In the presence of IL-3, PHA665752 had only little effect on cell growth of TPR-MET-transformed cells or BCR-ABL-transformed cells (Fig. 1B, top). In contrast, PHA665752 completely blocked cell growth in the absence of IL-3 in BaF3.TPR-MET and even reduced the number of viable cells (Fig. 1B, bottom). This suggests that IL-3 partially rescues the BaF3.TPR-MET cells from PHA665752-dependent growth inhibition. We did not observe a significant growth inhibitory effect of PHA665752 at 0.2 μmol/L in IL-3 stimulated parental BaF3 cells in a 72-hour culture (data not
shown). TPR-MET is therefore implicated in the deregulation of pathways that are independent of or opposed by growth pathways regulated by the IL-3 receptor, similar to the relation between the ABL inhibitor STI-571 and the BCR-ABL oncoprotein in IL-3-treated BaF3 cells. PHA665752 (0.2 μmol/L, 18 hours) also did not inhibit cell growth of BaF3 cells transformed by other oncogenic tyrosine kinases, including BCR-ABL, TEL-JAK2, TEL-ABL, and TEL-PDGFBR (Fig. 1C).

Untransformed BaF3 cells do not migrate through a transwell membrane. However, cells transformed by TPR-MET display spontaneous transwell migration consistent with enhanced cell motility. Migration of BaF3.TPR-MET cells was inhibited with 0.2 μmol/L PHA665752 (92.5 ± 3% inhibition of the cell migration) compared with DMSO-treated cells indicating the ability to inhibit this phenotype of malignant transformation. This shows that the TPR-MET kinase activity regulates cell growth, motility, and migration of the transformed BaF3 cells.

**Inhibition of c-MET Kinase Activity by PHA665752 Induces Apoptosis and Cell Cycle Arrest in TPR-MET-Transformed BaF3 Cells.** Apoptosis is a complex cellular function that is regulated in part through the c-MET tyrosine kinase activity in TPR-MET-transformed cells and inhibition of c-MET kinase is therefore expected to induce an increase in apoptosis. We measured the change in Annexin V positive

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Fig. 1  PHA665752 inhibits cells growth of TPR-MET-transformed BaF3 cells. BaF3 cells lines transformed by tyrosine kinase oncogenes were used to determine cell growth (A–C) or transwell migration (D) in response to the small molecule c-MET kinase inhibitor PHA665752. A, relative growth of BaF3 cells transformed by TPR-MET in response to different concentrations of PHA665752 was determined after 18 hours (n = 3). B, TPR-MET-transformed BaF3 cells were either left untreated (○) or treated (▲) with PHA665752 (1 μmol/L) for the indicated time in the presence or absence of IL-3 (n = 3). C, BaF3 cells transformed by tyrosine kinase oncogenes were treated for 18 hours with PHA665752 (1 μmol/L; n = 3). D, cells were treated for 18 hours with the indicated dose of PHA665752 and the spontaneous transwell migration relative to DMSO-treated cells determined (n = 4).
staining of cells, an indication for increased exposure of phosphatidylserine to the outer cell membrane during apoptosis. Using TPR-MET-transformed BaF3 cells, we found that treatment with PHA665752 (0.2 μmol/L, 18 hours) led to an increase in Annexin V–positive cells compared with DMSO-treated cells (Fig. 2A, top left). In the control cells, 5% of the total population showed signs of apoptosis; however, the number of apoptotic cells increased to 33.1% after PHA665752 treatment. On average, 13.9 ± 1.0% of the cells were in early apoptosis (Annexin V positive) and 19.2 ± 1.8% of the cells were in late apoptosis (Annexin V plus propidium iodide positive). To confirm this observation, we thereafter measured the activation status of caspase-3, a downstream effector of the proapoptotic caspase-9. Similar to the previous data, we observed a consistent increase in caspase-3 activity (3.5 ± 0.7-fold increase; n = 3; P < 0.03) compared with DMSO-treated cells (Fig. 2B).

We also determined if inhibition of the TPR-MET tyrosine kinase would induce cell cycle arrest. Cells were treated with DMSO or increasing concentrations of the c-MET kinase inhibitor and the different phases of cell cycle distribution were then determined by propidium iodide staining and fluorescence-activated cell sorting (Fig. 2C). The percentage of cells in G1 phase increased from 42.4% to 77.0% in PHA665752-treated cells (0.2 μmol/L), whereas the percentage of cells in S phase (reduced from 45.4% to 17.5%)

Fig. 2 PHA665752 induces apoptosis and cell cycle arrest in TPR-MET-transformed BaF3 cells. TPR-MET-transformed BaF3 cells were treated for 18 hours with either DMSO or the indicated amount of PHA665752 (n = 3). A, Annexin V and propidium iodide staining was determined by flow cytometry. B, activity of caspase-3 was determined in cell lysate (n = 3). C, % cells in different cell cycle phases was determined by flow cytometry after propidium iodide staining (n = 3).
PHA665752 Inhibits Tyrosine Phosphorylation of Cellular Proteins in TPR-MET-Transformed BaF3 Cells. To determine the biochemical consequences of c-MET kinase inhibition by PHA665752 in BaF3.TPR-MET cells, changes in tyrosine phosphorylation of cellular proteins were evaluated. The tyrosine phosphorylation sites in TPR-MET with the corresponding sites in the tyrosine kinase domain of c-MET are described in Materials and Methods. The extracellular and juxtamembrane domains of c-MET are deleted as a result of the chromosomal translocation resulting in the TPR-MET fusion oncprotein. Treatment of BaF3.TPR-MET cells with PHA665752 reduced tyrosine phosphorylation of cellular proteins in a dose-dependent manner (Fig. 3B) but did not alter tyrosine phosphorylation of cellular proteins in BCR-ABL-transformed BaF3 cells (data not shown). These data are consistent with the dose-dependent reduction of cell growth in the present studies and suggest that PHA665752 specifically inhibits TPR-MET-induced tyrosine phosphorylation but not BCR-ABL. In addition, using phosphospecific antibodies against tyrosine phosphorylation sites in c-MET, we found that PHA665752 inhibits autophosphorylation in the catalytic tyrosine kinase domain at Tyr361/365/366 (autophosphorylation site), Tyr360 (growth factor receptor binding protein 2 binding site), and Tyr366 (important in cell morphogenesis; Fig. 3B).

In addition, we sought to determine if inhibition of TPR-MET would reduce the phosphorylation and alter the activation status of pathways that are involved in cell growth and proliferation. We found that the dose-dependent reduction in tyrosine phosphorylation of cellular proteins after PHA665752 treatment correlated with reduced serine phosphorylation of AKT[Ser473] as well as the reduced phosphorylation of the mTOR substrate p70-S6K[Thr389/Ser424] (Fig. 3C). This would suggest that inhibition of c-MET kinase activity leads to reduced activation of the PI3K/AKT/mTOR pathway in these transformed cells.

PHA665752 Cooperates with Rapamycin to Inhibit Cell Growth in TPR-MET-Transformed BaF3 Cells through Mammalian Target of Rapamycin-Dependent Pathway. Finally, we determined the significance of mTOR regulation by c-MET in the cells with the specific mTOR inhibitor rapamycin. In the absence of PHA665752, rapamycin reduced cell growth of the BaF3.TPR-MET cells in a dose-dependent manner. In the presence of PHA665752 (0.05 μmol/L), rapamycin cooperated with the c-MET inhibitor in inhibiting cell growth of the TPR-MET-transformed cells (Fig. 4).

PHA665752 can also Cooperate with Rapamycin in Inhibiting Non-Small Cell Lung Cancer H441 Cells. The significance of the regulation of mTOR pathway by c-MET and its potential for therapeutic targeting in NSCLC was assessed. PHA665752 was active against NSCLC H441 cells in vitro (22). H441 cells were recently identified as sensitive to PHA665752 inhibition in soft agar colony growth, cell proliferation, and cell migration, abrogating signaling through extracellular signal-regulated kinase 1/2, AKT, and FAK (22). Here, we show that PHA665752 (0.5 μmol/L) can cooperate with rapamycin in inhibiting cell viability of H441 cells (Fig. 5), similar to that seen with BaF3.TPR-MET cells.

DISCUSSION

c-MET RTK has been convincingly shown in many human cancers to be involved in oncogenesis, tumor progression with enhanced cell motility and invasion, and metastasis (1, 23). c-MET can be activated through overexpression or mutations in various human cancers, including SCLC (4) and NSCLC.4 We have characterized here a novel small molecule–selective c-MET inhibitor, PHA665752, that inhibited cell growth, motility and migration of BaF3 cells transformed with TPR-MET. We had previously shown that the BaF3 cell line

4 Unpublished data.
system can serve as an excellent model for studying transforming tyrosine kinase oncogenes (21). The specificity of PHA665752 was further shown by its inability to inhibit growth mediated by transforming forms of the ABL, JAK2, or PDGFB receptor. Inhibition of c-MET kinase activity by the small molecule drug induced apoptosis and cell cycle arrest in TPR-MET-transformed BaF3 cells. We also found that the dose-dependent reduction in tyrosine phosphorylation of cellular proteins after PHA665752 treatment correlated with reduced activation of the PI3K/AKT/mTOR pathway in these cells. The specific mTOR inhibitor rapamycin was found able to cooperate with the c-MET inhibitor PHA665752 in the reduction of cell growth of BaF3.TPR-MET and H441 NSCLC cells. PHA665752 serves as an example of specific small molecule c-MET inhibitor.

Recently, we have reported the effects of the c-MET inhibitor SU11274 on the TPR-MET-transformed BaF3 cell system and showed that this constitutively activated oncogenic c-MET allows the identification and characterization of small molecule–selective c-MET inhibitors (21). Unlike Gleevec for chronic myelogenous leukemia, (targeting BCR/ABL) and gastrointestinal stromal tumor (targeting c-KIT), targeted small molecule inhibitors against c-MET have not yet come to clinical fruition. In our previous study with BaF3.TPR-MET cells, SU11274 inhibited the IL-3-independent cell growth in a dose-dependent manner with an IC50 of <3 μmol/L (21), compared with studies in this report with PHA665752 that lead to an IC50 of <0.06 μmol/L. Evidently, in the current results, PHA665752 is more potent than SU11274. Similar to our current findings with PHA665752 in this study, we also found dramatic inhibitory effects on cell motility and migration of BaF3.TPR-MET cells when treated with SU11274. However, the effects were observed at a higher concentration of SU11274 (44.8% inhibition at 1 μmol/L and 80% inhibition at 5 μmol/L) when compared with that observed here with PHA665752 (>90% inhibition at 0.2 μmol/L). Cell motility and migration of a number of cell systems is tightly controlled by PI3K. In this study, we show that PI3K pathway is dramatically affected by c-MET inhibition.

Molecular targeted therapies against protein tyrosine kinases, such as BCR-ABL (non-RTK) in chronic myelogenous leukemia and epidermal growth factor receptor (RTK) in NSCLC, have gained much progress in recent years. However, resistance to tyrosine kinase inhibitors has also become an important emerging issue. The mechanisms of tyrosine kinase inhibitor resistance includes oncogene amplification and overexpression, as well as resistant mutations in the kinase oncoprotein, not limited to the kinase domain (24). Hence, attempts to combine additional inhibitory agents together with tyrosine kinase inhibitors in the quest for curative therapy, or at least a more durable response, have taken on new momentum (25). The PI3K/AKT/mTOR pathway is an attractive target for this combinational approach because it has been shown activated by c-MET signaling, among other oncogenic tyrosine kinases. Safe and effective inhibitors of PI3K or AKT have not been well established yet at this time, whereas inhibitors of mTOR such as rapamycin or its analogue CCI-779 are available and have shown antitumor activity (26). Rapamycin acts by binding to the immunophilin FK506-binding protein (FKBP12), with the resultant complex inhibiting the target of rapamycin (TOR).

We have recently shown that PI3K is an important signaling pathway downstream of c-MET (27). PI3K is responsible for diverse cellular regulation, including cell adhesion, motility and migration, proliferation, reduced apoptosis, anchorage independence, and intracellular vesicle trafficking/secretion (28, 29). AKT (30, 31) and FKHR (32, 33) are two downstream targets of PI3K, and phosphorylation of them leads to enhanced cell survival. Constitutive activation of PI3K signaling pathway has been reported in SCLC, mediating anchorage-independent proliferation via protein kinase B/AKT and p70-S6K-dependent pathway (34). Upon activation, c-MET can recruit and associate with PI3K, which eventually lead to downstream pathway

![Fig. 4 PHA665752 cooperates with rapamycin in regulating growth through mTOR-dependent pathway. The relative growth of BaF3 cells transformed by TPR-MET in response to different concentrations of rapamycin (0.01-10 nmol/L) was determined in the presence (▲) or absence (■) of PHA665752 (0.05 μmol/L) after a 3-day culture (n = 3). Bars, SE.](image)

![Fig. 5 PHA665752 cooperates with rapamycin in inhibiting growth of NSCLC H441 cells through mTOR-dependent pathway. The cell growth of NSCLC H441 cells, in culture medium containing 2% FCS and HGF (40 ng/mL), in response to different concentrations of rapamycin (0.01-20 nmol/L) was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in triplicates in the presence (▲) or absence (■) of PHA665752 (0.5 μmol/L) after a 3-day culture (n = 3). Bars, SE.](image)
activation of AKT (35). AKT in turn regulates cell survival by inhibiting caspase-9, Bad, and the forkhead (FKHR) transcription factors. Here, we show that the c-MET inhibitor PHA665752 treatment of BaF3.TPR-MET cells leads to decreased phosphorylation of AKT at Ser473 (phosphorylated by the kinase PDK2), thereby leading to apoptosis. It has been shown that c-MET/hepatocyte growth factor activation protects against cell death via PI3K- and AKT-dependent pathway in human glioblastoma cells treated with cytotoxic agents (36). It would therefore be useful to determine whether there is any cooperation or synergism between PHA665752 and PI3K inhibition.

mTOR is an important signaling intermediate molecule downstream of the PI3K/AKT pathway that inhibits apoptosis and is important in nutritional status checkpoint (37–40). mTOR is a large (Mr ~289,000) multidomain serine/threonine kinase and is a member of the PI3K family of protein kinases based on homology within its catalytic domain. Although signals that activate mTOR have not been well understood, AKT phosphorylation and protein interactions via the mTOR NH2-terminal multiple repeat HEAT motifs are possible mechanisms. The p70-S6K and the translation inhibitor 4E-BP-1 are the two best-characterized mTOR substrates. Growth factor activation of the PI3K pathway results in phosphorylation and activation of p70-S6K by mTOR or PDK-1. Rapamycin, initially approved by the Food and Drug Administration in 1999 as an immunosuppressant for prevention of allograft rejection, has been shown to have selective antitumor activity in a broad range of human cancers in vitro and in vivo with mutations in PTEN or up-regulation of the PI3K/AKT pathway (41).

In this report, we show that the c-MET inhibitor PHA665752 inhibited c-MET/hepatocyte growth factor pathway–mediated tyrosine phosphorylation of cellular proteins. In addition, there was also a dose-dependent inhibition of the serine phosphorylation of AKT[Ser473] as well as the phosphorylation of the mTOR substrate p70-S6K[Thr421/Ser424]. This provides an opportunity for testing the hypothesis that the hepatocyte growth factor/c-MET-PI3K/AKT/mTOR signaling axis can be modulated and inhibited with a combination of both the specific c-MET inhibitor (PHA665752) and also the downstream specific inhibitor of mTOR (rapamycin). Attempts to combine rapamycin and tyrosine kinase inhibitors to improve the treatment of primary/relapsed chronic myelogenous leukemia and/or acute myelogenous leukemia caused by FLT3 mutations have shown some promise in preclinical models (25). Here, we show that this combinational strategy is functional in vitro with the rapamycin cooperating with the c-MET inhibitor in reducing the cell viability of the BaF3.TPR-MET cells as well as NSCLC H441 cells. The c-MET inhibitor PHA665752 may exert its effects downstream of c-MET by modulating the AKT/mTOR pathway. In addition, cooperative effects of PHA665752 and rapamycin seen here may be a result of inhibition of complementary pathways downstream of c-MET that are independent of mTOR. It would now be useful to further test this strategy in an in vivo mouse model. Current data suggest that PHA665752 does have potent in vivo cytoadhesive antitumor activity shown in a gastric carcinoma xenograft model (22). Rapamycin or specific drugs similar to it against mTOR pathway may be attractive therapeutic agents to be used in combination therapy with c-MET inhibition by prototype c-MET inhibitors in c-MET expressing cancers.

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