**The Fibroblast Growth Factor Receptor Inhibitor PD173074 Blocks Small Cell Lung Cancer Growth In vitro and In vivo**

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**Abstract**

Lung cancer is the commonest cancer killer. Small cell lung cancer (SCLC) is initially chemosensitive, but rapidly relapses in a chemoresistant form with an overall survival of <5%. Consequently, novel therapies are urgently required and will likely arise from an improved understanding of the disease biology. Our previous work showed that fibroblast growth factor receptor (FGFR) inhibitor PD173074 blocks H-510 and H-69 SCLC proliferation and clonogenic growth in a dose-dependent fashion and prevents FGF-2–induced chemoresistance. Here, we show that the selective fibroblast growth factor receptor (FGFR) inhibitor PD173074 blocks H-510 and H-69 SCLC proliferation and clonogenic growth in a dose-dependent fashion and prevents FGF-2–induced chemoresistance. These effects correlate with the inhibition of both FGFR1 and FGFR2 transphosphorylation. We then determined the efficacy of daily oral administration of PD173074 for 28 days in two human SCLC models. In the H-510 xenograft, tumor growth was impaired similar to that seen with single-agent cisplatin administration, increasing median survival compared with control sham–treated animals. Crucially, the effect of cisplatin was significantly potentiated by coadministration of PD173074. More dramatically, in H-69 xenografts, PD173074 induced complete responses lasting >6 months in 50% of mice. These effects were not a consequence of disrupted tumor vasculature but instead correlated with increased apoptosis (caspase 3 and cytokeratin 18 cleavage) in excised tumors. Moreover, in vivo imaging with 3′-deoxy-3′-[18F]fluorothymidine–positron emission tomography ([18F]FLT-PET) showed decreased intratumoral proliferation in live animals treated with the compound at 7 to 14 days. Our results suggest that clinical trials of FGFR inhibitors should be undertaken in patients with SCLC and that [18F]FLT-PET imaging could provide early in vivo evidence of response. [Cancer Res 2009;69(22):8645–51]

**Introduction**

Small cell lung cancer (SCLC) accounts for 20% of all lung malignancies. Despite initial chemosensitivity, the disease recurs rapidly in a form usually resistant to further therapy and so overall survival is <5% at 3 years. Consequently, novel therapies are urgently required and these will most likely arise from an improved understanding of the disease biology. There is now considerable evidence implicating multiple growth factors that act in an autocrine or paracrine fashion to drive SCLC proliferation (1–5). In particular, our laboratory has established the role of basic-fibroblast growth factor (FGF-2) in this disease (6–9).

FGF-2 is a mitogen and a survival factor in many experimental models and is involved in neoangiogenesis in vivo (10–12). These effects are of potential relevance in cancer biology, and the inhibition of FGF-2 signaling may provide a valuable target to control tumor development. PD173074, a previously described ATP pocket inhibitor (13), is reported to show both high affinity and selectivity for the FGFR receptor (FGFR) family. However, its selectivity has only been examined against a limited number of kinases. When used in vivo, this compound was shown to inhibit FGF-driven neoangiogenesis while being exempt of general toxicity (13, 14). Therefore, PD173074 is perceived as a promising compound for in vivo inhibition of FGF signaling.

We have previously shown that FGF-2 induces SCLC cell proliferation and resistance to cytotoxic drugs (7, 9). Here, we show that both these effects are blocked by PD173074. This correlated with inhibition of FGFR1 and FGFR2 autophosphorylation and the subsequent phosphorylation of Erk1/2 in response to FGF-2. An extended in vitro kinase screen (78 kinases) confirmed selectivity of the inhibitor. Crucially, PD173074 inhibited tumor growth in two different SCLC xenograft models (H510 and H69), with 50% disease-free survival at 1 year in the case of H69 and tumor control similar to that achieved with chemotherapy (cisplatin) in H510 tumors. Indeed, PD173074 treatment decreased intratumoral proliferation as shown by in vivo [18F]fluorothymidine–positron emission tomography ([18F]FLT-PET) studies while increasing the appearance of apoptotic cell death markers in tumor biopsies. Moreover, coadministration of the drug enhanced response to cisplatin in vivo. Taken together, our results suggest that inhibition of FGFR kinase activity, while improving response to classic therapeutic agents, could be efficient as a monotherapy for a subset of lung cancer patients.

**Materials and Methods**

**Cell culture.** H510 and H69 SCLC cell lines were maintained as previously described (7). For experimental purposes, the cells were grown in serum-free medium (SITA: RPMI 1640 supplemented with 5 μg/mL insulin, 10 μg/mL transferrin, 30 nmol/L sodium selenite, and 0.25% bovine serum albumin) and used after 3 to 7 d.

**FGFR transphosphorylation.** H510 cells in SITA were treated in the presence or absence of 10 nmol/L PD173074 for 1 h before stimulation with or without 0.1 ng/mL FGE-2 for 2 min. Immunokinase assay and autoradiography were performed as described (7).
FGF-2 for 4 h and subsequent treatment with 0.1 μg/mL etoposide (VP16) for 4 d. Viable cell numbers were determined under microscopic observation using trypan blue exclusion.

Clonogenic assay. H510 cells in SITA were disaggregated into a single-cell suspension and mixed with 0.1 ng/mL FGF-2 and PD173074 at the concentration indicated. Clonogenic assay was then performed over a period of 3 wk as described (7).

Cell proliferation assay. H510 and H69 (1.5 × 10⁴ cells/mL) in SITA were treated with or without FGF-2; PD173074 for the duration and at the concentration highlighted in the figure legends. Cell number was determined by cell counting as described (7).

Cell death assay. H510 cells (5 × 10⁴ cells/mL) were pretreated with or without 10 nmol/L PD173074 for 1 h before addition of 0.1 ng/mL FGF-2 for 4 h and subsequent treatment with 0.1 μmol/L etoposide. Cells were then incubated at 37°C for 96 h. Cell number was determined by cell counting under the microscope using trypan blue exclusion.

RNAi transfection. FGFRI/2 knockdown was achieved using Dharmacon Smartpools and electroporation as described (9).

Xenografts and immunohistochemistry. H510 (1:1 cell suspension; Matrigel) or H69 cells were implanted into the flank of nude mice. When tumors became measurable, 50 μg/kg PD173074/mice or equivalent volume of buffer alone were administered daily for 14 or 28 d. In addition, mice received or did not receive two doses of 5 mg/kg cisplatin. Tumor volume was monitored using a calliper. Animals were sacrificed when tumor burden reached 15 mm in any dimension and survival recorded as a Kaplan-Meier plot. Tissues were formalin fixed and paraffin embedded before staining as indicated in the figure legends. For the endomucin experiments, pictures were acquired using a ×10 objective and analyzed using ImageJ. For activated Caspase 3 and cytokeratin 18 scoring, the number of positive cells in five high-power field views/tumor (five tumors per condition) was determined and results represented as bar graphs (Fig. 5C, bottom). The total number of nuclei per field was determined by manual counting using event flagging in Metamorph. Nuclei partly outside the field of view were excluded.

[¹⁸F]FLT-PET imaging. Animals with subcutaneous H-69 xenografts in the neck were used when the tumors reached ~150 mm³. The tumor-bearing mice were given vehicle or PD173074 once daily by oral gavage and imaged with [¹⁸F]FLT-PET on days 0, 7, and 14 of treatment. Dynamic [¹⁸F]FLT-PET studies were carried out on a dedicated small animal PET scanner, quad-HIDAC (Oxford Positron Systems; ref. 15). Scanning was performed as previously described (16). [¹⁸F]FLT (80–100 μCi; 2.96–3.7 MBq) was injected into the tail veins of anesthetized mice positioned prone within the scanner. Dynamic scans were acquired in list-mode format over a 60-min period and sorted into 0.5-mm sinogram bins and 19 time frames (0.5 × 0.5 × 0.5 mm voxels; 4 × 15 s, 4 × 60 s, and 11 × 300 s) for image reconstruction. Cumulative images comprising of 30 to 60 min of the dynamic data were used for visualization of radiotracer uptake and to draw regions of interest. Regions of interest were defined on five tumors and five heart slices (each was 0.5 mm thick). Dynamic data from these slices were averaged for each tissue and at each of the 19 time points to obtain time versus radioactivity curves for these tissues. Tumor radioactivity was corrected for physical decay and normalized to that of heart to obtain a standardized uptake value. The fractional retention of tracer was calculated as the normalized uptake in tumors 60 min relative to that at 1.5 min (16).

Fluorescent in situ hybridization. Paraffin-embedded sections were dewaxed for 10 min in xylene then treated with 100% methanol for 5 min. Endogenous peroxidase was blocked with 0.6% hydrogen peroxide in methanol for 10 min. Sections were then washed in PBS for 5 min at room temperature and rinsed in PBS. Postfixation was performed using 4% PFA for 2 min then washed in PBS. Sections were dehydrated through graded alcohols to absolute and air dried. Probe mix (10 μL) was applied to each section then covered slipped and sealed with luber cement. The sections were denatured at 80°C for 10 min before hybridizing overnight at 37°C in a sealed humid chamber. The rubber

![Figure 1](image1.png)

**Figure 1.** Expression of FGFRI and FGFRII in a panel of SCLC cells and inhibition by PD173074. A, equal protein amounts from SCLC cell lines were analyzed by SDS-PAGE/Western blotting for their FGFRI and FGFRII expression levels. B, H510 cells were treated with 100 nmol/L PD173074 for 2 wk in agar (A) or 1 wk in liquid culture (B). Cell or colony numbers were determined using microscopic observation. C, H510 cells were pretreated or not with 10 nmol/L PD173074 for 1 h before incubation with or without 0.1 ng/mL FGF-2 and subsequent treatment with 0.1 μg/mL etoposide (VP16) for 4 d. Viable cell numbers were determined under microscopic observation using trypan blue exclusion. Points and columns, averages of quadruplicates; bars, SD. All data shown are representative of at least three independent experiments.

![Figure 2](image2.png)

**Figure 2.** PD173074 inhibits clonogenic growth, proliferation, and chemoresistance of H510 SCLC cells. A and B, cells, treated with increasing concentrations of PD173074, were grown in the presence or absence of 0.1 ng/mL FGF-2 for 2 wk in agar (A) or 1 wk in liquid culture (B). Cell or colony numbers were determined using microscopic observation. C, H510 cells were pretreated or not with 10 nmol/L PD173074 for 1 h before incubation with or without 0.1 ng/mL FGF-2 and subsequent treatment with 0.1 μg/mL etoposide (VP16) for 4 d. Viable cell numbers were determined under microscopic observation using trypan blue exclusion. Points and columns, averages of quadruplicates; bars, SD. All data shown are representative of at least three independent experiments.
cement and coverslips were then removed and the slides immersed in 0.5× SSC for 5 min at 37°C. The slides were then washed 5× in PBS at room temperature for 10 min then mounted in Vectashield (Invitrogen) with 4′,6-diamidino-2-phenylindole (DAPI) before examination with a fluorescent microscope.

Reagents. Etoposide was from Sigma-Aldrich. The inhibitor PD173074 was from Park Davies. Antibodies for FGFR2 and endomucin were from Santa Cruz Biotechnology. The active caspase 3 antibody was from R&D Systems and the cytokeratin 18 antibody was from Dako. The FGFR1 antibody was a kind gift from Dr. Caroline Johnston. 32Pγ-ATP and enhanced chemiluminescence reagents were from Amersham-Pharmacia. Human recombinant FGF-2 and cisplatin were from Calbiochem.

Results

Addition of PD173074 inhibits FGF-2–mediated growth, proliferation, and chemoresistance of H510 cells. We first verified that FGFR1 and FGFR2 are expressed in SCLC cells. Equal protein amounts from eight SCLC cell lines were probed for the presence of these two high-affinity FGF-2 receptors. Both were expressed in all lines although to a varying extent (Fig. 1A). Activation of FGFR tyrosine kinases can be assessed by their transphosphorylation upon ligand binding. In vitro transphosphorylation assays showed that addition of FGF-2 to H510 cells increased both FGFR1 and FGFR2 kinase activity (Fig. 1B), demonstrating that these receptors are functional. Indeed, this resulted in an increase in clonogenic growth and liquid culture cell proliferation that was blocked in a dose-dependent fashion by the FGFR inhibitor PD173074 (Fig. 2A and B). This correlated with the ability of the compound to impair FGF-2–induced receptor transphosphorylation (Fig. 1B). Similar results were obtained in H69 cells (data not shown; Fig. 4A). Inhibition of FGF-2–mediated proliferation was also observed in three additional SCLC cell lines, SW2, H209, and H2171 (Supplementary Fig. S1).

We have previously shown that FGF-2 induces chemoresistance in a MAP/ERK kinase (MEK)/Erk–dependent fashion in SCLC cells (6). Therefore, we next investigated whether the compound could impair this effect. Figure 2C shows that addition of 10 nmol/L PD173074 blocked FGF-2–triggered resistance to etoposide killing.
of H510 cells. This correlated within inhibition of MEK/Erk signaling (data not shown). Altogether, our results show that PD173074 successfully inhibits the biological effects of FGF-2 on H510 and H69 cells.

PD173074 is a selective inhibitor of FGFR. Previous work has shown that PD173074 is specific to FGFR, with a 30-fold higher IC50 against the related kinasedomain of vascular endothelial growth factor receptor (13). However, only five other kinases were examined. Here, we have tested the inhibitory effect of this molecule against a panel of 78 kinases at two concentrations (0.1 and 1 μmol/L). This confirmed that FGFR1 was by far the most efficiently inhibited kinase examined (Table 1). The only other kinase to show significant impairment of activity was Lck. However, a dose response study (Supplementary Fig. S2) showed that the IC50 for this kinase was ∼15 fold greater than that previously reported for FGFR1 (13).

Oral administration of PD173074 inhibits H510 and H69 tumor growth and potentiates cisplatin effects in nude mice. H510 cells in Matrigel were injected into the flank of nude mice, and once tumors were measurable, animals were randomized to receive carrier buffer in the absence or presence of PD173074 (50 mg/kg) by oral gavage daily for 28 days with or without cisplatin (5 mg/kg) i.v. on days 1 and 10. Figure 3A shows that PD173074 as a single agent was as efficient as cisplatin at prolonging survival of tumor-bearing animals compared with control treatment. However, combined cisplatin and PD173074 exerted an additional survival benefit. This correlated with each agent independently, reducing tumor growth compared with control

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<th>Table 1. PD173074 shows a high degree of selectivity for FGFR tyrosine kinase</th>
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<tr>
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NOTE: The inhibitory potential of PD173074 (0.1 and 1 μmol/L) was tested in an in vitro kinase assay using a panel of tyrosine and serine/threonine kinases as previously published (21). Results are expressed as a percentage of initial activity ± SEM.

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treatment (Fig. 3B). Moreover, the combination of cisplatin and PD173074 was significantly better at impairing growth compared with either agent alone (Fig. 3B).

As PD173074 reduced but did not completely eliminate H510 primary tumors, we wondered whether it could nevertheless inhibit tumor dissemination. Following 28 days of PD173074 or buffer treatment, the lymph nodes proximal to each xenograft were extracted and processed by fluorescent in situ hybridization (FISH) for murine and human centromeres. Although murine pan-centromeric probes allowed visualization of the mouse lymph node tissue, the appearance of human pan-centromeric staining was used to identify and count invading human H510 cells (Fig. 3C, left). Our results show that PD173074 did not block tumor cell metastasis (Fig. 3C, right). Because FGFR-2 is known to mediate neoangiogenesis, we next examined whether the inhibitor might disrupt this process in the xenografts. This was assessed by imaging blood vessels in tumor sections after endomucin staining (Fig. 3D, left top) and subsequent Image J analysis of the vascular area (Fig. 3D, left bottom). Our data revealed that FGFR inhibitor treatment had no effect on tumor neoangiogenesis (Fig. 3D, right). In contrast, animals treated with cisplatin, used here as a positive control (17), showed a marked decrease in the vascular area. Finally, coadministration of PD173074 did not enhance disruption of the vasculature by cisplatin. This suggests that the increased efficacy of coadministered cisplatin and PD173074 in H510 xenografts is not due to vascular targeting.

To establish whether the inhibitory effect of this compound was applicable to other SCLC xenografts, we next examined its effects on H69 cells. In agreement with our earlier observations, PD173074 significantly blocked the growth of these cells in vitro (Fig. 4A). Administration of the compound to mice bearing H-69 xenografts significantly impaired tumor growth compared with control (Supplementary Table; Fig. 4B). This resulted in complete remission and long-term survival beyond 6 months in 50% of the treated animals, whereas all controls had to be sacrificed within 2 months (Fig. 4C). Indeed, the surviving animals were subsequently sacrificed 1 year later and extensive histology was negative for disease (data not shown).

**[18F]FLT-PET is an early predictor of response to PD173074 in vivo.** To determine if we could predict response to PD173074 using an in vivo imaging technique applicable to patients in the clinic, we next used [18F]FLT-PET to monitor intratumoral proliferation. Animals bearing subcutaneous H69 xenografts in the neck were given diluent with or without PD173074 by oral gavage daily and injected with [18F]FLT-PET before imaging at day 8 and 14. Figure 5A shows representative [18F]FLT-PET imaging from one control and one PD173074-treated animal before and 14 days into administration of the treatment. Analysis of [18F]FLT-PET results by the fractional retention time, a parameter independent of tumor size, and less dependent on perfusion, showed that PD173074 administration reduced cellular proliferation (Fig. 5B). In the same tumors, growth inhibition was shown by calliper measurements (Fig. 5A, bottom). This suggests that [18F]FLT-PET might provide a noninvasive way to predict early tumor response in patients treated with an agent like PD173074.

**PD173074 induces intratumoral apoptosis.** Since a significant proportion of treated tumors disappeared (Fig. 4C), we wondered whether administration of PD173074 might also induce apoptosis in the H69 SCLC xenografts. Figure 5C (top) shows immunohistochemical staining of representative tumor tissue sections from a control and PD173074-treated animal at day 14. PD173074 increased caspase 3 activation and cleavage of one of its well-known substrates, cytokeratin 18 (Fig. 5C). The effects seen were statistically significant across all 10 control and treated tumors (Fig. 5C, bottom; P < 0.005 and P < 0.0004 for caspase-3 and cytokeratin-18, respectively). The increased cell death may be linked to inhibition of FGFR1 rather than FGFR2 because Smartpool siRNA targeting FGFR1 but not FGFR2 increased the proportion of H-69 cells in sub-G1, as assessed by flow cytometry (Fig. 5D, left). Both FGFR1 and 2 levels were similarly downregulated by the respective RNAi treatment in H69 cells, excluding the possibility that differences in targeting efficiency were responsible for this differential response (Fig. 5D, right). Moreover, our results were validated by repeating the experiments with deconvoluted single targeting sequences (data not shown). Concomitant silencing of both FGFR1 and FGFR2 did not decrease cell viability beyond FGFR1 downregulation alone (Fig. 5D, left). Taken together, these results suggest that the FGFR inhibitor induces apoptosis in H-69 cells and that monitoring of apoptotic markers in patients might be another way to assess response.

**Figure 4.** Administration of PD173074 inhibits tumor growth and leads to long-term survival in H69 xenograft models. A. H69 cells were grown in liquid culture in the absence or presence of 10 nmol/L PD173074 and counted at the indicated times. Point, mean of triplicates; bars, SDM. B and C. PD173074 administration for 28 d inhibits tumor growth (B) and leads to long-term survival (C) in H69 xenograft models. VO, vector only; PD, PD173074 (A and B). C, arrow, end of PD173074 treatment. All data shown are representative of at least three independent experiments.
Figure 5. \[^{18}F\]FLT-PET and tumor staining for apoptotic markers can assess the therapeutic response to PD173074 in H69 xenografts. A and B, \[^{18}F\]FLT PET imaging data. A, representative scans of tumor-bearing mice treated with PD173074 or vector only (VO) on days 0 and 14 (top) and subsequent tumor volume measurements (bottom). B, bladder; T, tumor; Br, brain. B, fractional retention data. A (bottom) and B, measurements were determined from the imaging of eight mice per group. Columns, mean; bars, SD. C, top, representative primary tumors tissue sections from PD173074 or vector only–treated animals at 14 d, stained for active caspase 3 and cleaved cytokeratin 18. Immunoreactivity was monitored using horse radish peroxidase–conjugated secondary antibodies. Red arrows, examples of horse radish peroxidase activity. The number of positive cells in five high-power field views/tumor (five tumors per condition) was determined and results represented as bar graphs (bottom). ***, P ≤ 0.005. D, H69 cells were transfected with Smartpool siRNA oligonucleotides targeting FGFR1 and/or FGFR2 or a scrambled sequence (Sc). The sub-G1 population was determined by fluorescence-activated cell sorting analysis after propidium iodide staining (left). Columns, mean triplicate samples; bars, SD. Downregulation of the corresponding protein was assessed by Western blotting for the relevant protein and the blots were analyzed by optical densitometry using image J (right). Actin detection was used as a loading control. All data shown are representative of at least three independent experiments.
Discussion

The development of novel therapies for SCLC is urgently required. Our previous work has suggested that targeting FGFR might be useful as FGF-2 induces both proliferative and prosurvival signaling in this tumor type (6, 7). Here, we have examined the in vitro and in vivo effects of the selective FGFR inhibitor PD173074 on SCLC tumor growth and response to chemotherapy.

Our results show that PD173074 impairs the growth of two SCLC cell lines both in vitro and in vivo. Moreover, in one model, it led to full tumor regression and disease-free long-term survival in 50% of animals bearing this xenograft. These effects on tumor growth could be explained by a decrease in intratumoral proliferation, as shown by [18F]FLT-PET, and an increase in apoptotic cell death, as shown by the appearance of two well-characterized apoptotic markers. FGF signaling has been involved in angiogenesis, and inhibiting FGFRs could be expected to lead to neoangiogenesis defects (14). However, macroscopic changes to the tumor vasculature could not be detected, suggesting that changes in blood supply was unlikely to be involved in the tumor regression. Also, FGF signaling has been shown to regulate cell motility in a variety of cellular contexts (18). Therefore, it would be reasonable to expect that these effects would impair tumor dissemination to distant sites in our in vivo models. Surprisingly, however, no statistically significant changes to lymph nodes metastasis was detected in animals treated with the drug compared with those treated with solvent alone.

Taken together, these data suggest that FGFR inhibition could provide a powerful therapeutic strategy for SCLC treatment in clinical settings. Our previous results highlighted protein kinases involved in the growth promoting effects of FGF-2 in SCLC. These included MEK, B-Raf, PKCε, and S6 Kinase 2 (9). Except for S6 Kinase 2, compounds targeting these molecules exist, but their use in vivo has been disappointing owing mainly to the general toxicity of target inhibition (19, 20). The drug used here is orally available and no sign of toxicity was observed in treated animals following 28 daily administrations of the compound. Our animal imaging data using [18F]FLT-PET show that changes to tumor biology can be detected early following PD173074 treatment. This approach could be used in the clinic to determine early responsiveness to the drug. Similarly, the cytokeratin 18 cleavage that increased in response to PD173074 in the present study could potentially be used in patient serum samples to follow antitumor effects.

In short, our results suggest that FGFR inhibition, while improving response to classic therapeutic regimens, could be efficient as a monotherapy for a subset of lung cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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