Enhancement of the Therapeutic Efficacy of Taxol by the Mitogen-Activated Protein Kinase Kinase Inhibitor CI-1040 in Nude Mice Bearing Human Heterotransplants

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Abstract
Taxol may contribute to intrinsic chemoresistance by activating the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) cytoprotective pathway in human cancer cell lines and tumors. We have previously shown additivity between Taxol and the MEK inhibitor, U0126 in human cancer cell lines. Here, the combination of Taxol with an orally bioavailable MEK inhibitor, CI-1040, was evaluated in human lung tumors heterotransplanted into nude mice. Unlike xenograft models that are derived from cells with multiple genetic alterations due to prolonged passage, heterotransplanted tumor models are more clinically relevant. Combined treatment with both drugs resulted in inhibition of tumor growth in all models and tumor regressions in three of four models tested, supporting our previous observation that Taxol’s efficacy is potentiated by MEK inhibition. Concurrent administration was superior to intermittent dosing. Pharmacodynamic assessments of tumors indicated that suppression of MEK was associated with induction of S473 phosphorylated Akt and reduced proliferation in the combination groups relative to single agents, in contrast to complete abrogation of the MEK/ERK pathway in the combination groups relative to single agents, in contrast to complete abrogation of the MEK/ERK pathway.

Introduction

Aberrant signaling is a hallmark of tumorigenesis and there has been an explosion in the search for molecular targets that are amenable for pharmacologic intervention (1). It is anticipated that optimal use of molecular targeted therapies will be in combination therapy, whether with classic cytotoxic drugs, or with other targeted therapies. Cytotoxic or molecular targeted therapies may inappropriately induce the expression of cytoprotective signaling cascades that can mediate resistance. The use of a novel combination to circumvent this possibility is explored herein, and this strategy may be effective in immobilizing the survival and proliferative capacity of tumors.

The mitogen-activated protein kinase (MAPK) signaling family transduces signals that regulate embryogenesis, proliferation, homeostasis, and cell death (2), primarily via phosphorylation. Three major MAPK pathways have been identified in mammalian cells. Constitutive activation of the extracellular signal-regulated kinase (ERK) pathway results from defects in multiple oncogenic pathways including the epidermal growth factor receptor family (EGFR and HER-2/neu), vascular endothelial growth factor (VEGF), platelet derived growth factor, and mutated Ras (3) or Raf (4). Hence, inhibition of the MAPK kinase (MEK)/ERK pathway is a validated target in cancer treatment. MEKs (MEK1 and MEK2) are attractive chemotherapeutic targets because of their unusually restrictive substrate specificity (5). Signal amplification of the ERK cascade occurs at the Raf-MEK stage (6) and aberrant MEK/ERK activity has been observed in a number of primary human tumors (7, 8). The development of MEK inhibitors such as CI-1040, an orally active and highly potent inhibitor that has completed phase I/II clinical evaluation, has progressed rapidly (9, 10). CI-1040 and its analogues have a unique inhibitor-binding pocket adjacent to the MgATP-binding site of MEK1 and MEK2 (11).

The role of MEK/ERK in the control of the G1 phase of the cell cycle has been well documented (12); however, MAPKs are also required for mitosis and meiosis (4, 13–16). Activated ERKs localize at kinetochores, asters, and the midbody during mitosis (17), presumably to facilitate interaction with proximal substrates that are involved in mitotic spindle function. It has also been shown that some 40% of ERKs colocalize to microtubules (18) and that ERK2 specifically interacts with the actin-microtubule cytoskeleton (19). In view of the association between microtubules and MEK/ERKs, one would predict that perturbation of microtubules by Taxol alters the expression of proteins that are associated with microtubules. We have previously shown that Taxol activates the MEK/ERK pathway (20) and that the efficacy of Taxol is potentiated by MEK inhibition. Similar findings have been reported by other groups (21, 22).

This report describes enhanced activity of Taxol by CI-1040 in vivo using human heterotransplanted non–small cell lung cancer (NSCLC) tumors grown in nude mice (23). This potentiation of Taxol activity was associated with altered expression of specific proteins, including proliferating cell nuclear antigen (PCNA), VEGF, and S473 phosphorylated Akt (protein kinase B). These in vivo studies support our previous findings and show activity of this combination in a number of diverse NSCLC tumor models with differential intrinsic sensitivity to Taxol.

Materials and Methods

Animals and tumor implantations. The human NSCLC cell line, A549 was maintained as previously described (20). Either 4 × 106 A549 NSCLC cells (xenograft), or minced fragments of NSCLC tumors from...
patients (heterotransplants) were injected s.c. into the right axillary region of 6-week-old athymic (nu/nu) female mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN). Tumors were collected from consenting patients under institutional review board approval. Tumors were allowed to grow until a volume of ~100 mm³ and mice were randomized into balanced groups.

Clinical grade Taxol was given by i.p. injection in five separate doses 3 days (q3dX5). CI-1040 (PD184352) was obtained from Pfizer (Ann Arbor, MI) and was given orally (p.o.) three times a day (TID), as a suspension in 0.5% hydroxypropylmethylcellulose/0.2% Tween 80 (see Supplementary Fig. S1 for determination of optimal dosing). Maintenance dosing of CI-1040 twice daily (BID), including weekends, was done in mice receiving CI-1040 alone and in combination groups from day 16 after drug treatment initiation (when Taxol administration was complete) until the termination of each experiment. End points (body weight, tumor volume, and toxicities) were measured weekly for the duration of experiments, which were done with Animal Institute Committee approval and in compliance with policies for ethical animal use and federal, state and local regulations. Tumor volumes were calculated from the formula \( V = \frac{1}{2} \times l \times w^2 \), where \( l \) and \( w \) are length and width of the tumor, respectively. Antitumor activity was expressed as relative tumor volume, \( \frac{T}{T_0} \times 100 \), where \( T \) is the mean tumor volume at a particular time and \( T_0 \) is the mean tumor volume at day 0. Statistical significance was evaluated using either a one-tailed Student's \( t \) test, or a repeated measure one-way ANOVA test with Newman-Keuls multiple comparison post test.

Matrigel plug analysis and Drabkins assay. Nude mice were anesthetized and injected s.c. in the lower abdomen with 0.5 mL of matrigel basement membrane matrix (BD Biosciences, San Jose, CA) supplemented with 1 ng/mL bovine fibroblast growth factor (FGF). Groups of mice (n = 4) were randomized and treated 24 hours later with drugs. Animals were euthanized at day 14 after dosing and matrigel plugs excised and processed as previously described (24).

Immunohistochemistry. H&E-stained, and immunoperoxidase antigen localization were performed on paraffin-embedded sections. Sections were incubated in primary antibody to phospho-p44/42 MAPK (Thr202/Tyr204), Phospho-AKT (Ser473), Phospho-Erk5 (Thr218/Tyr220), ERK5 (all from Trevigen, Gaithersburg, MD); and glyceraldehyde 3-phosphate dehydrogenase, G3PDH (Cell Signaling Technologies, Beverly, MA); PCNA (NeoMarkers, Vermont, USA). Immunostaining was visualized with 3,3′-diaminobenzidine and counterstained in hematoxylin. The mitotic index (the mean number of mitotic figures per 10 high power fields) was determined from H&E-stained sections at 40× magnification.

Results

Concurrent administration of CI-1040 potentiates the efficacy of Taxol in an A549 xenograft model of non–small cell lung cancer. A549 NSCLC xenografts were chosen for optimization of dosing and schedules in vivo because (i) this cell line was used in our in vitro studies (20) and (ii) A549 cells proliferate rapidly in vitro; therefore, dose optimization potentially be applicable to other tumor models with comparable or reduced proliferation. Eight animals per treatment group were used. Control animals received normal saline i.p. and 0.5 mL hydroxypropylmethylcellulose/0.2% Tween 80 p.o. CI-1040 was given at 150 mg/kg TID (Supplementary Fig. S1). Initially, two different doses of Taxol, either 20 mg/kg q3dX5, or 50 mg/kg qweek ×2 i.p., for a total dose of 100 mg/kg, were evaluated (Fig. 1A). The dose of Taxol was determined from previous studies (25). Justification for weekly dosing was from ongoing clinical trials of weekly Taxol in a number of disease types, including NSCLC (26). Concurrent dosing of Taxol with CI-1040 was evaluated initially, because our in vitro studies determined this to be the most effective (20). Tumor volumes in mice that received either CI-1040 or Taxol, at either dose, were significantly smaller than tumor volumes in control mice (Fig. 1A; P < 0.05, ANOVA). None of the single agent treatments resulted in tumor regression, although all inhibited tumor growth to varying degrees. Mice that received the combination of CI-1040 with either schedule of Taxol had tumor regressions and tumor volumes were significantly smaller than those treated with single agents (P = 0.027, ANOVA). Combination group A (concurrent combination of CI-1040 and 20 mg/kg q3dX5 Taxol) was superior to combination B (concurrent combination of CI-1040 and 50 mg/kg qweek Taxol), in terms of the duration of tumor regression (time for actual mean tumor volumes to reach 300 mm³ was ~23 days for combination B versus 29 days for combination A). Combination A was associated with transient gastrointestinal toxicities (diarrhea with hematochezia) in two of eight mice that resolved upon completion of Taxol dosing. Weight
loss data are available in Supplementary Fig. S2. Animals from the experiment depicted in Fig. 1A were sacrificed at day 8 post-treatment initiation and the xenografts analyzed by immunoblotting (Fig. 1B). Treatment with CI-1040 alone, or in combination with Taxol, suppressed phosphoERK relative to untreated tumors. Consistent with previous data (20), we observed a slight activation of phosphoERK in the lysates of Taxol-treated xenografts.

The effect of schedule on antitumor efficacy and toxicity was assessed by intermittent dosing (defined as stopping and starting CI-1040 at intervals), illustrated in Fig. 1C. Two dosing strategies were evaluated. In the first, Taxol was given on days 1, 4, 7, 10, and 13 and CI-1040 was given 24 hours later on days 2, 5, 8, 11, and 14. Neither drug was given on day 3, 6, 9, or 12 and maintenance dosing of CI-1040 was initiated on day 16. Compared with concurrent administration that resulted in sustained tumor regressions up to days 22 to 23 post-treatment initiation, this schedule was less effective \((P = 0.04, t \text{ test})\) and tumor regressions were not observed. In the second dosing strategy, both Taxol and CI-1040 were given on days 1, 4, 7, 10, and 13. This was even less effective in terms of antitumor efficacy and xenograft growth in this group was not statistically different from the growth of tumors treated with Taxol alone. No weight loss or gastrointestinal toxicities were observed with either sequencing regimen. The time for actual mean tumor volumes to reach 300 mm\(^3\) was \(\sim 27\) days for the concurrent combination versus 22 days for either intermittent dosing regimen. Sequential administrations were also evaluated, whereby each drug was given after one another (data not shown); for example, CI-1040 was given 13 days post-treatment initiation when Taxol dosing was complete. However, no enhancement of antitumor efficacy relative to single agents was observed. Therefore, the most effective enhancement of Taxol’s cytotoxic potency was obtained via continual suppression of MEK through concurrent administration of both drugs, which was used in all subsequent experiments. Any form of intermittent dosing was associated with reduced antitumor efficacy.

**CI-1040 potentiates the efficacy of Taxol in heterotransplanted models of lung cancer.** The concurrent combination of Taxol with CI-1040 that was optimized in A549 xenografts was evaluated in heterotransplanted tumor models (Fig. 2). These included two adenocarcinomas (HTL-13 and HTL-25) derived from patients with stage II and stage I metastatic disease, respectively. A squamous tumor model (HTL-34) that was derived from a patient with stage I disease was also evaluated. Tumors that were grown for three passages in nude mice were used in all experiments. Standard H&E staining of archived tissue from heterotransplants grown in mice at each passage was done and these analyses confirmed retention of the original tumor histology (data not shown). Approximately four to six animals per treatment group were used. All experiments used concurrent dosing of the combination (150 mg/kg CI-1040 TID with 20 mg/kg Taxol q3dX5) and BID maintenance dosing of CI-1040 was done from day 16, until the termination of each experiment.

The adenocarcinoma tumor model, HTL-13, was resistant to treatment with either Taxol or CI-1040 as single agents (Fig. 2A). Despite this, the concurrent combination decreased the proliferation rate of tumors \((P < 0.05, \text{ANOVA})\) relative to single agents (time for actual mean tumor volumes to reach 300 mm\(^3\) was \(\sim 7\) days for single agents versus \(\sim 15\) days for the combination). No tumor regressions were observed. The combination treatment was associated with \(\sim 5\%\) weight loss and transient diarrhea and hematochezia in one of six mice.

HTL-34 was a chemosensitive tumor model (Fig. 2B), in that treatment with single agents induced statistically significant suppression of tumor growth compared with untreated control tumors \((P < 0.01, \text{ANOVA})\). The concurrent combination of Taxol and CI-1040 induced tumor regressions that were significantly different \((P < 0.01, \text{ANOVA})\) from single agents (time to reach actual mean tumor volumes of 300 mm\(^3\) was \(\sim 44\) days for the concurrent combination, versus 10 days for Taxol and 13 days for CI-1040). The combination treatment was associated with \(\sim 5\%\) to \(8\%\) weight loss and transient diarrhea in two of six mice.

HTL-25 was a slowly proliferating, chemosensitive tumor model and the duration of this experiment exceeded 120 days (Fig. 2C). Similar to HTL-34, treatment with either Taxol or CI-1040 induced significant reductions in tumor growth relative to untreated control tumors \((P < 0.05, \text{ANOVA})\). The concurrent combination of Taxol and CI-1040 induced tumor regressions that were significant at defined times only (at day \(\sim 20\) and again after day 60; \(P = 0.05, t\text{-test}\)) relative to single agents. This is likely due to the fact that Taxol and CI-1040 alone induced regressions of \(\sim 2\%\) and \(7\%\), respectively, of the initial tumor volumes. The time to reach actual mean tumor volumes of 300 mm\(^3\) was \(\sim 48\) days for control tumors versus 88 days for Taxol,
100 days for CI-1040, and 130 days for the concurrent combination. The combination treatment was associated with ~5% weight loss and transient diarrhea in one of six mice.

Analysis of drug-treated tumors by immunoblotting. Animals from each treatment group were euthanized and tumors were excised and analyzed by immunoblotting. Representative data for HTL-13 are shown in Fig. 3A. Phospho-ERK was suppressed in tumors treated with CI-1040 alone and in combination, and there was no increase in the expression of phospho-ERK in Taxol-treated tumor cells. Consistent with the decreased proliferation of tumors undergoing concurrent treatment (Fig. 2A), tumor cells had decreased expression of the proliferation specific antigen, PCNA, relative to single agents. Decreased expression of vascular endothelial growth factor, VEGF, relative to single agent treatment was also observed. A recent report has shown suppression of VEGF by CI-1040 (27), although both CI-1040 and Taxol as single agents increased VEGF in HTL-13. Furthermore, CI-1040 treatment whether alone, or in combination, increased expression of S473 phosphorylated Akt, which together with phosphorylation of T308, is associated with constitutively active Akt (28). Reciprocal signaling between the MEK/ERK and PI3K/Akt pathways (29–31) has been reported. The combined treatment of Taxol with U0126 has been reported to suppress Akt kinase activity and this may contribute to the cell death–inducing potency of this combination (32). Although these findings are not supported for HTL-13, it should be noted that tumor regressions also were not observed.

Representative data from HTL-34 (Fig. 3B) showed suppression of phospho-ERK, relative to the untreated control tumors in all groups, as well as decreased expression of PCNA. VEGF expression was dramatically suppressed in CI-1040 and Taxol-treated tumors and also decreased with concurrent treatment. CI-1040 alone increased the expression of S473 phosphorylated Akt, whereas the concurrent combination, or Taxol alone had levels of expression comparable to control tumors.

Representative data from HTL-25 indicated suppression of phospho-ERK by CI-1040 and the combination treatments (Fig. 3C). PCNA expression did not substantially change during the course of treatment with either single agents or the combination, presumably due to the slow proliferation rate of this tumor. VEGF expression was suppressed in both CI-1040- and Taxol-treated tumor cells and was dramatically suppressed by the concurrent combination treatment (Fig. 3C). Similar to the data presented in Fig. 3B, S473 phosphorylated Akt expression increased in CI-1040 and in combination treated tumors (Fig. 3C).

Immunohistochemical analysis of phospho–extracellular signal-regulated kinase expression in heterotransplants. As a complement to the immunoblot analysis of tumors, immunohistochemical staining of fixed tumors was done (Fig. 4). Staining localized to both nuclear and cytoplasmic regions, with intense immunoreactivity in nuclei. Infiltrating mouse stromal tissue was unreactive. HTL-13 (A–D) showed primarily strong nuclear staining. Consistent with Fig. 3A, CI-1040 treatment resulted in decreased staining. Although the expression of pERK decreased overall in the combination treatment, there were a number of strongly immunoreactive multinucleated cells (D). Taxol-induced activation of phospho-ERK was evident in HTL-34 and A549 tumor models (F and N), whereas suppression of phospho-ERK by CI-1040 was apparent in HTL-13, HTL-25, and A549 tumor models (C, K, and O). Interestingly, the only tumor model to exhibit conclusive diminished phospho-ERK immunoreactivity with the combination treatment was HTL-25 (I). Both HTL-34 and A549 retained strong phospho-ERK staining with the combination treatment (H and P) with intense staining of multinucleated, or aberrant cells, similar to what was observed in HTL-13.

Decreased proliferation by the combination treatment was confirmed from the mean mitotic indices determined from the quantitation of mitotic cells in H&E-stained tumor sections. All tumors models treated with the concurrent combination had a reduction in mitotic cells consistent with the decreased expression of PCNA presented in Fig. 3, although the effects of drug treatments were minimal in HTL-23. Ki-67 staining was also decreased by the combination treatments, consistent with decreased mitotic index. Cell death, as determined by terminal deoxynucleotidyl transferase–mediated nick end labeling staining, was increased (Supplementary Fig. S3).

Inhibition of fibroblast growth factor–stimulated angiogenesis by CI-1040 and Taxol. The matrigel plug assay was used to determine the angiogenic response to treatment. The Drabkins method provides an index of blood containing vessels by quantifying the hemoglobin content of the matrigel. There was little angiogenesis in control plugs that had not been supplemented with FGF (data not shown). FGF-supplemented plugs had a moderate angiogenic response that was not significantly inhibited by treatment with Taxol alone but was significantly inhibited by CI-1040 (P = 0.02, t test; Fig. 5). Those matrigels treated with the concurrent combination of Taxol and CI-1040 showed suppression of angiogenesis that was significantly different from single agents, CI-1040 (P = 0.05, t test) and Taxol (P = 0.01, t test).

Discussion

Here we describe significant potentiation of Taxol-induced efficacy by the MEK inhibitor, CI-1040, that is optimal with concurrent dosing in human heterotransplanted NSCLC tumor...
models. These tumor models are more clinically relevant compared with cell lines that are grown as xenografts. They recapitulate the distinct pharmacogenic profiles and differential chemotherapeutic responses of patients as well as histology, chromosome complement, antigen expression, and gene expression. It previously has been shown that human tumor heterotransplant models have patient response prediction rates of 90% and 97% for chemosensitivity and chemoresistance, respectively (33). The studies described here used s.c. "early-stage" tumors, in that treatments were initiated when tumors were palpable (around 100 mm³), which mimics adjuvant chemotherapy of patients with early stage or resected disease. In phase I, CI-1040 was well tolerated BID or TID, with stable disease in 25% of patients (9). Phase II studies showed excellent tolerability and minimal single agent activity, which was attributed to insufficient drug exposure (10), possibly due to metabolic degradation (34). Another MEK inhibitor, PD0325901, has exquisite specificity for MEK1 and an improved pharmaceutical profile culminating in enhanced solubility and bioavailability (10). The chemotherapeutic merit of MEK inhibitors will be explored by the ongoing clinical trials of PD0325901, although the true potential of these drugs may be in combination chemotherapy.

Each heterotransplanted tumor model described here had a differential response to either Taxol or CI-1040 as single agents, but all tumors showed potentiation of Taxol's antitumor efficacy by CI-1040. The antitumor efficacy of combined Taxol and MEK inhibition was superior in A549, HTL-34, and HTL-25 models where tumor regressions were obtained. Two of the six HTL-34 mice in the combination group had sustained regressions 10 months after dosing of CI-1040 and Taxol had terminated. In the chemoresistant tumor model, HTL-13, treatment with CI-1040 or Taxol alone was ineffective, yet the combination treatment had increased efficacy, relative to either single agent that was associated with suppressed proliferation. This suggests a broad therapeutic application for this drug combination in NSCLC. One of the potential mechanisms for the interaction between Taxol and MEK inhibition is inadvertent induction of MEK/ERK signaling by Taxol treatment, due to the accumulation of cells in the G2-M phase of the cell cycle. This activation increases the abundance of MEK, the target of CI-1040, leading to enhanced efficacy of the combination. In addition, it may increase the rate of mitotic catastrophe, due to the absolute requirement of ERK/MEK for the successful execution of mitosis. Our previous study (20) suggested that the nature of the interaction between Taxol and MEK inhibition in cancer cells may be dependent on the level of constitutive ERK activation and the degree to which cells undergo Taxol-induced ERK activation. Interestingly, those tumor models that showed Taxol-induced ERK activation by immunohistochemistry (HTL-34, HTL-25, and A549) also showed the most promising antitumor response, although the significance of this should not be overstated, taken the small sample number evaluated.

Numerous Taxol and CI-1040 sequencing regimens were analyzed in this study due to the attention surrounding the INTACT trials (35, 36). Despite 5% to 10% response rates for the EGFR tyrosine kinase inhibitor, gefitinib, in discrete populations of NSCLC, there was no benefit to adding it to existing taxane or platinum containing chemotherapy (37). The failure of gefitinib in combination therapy may have been due to inappropriate scheduling, or perturbations of the cell cycle that impeded the efficacy of cytotoxic drugs (38). Ongoing combination trials of another EGFR inhibitor, erlotinib with carboplatin and paclitaxel (TRIBUTE) and gemcitabine and cisplatin (TALENT) reported no clinical benefit (39). Furthermore, a recent clinical trial showed no clinical benefit for the administration of Trastuzumab in combination with cisplatin and gemcitabine in NSCLC, although it has been suggested that this was due to the predominantly low to intermediate HER expression status of patients accrued (40). The foundations for these clinical trials were based on in vivo xenograft data and there are numerous reasons for the disparity between the two. The most obvious is that the schedule used in the animal studies was different from the schedule used in the clinical trial. In addition, preclinical drug combination studies are usually done between two agents, whereas these clinical trials used...
molecular-targeted therapies added to a chemotherapy doublet. Finally, patients enrolled on these studies were not preselected for overexpression of receptors or mutational status, the very factors that may be the most important determinants of response rates. The optimal administration of cytotoxic and molecular therapeutic strategies continues to be a subject of debate; however, we have determined concurrent administration to be most favorable for Taxol and CI-1040.

Phospho-ERK status was evaluated by immunoblotting and immunohistochemistry as a correlate of response to CI-1040. All of the four tumor models showed suppression of phospho-ERK by CI-1040 (where data was complementary by immunoblotting and immunohistochemistry for three of four models). The response to CI-1040 was somewhat related to the degree of constitutive expression of the ERK/MEK signaling cascade, although HTL-13 had moderate expression of this pathway yet had no therapeutic response to CI-1040, despite suppression of phospho-ERK. Thus, it is our contention that MEK/ERK overexpression alone is not a sufficiently accurate predictor of response to MEK inhibition therapies. The Akt signaling pathway has been shown to be deregulated in a number of cancer types and there is evidence to suggest that phospho-Akt overexpression in NSCLC confers a stage-independent survival disadvantage (41). Activated Akt promotes cell survival by inhibiting cell death via phosphorylation and inactivation of several targets (42, 43). Moreover, cross-talk has been shown between MAPK and PI3K at the level of Raf and Akt (29). This finding is supported by our data as all three heterotransplanted tumor models showed phosphorylation of AKT at S473 by CI-1040. Phosphorylation of Akt by MEK inhibitors have been noted elsewhere (44, 45). A recent study attributes resistance to trastuzumab, the humanized monoclonal antibody against ErbB2, to loss of PTEN function (46). It is plausible that a similar explanation for chemoresistance to MEK inhibitors exists. CI-1040 alone, and in combination with Taxol, suppressed FGF-mediated angiogenesis in vivo and decreased VEGF expression. It has been suggested that Erk5 may play an important role in controlling angiogenesis (47, 48). It is conceivable that the suppression of VEGF and FGF-induced angiogenesis are mediated via CI-1040-induced suppression of ERK5. CI-1040 and other commercially available MEK inhibitors are known to inhibit ERK5, albeit at 10-fold higher concentrations than that required for MEK1/MEK2 inhibition (49). We did not find any evidence to support altered expression of phospho-ERK5 in this study or in in vitro analyses of A549 cells (data not shown). Therefore, it is unlikely that the anti-angiogenic effects of CI-1040 are mediated via ERK5 suppression. Furthermore, it has been suggested that MEK inhibition also suppresses cyclooxygenase 1 and 2 (50). Although this may be the case for PD98059 (51), we found no evidence of altered cyclooxygenase 1 or 2 expression with CI-1040.

Activation of cytotoxic protective signaling in tumor cells, whether intrinsic or chemotheraphy induced, is a phenomenon that may be more prevalent than previously thought. Thus, acquired resistance to drugs, such as Taxol, may be due to acute activation of survival pathways such as MEK, during chemotherapy administration. We have shown impressive potentiation of Taxol's antitumor efficacy in vivo in human xenograft tumor models by coadministration with CI-1040, an orally bioavailable MEK inhibitor. Enhanced antitumor activity was associated with reduced proliferation and suppression of angiogenesis. We propose that this drug combination and the principle of suppressing cytoprotective signaling during chemotherapy administration will have broad therapeutic applications for lung cancer treatment.

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