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Tumor and Stem Cell Biology

Elimination of Human Lung Cancer Stem Cells through Targeting of the Stem Cell Factor–c-kit Autocrine Signaling Loop

Vera Levina1,2, Adele Marrangoni1, Tingting Wang1, Simul Parikh3, Yunyun Su4, Ronald Herberman1,2, Anna Lokshin1,2, and Elieser Gorelik1,4

Abstract

Cancer stem cells (CSC) are thought to be responsible for tumor initiation and tumor regeneration after chemotherapy. Previously, we showed that chemotherapy of non–small cell lung cancer (NSCLC) cells lines can select for outgrowth of highly tumorigenic and metastatic CSCs. The high malignancy of lung CSCs was associated with an efficient cytokine network. In this study, we provide evidence that blocking stem cell factor (SCF)–c-kit signaling is sufficient to inhibit CSC proliferation and survival promoted by chemotherapy. CSCs were isolated from NSCLC cell lines as tumor spheres under CSC-selective conditions and their stem properties were confirmed. In contrast to other tumor cells, CSCs expressed c-kit receptors and produced SCF. Proliferation of CSCs was inhibited by SCF-neutralizing antibodies or by imatinib (Gleevec), an inhibitor of c-kit. Although cisplatin treatment eliminated the majority of tumor cells, it did not eliminate CSCs, whereas imatinib or anti-SCF antibody destroyed CSCs. Significantly, combining cisplatin with imatinib or anti-SCF antibody prevented the growth of both tumor cell subpopulations. Our findings reveal an important role for the SCF–c-kit signaling axis in self-renewal and proliferation of lung CSCs, and they suggest that SCF–c-kit signaling blockade could improve the antitumor efficacy of chemotherapy of human NSCLC. Cancer Res; 70(1); 338–46. ©2010 AACR.

Introduction

Cancer stem cells (CSC) are a rare subpopulation of undifferentiated cells that are responsible for tumor initiation, maintenance, and spreading (1–3). They are drug resistant and display the ability to self-renew and generate a progeny of the differentiated cells that constitute a large majority of the cells in tumors. CSCs have been identified in various human malignancies, including breast, brain, prostate, pancreatic, colon, and lung cancer (1, 4–10). CSCs can be grown in vitro as tumor spheres under non-adherent conditions using a serum-free medium that is supplemented with growth factors (11, 12). A universal marker for CSCs has not been identified, but, in many tumors, CSCs exhibit CD133 (5, 7, 9, 10, 13, 14). We have found that human lung CSCs also express c-kit receptors, embryonic markers (SSEA-3, TRA-1-81, Oct-4, and nuclear β-catenin), and low levels of the cytokeratins 8/18 (CK8/18; ref. 10).

We have previously shown that treating tumor cells with chemotherapeutic drugs selectively enriches the survival of CSCs. Furthermore, the drugs prevent the differentiation of CSCs, thus maintaining the proliferation of the drug-resistant CSCs. We proposed that the highly tumorigenic and metastatic properties of CSCs are based on their advanced cytokine network. Our findings revealed an upregulated level of major human angiogenic and growth factors [vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin (IL)-6, IL-8, hepatocyte growth factor, platelet-derived growth factor (PDGF), granulocyte colony-stimulating factor (G-CSF), and stem cell growth factor-β (SCGF-β)] and VEGF receptor 2, FGF receptor 2, and CXCR1, CXCR2, and CXCR4 receptors in lung CSC–derived tumors (10). We also showed that drug treatment stimulates the production of various cytokines, chemokines, and angiogenic and growth factors. Blocking one or more of these factors with neutralizing antibodies could potentially increase tumor cell sensitivity to chemotherapy drugs (15).

We hypothesize that the proliferation of CSCs can also be stimulated by these growth factors. We have previously shown that lung CSCs express c-kit receptors and produce elevated levels of stem cell factor (SCF; ref. 10). This suggests a possible role the SCF–c-kit axis plays in the self-renewal and proliferation of CSCs in solid tumors.
Human c-kit, which has been shown to function as a SCF receptor, is a transmembrane receptor with a protein tyrosine kinase, an extracellular ligand binding domain, a single transmembrane segment, and a cytoplasmic kinase domain. C-kit stimulation by SCF results in dimerization, autophosphorylation, and a subsequent activation of downstream effector proteins, including the phosphoinositide 3-kinase (PI3K)/Akt; phospholipase C, gamma 1 (PLCG1); signal transducer and activator of transcription (STAT); and RAS/mitogen-activated protein kinase pathways (16–18). SCF is a major cytokine for the self-renewal, proliferation, and differentiation of numerous embryonic, adult hematopoietic, neural, and primordial stem cells (19, 20).

Accumulating data indicate that SCF is a mitogenic and angiogenic factor involved in carcinogenesis. Binding SCF to c-kit results in the activation of its intrinsic tyrosine kinase activity and promotes tumor growth (21–23). SCF and c-kit are overexpressed in some human malignancies, including gastrointestinal stromal tumors (GIST), breast cancer, SCLC, acute myelogenous leukemia (AML), and glioma (16, 18, 24). Cancer patients with either overexpression or mutations of c-kit in their tumors have poor prognosis and lower survival rates and show resistance to chemotherapy (25).

We hypothesize that blocking the SCF–c-kit signaling loop would lead to an interference with the proliferation or survival of c-kit–positive CSCs. It is considered that the survival of drug-resistant CSCs is a major obstacle for successful chemotherapy (2). Thus, inhibition or even elimination of CSCs by disruption of the SCF–c-kit loop might inhibit the regeneration of tumor cells from CSCs and thereby increase the efficacy of chemotherapy.

To test this hypothesis, we isolated CSCs from human non–small cell lung cancer (NSCLC) cell lines and analyzed the effect recombinant SCF and an antibody-neutralizing SCF had on the proliferation of CSCs. The tyrosine kinase inhibitor imatinib (also known as STI571 or Gleevec) is a small molecule belonging to the 2-phenylaminopyrimidine classes, which selectively inhibits c-kit, BCR/ABL, and PDGF receptor (PDGFR) signaling (26). Imatinib is approved by the Food and Drug Administration for the treatment of AML and GIST (27, 28). Imatinib as monotherapy was used in clinical trials against different solid tumors, including breast, ovarian, and small cell lung cancer; however, no clinical responses were detected (29–31).

We investigated whether imatinib or anti-SCF antibody affects the survival of lung CSCs and potentiates the efficacy of cisplatin against NSCLC cells. We found that SCF stimulates the efficacy of cisplatin, whereas anti-SCF antibody inhibited the proliferation of lung CSCs. Imatinib did not affect bulk cell proliferation. Thus, the disruption of the SCF–c-kit autocrine loop can be a useful approach against CSCs. A combination of imatinib or anti-SCF antibody with cisplatin treatment resulted in a destruction of tumor cells and blocked the selection of CSCs. Our data indicate that imatinib might be helpful when used in combination with chemotherapy for a more efficacious treatment for NSCLC patients.

### Materials and Methods

**Cell lines.** NSCLC cell lines H460 and A549 were obtained from the American Type Culture Collection (ATCC). Cells were grown in culture medium, as recommended by the ATCC.

**Reagents.** Cisplatin, doxorubicin, Hoechst 33342, insulin, bFGF, antibody-neutralizing PDGFRα–β, and PDGFAA-BB were from Sigma-Aldrich. Methylcellulose (MC)–based medium was from Stem Cell Technologies. Imatinib was from LC Laboratories (PKC Pharmaceutical, Inc.). Fluorochrome-conjugated antibodies against human c-kit were from Beckman Coulter. Recombinant human SCF and antibodies against PDGFRα and PDGFRβ were from R&D Systems, Inc. Antibodies against SCF, c-kit, CD133, and CK8/18 were from Abcam, Inc. Epidermal growth factor (EGF); Alexa Fluor 488–conjugated anti–TRA-1-60, anti–TRA-1-81, and anti–SSEA-1-4; and the antibody against human β-catenin were purchased from BD Biosciences, Inc. Secondary Alexa Fluor 488–conjugated and Alexa Fluor 680–conjugated antibodies were from Molecular Probes (Invitrogen).

**Cultured lung cancer spheres.** Suspension growth was assessed as described (11, 12). Briefly, cells were suspended in 0.8% MC-based serum-free medium supplemented with EGF, bFGF, 20 ng/mL, and 4 μg/mL insulin and plated at 500 to 10,000 per milliliter in ultralow-attachment 24- and 96-well plates (Corning). The medium was replaced or supplemented with fresh growth factors twice a week. To assess the self-renewing potential of the cells, first-generation spheres were collected by gentle centrifugation, dissociated into single-cell suspensions, and cultured under the conditions described above. The same procedures were repeated with the second- and third-generation spheres.

**Differentiation.** Cells that were dissociated from spheres were grown in adherent conditions in a culture medium supplemented with 10% fetal bovine serum (FBS) as described (10). To test the self-renewing potential of differentiated cells, the cells were transferred to stem cell–selective conditions and their ability to form tumor spheres was evaluated as described above. To perform a phenotypic characterization of cells from spheres and cells after differentiation, cells were seeded in 96-well plates (5 × 103 per well) and stained with the various antibodies described above.

**Effects of human recombinant SCF and neutralizing SCF antibody on the proliferation of lung cells.** CSCs were dissociated into a single-cell suspension, plated (1,000 cells/mL) in ultralow-attachment plates, and cultured in stem cell–selective conditions. Human recombinant SCF (0–100 ng/mL) or neutralizing SCF antibody (0.1–1.0 μg/mL) was added for 3–12 days. Tumor spheres were counted and measured under the microscope.

NSCLC cells were plated onto 96-well plates precoated with collagen and cultured in the presence/absence of SCF or neutralizing SCF antibody for 72 h. Cells were counted using the Cellomics ArrayScan as described (10, 32).

**Drug resistance studies.** CSCs were dissociated into a single-cell suspension and plated (1,000 cells/mL) in ultralow-attachment plates in stem cell–selective medium.
Doxorubicin (0.016–0.25 μg/mL), cisplatin (0.016–0.25 μg/mL), imatinib (0.010–15 μmol/L), or combinations of the above drugs were added to the culture medium. Tumor spheres were counted and measured under the microscope.

NSCLC cells were plated onto 96-well plates and cultured in a medium supplemented with 10% FBS. After 24 h, doxorubicin (0.016–0.25 μg/mL), cisplatin (0.016–0.25 μg/mL), imatinib (0.010–15 μmol/L), or combinations of the above drugs were added to the culture medium. To test the effects of neutralizing SCF antibody on tumor cell proliferation and sensitivity to drugs, cells were pretreated with monoclonal antibody–neutralizing SCF (100 ng/mL) for 2 h followed by drug treatment in the manner described above. After 3, 6, 12, and 20 d of treatment, the cells were fixed and the number of cells per well was counted using the Cellomics ArrayScan.

To test the proportion of CSCs in tumor cell cultures, untreated cells or cells treated for 12 d with cisplatin, imatinib, or combination cisplatin-imatinib were harvested, counted, and plated as single-cell suspensions and grown in stem cell–selective conditions. The tumor spheres were counted and CSC markers were evaluated as described (10).

**Cell staining procedure for cellomics arrayscan automated imaging.** Cells were fluorescently stained and analyzed using the Cellomics ArrayScan HCS Reader (Cellomics/Thermo Fisher) as described (32). Cell nuclei were stained with Hoechst 33342 to identify individual cells and to

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**Figure 1.** Isolation of lung CSCs. A, generation of CSC tumor spheres. A549 and H460 cells were plated and cultivated in stem cell–selective conditions. Tumor spheres were counted after 3 wk. ****, P < 0.001. B, expression of CD133. H460, A549, CSC/H, and CSC/A cells were stained for CD133. Images were acquired using the Cellomics ArrayScan HCS Reader and analyzed using the Target Activation BioApplication Software Module. Fluorescence intensity was plotted against the object area. The red lines in all histograms show the boundaries of the fluorescence intensity of isotype control. C, cytokeratin expressions. Cells were incubated with an anti–CK8/18-FITC (green) antibody or an isotype IgG-FITC antibody. Blue, nuclei were stained with Hoechst 33342. The images were acquired using the Cellomics ArrayScan HCS Reader. D, loss of stem cell markers by differentiated progenitors. CSC/H cells were grown in adherent conditions in differentiating culture medium for 4 wk. Then, bulk H460, CSC/H, and differentiated cells were stained for CD133. Images were acquired using the Cellomics ArrayScan HCS Reader and analyzed using the Target Activation BioApplication Software Module.
optimize focusing. Briefly, bulk tumor cells and CSCs were grown in collagen-precoated 96-well plates and incubated with anti-CD133 and secondary Alexa Fluor 488 antibodies or primary antibodies against c-kit, TRA-1-60, TRA-1-81, β-catenin, PDGFRα, or PDGFRβ conjugated with FITC, phycoerythrin, or Alexa Fluor 488. To detect intracellular proteins, cells were incubated with primary antibodies against SCF, β-catenin, Oct-4, or CK8/18 and with secondary antibodies conjugated with Alexa Fluor 488 or Alexa Fluor 680. To stain tumor spheres, all manipulations were done under microscopic control.

**Analysis of SCF in tumor cell conditioned medium.** Cells were cultivated in RPMI 1640 supplemented with 10% FBS for 48 h. Samples of conditioned medium were collected and the cells were counted. Concentrations of human SCF were analyzed using xMAP technology (Luminex Corp.). Concentrations of SCF (pg/10^6 cells/mL) were calculated.

**Statistical analysis.** All experiments were repeated at least thrice. Comparisons between values were performed using a two-tailed Student’s t test. For the comparison of multiple groups, a one- or two-way ANOVA test was applied. For all statistical analyses, the level of significance was set at a probability of <0.05 (*, *P* < 0.05; **, *P* < 0.01; *** , *P* < 0.001).

**Results**

**Analysis of c-kit and SCF expression.** CSCs were isolated from NSCLC cells that were growing in stem cell–selective conditions. The H460 cell line had a 6-fold higher proportion...
of tumor sphere–forming cells in comparison with A549 (Fig. 1A). Cells that were derived from first-generation spheres in both lines showed a high and equal ability to form second-generation spheres. Third-generation spheres were used in our experiments. We tested the CSC nature of cells in tumor spheres as described (10). The Cellomics ArrayScan HCS Reader was used for the imaging and analysis of CSC expression and embryonic stem cell markers in lung tumor cells. Tumor spheres were enriched in CSCs and early progenitor cells; they consistently expressed lung CSC marker CD133 (Fig. 1B) and embryonic cell markers TRA-1-81, nuclear β-catenin, and Oct-4 (data not shown); and had a low expression of epithelial markers CK8/18 (Fig. 1C). CD133+ fractions varied from 1.4% in A549 cells to 1.7% in H460 bulk cells, whereas 31.7% cells in A549-derived tumor spheres and 70.9% cells in H460-derived tumor spheres were CD133+ (Fig. 1B). Cells in tumor spheres were self-renewing: cells dissociated from first-generation spheres formed the second-generation spheres. If tumor spheres were plated into adherent plastic plates with a culture medium supplemented with FBS and cultivated for 4 weeks, they generated a differentiated progeny with the phenotypic features of lung cancer cells and gradually acquired cytokeratins, whereas the CSC markers were lost during differentiation (Fig. 1D). Thus, our results showed that cells isolated from H460 and A549 cells and growing as tumor spheres have all features of CSCs: they express CSC markers (CD133 and c-kit) and embryonic stem cell markers (Oct-4, TRA-1-81, and nuclear β-catenin), have a low level of differentiated markers (CK8/18), and are able to self-renew and to differentiate losing CSC markers and gaining CK8/18. These results in combination with our previous in vivo data showed that CSCs isolated from H460 cells have high tumorigenic and metastatic abilities (10) and indicate that cells in lung tumor spheres have the characteristics of CSCs. In the absence of in vivo determination of tumorigenic properties of A549 cells isolated from tumor spheres, these cells cannot be unequivocally defined as lung CSCs. However, the findings that H460 and A549 cells growing in tumor spheres manifest identical phenotypic properties of CSCs as well as the ability of self-renewal and differentiation give us the ground to conclude that these cells derived from two NSCLC cell lines are CSCs. Henceforth, CSCs from H460 and A549 cell lines will be termed CSC/H and CSC/A, respectively.

CSCs in tumor spheres expressed c-kit (Fig. 2A). When CSCs were cultivated in collagen-precoated plates for 8 hours, >60% of lung CSCs expressed c-kit (Fig. 2B). If these cells were cultured in medium supplemented with FBS for 4 weeks, they differentiated and changed their morphology and the percentage of c-kit+ cells substantially decreased (1.9% and 1.1% for cells differentiated from CSC/A and CSC/H cells).

Next, the expression of SCF in bulk cells and CSCs was tested. Immunofluorescence staining revealed a high intracellular level of SCF in CSCs (Fig. 2C), whereas the SCF level in the bulk population was undetectable. Similarly, a low level of SCF was detected in culture medium conditioned by A549 and H460 cells, whereas CSCs produced higher levels of SCF (Fig. 2D). These findings indicate that SCF–c-kit axis is associated with the CSC phenotype in the NSCLC cells.

Stimulation of lung CSC growth by exogenous SCF. Recombinant SCF was used to test the importance of SCF–c-kit signaling for CSC growth. Dose-response analyses showed that SCF has a major proliferative effect at a concentration of 10 ng/mL. SCF-stimulated CSC proliferation was manifested...
in augmenting the yield of formed spheres and their sizes (Fig. 3A and B). To test whether SCF can stimulate the proliferation of already developed tumor spheres, SCF was first added on the 7th day of culturing, resulting in an increased size of spheres ($P < 0.05$) and the number of cells in the spheres by $20\%$ ($P < 0.05$). These data indicate that SCF stimulated the proliferation of CSCs but had no significant effect on bulk A549 and H460 cell proliferation (data not shown).

Next, the effect of neutralizing SCF antibody (0.1–1.0 μg/mL) on tumor sphere formation was analyzed. The neutralization of SCF resulted in a 4-fold reduction of the total number of growing CSC/A spheres and almost completely abrogated the growth of CSC/H tumor spheres (Fig. 3C). The remaining CSC/A tumor spheres that were growing with the anti-SCF antibody had a significantly smaller size ($P < 0.001$) than spheres growing in control wells (Fig. 3D).

**Effect of c-kit inhibitor imatinib on CSCs.** Imatinib abrogated CSC/H growth at nanomolar concentrations (10–100 nmol/L) and drastically reduced the size of tumor spheres from CSC/A cells (Fig. 4A and B). The average diameter of control CSC/A sphere was $1.74 \pm 0.34$ μm, whereas in the presence of imatinib the diameter was $0.66 \pm 0.21$ μm.

**Effect of combined imatinib and cisplatin treatment on growth of bulk tumor cells and CSCs.** Earlier, we found that treating H460 cells with drugs led to the elimination of the vast majority of the cells; however, CSCs formed visible colonies after 7 to 10 days of drug treatment (10). We hypothesized that a combined cisplatin-imatinib treatment could be more efficient than each drug used separately, perhaps leading to a virtually complete elimination of non-CSCs and CSCs.

CSCs were resistant to conventional chemotherapy, and only a partial reduction in sphere formation was observed. In contrast, imatinib alone or in combination with cisplatin or doxorubicin dramatically reduced CSC growth (Fig. 4A and B).

We assessed to see if imatinib could inhibit the proliferation of, or even eliminate, CSCs from the bulk cell population and thus increase the efficacy of therapy. Imatinib at 10 nmol/L to 2 μmol/L did not affect the growth of NSCLC cells (Fig. 4A). In contrast, cisplatin substantially reduced cell numbers, although A549 cells were more resistant than H460 cells (Fig. 5A). A combined cisplatin-imatinib treatment for 72 hours had a similar inhibitory effect as the cisplatin alone (Fig. 5A). This could be explained by the low proportion of CSCs in the bulk populations. After 3 to 6 days of treatment, a significant ($P < 0.001$) reduction in cell numbers was detected in the cultures treated with cisplatin or the combination of cisplatin-imatinib (Fig. 5B). During the next week of culturing, substantial differences appeared between the cells treated with cisplatin alone and the cisplatin-imatinib combination. A population of morphologically distinct small cells started to proliferate and form multiple, fast-growing colonies in the cultures treated with cisplatin alone. No growing clones of lung CSCs were found after cisplatin-imatinib treatment (Fig. 5C). A vast majority of the cells died; however, some of the cells resembled senescent cells with an enlarged and flattened morphology. These "senescent" cells grew larger in size and died during the next 4 to 10 weeks. No growing tumor spheres were found when these cells were harvested and plated into ultralow-attachment plastic plates with CSC-selective medium. No significant changes in cell number in control cultures and in cultures treated with imatinib alone were detected after 12 days (Fig. 5B). To examine the proportion of CSCs, we harvested cells from 12-day cultures and plated them in stem cell–selective conditions. As shown in Fig. 5D, low numbers of spheres grew from the nontreated H460 and A549 cells and a 10-fold number of spheres developed from the cells in post-cisplatin treatment. This indicates that cisplatin treatment enriched the population with CSCs. However, no tumor spheres were grown from the cells that were treated with the combination of cisplatin and imatinib. Thus, a combination of imatinib and cisplatin could be highly efficient against the regeneration of tumor cells by drug-resistant CSCs.

Next, we tested whether a treatment of bulk NSCLC cells with a combination of cisplatin and neutralizing SCF antibody would inhibit CSC selection. Treatment of H460 and A549 cells with cisplatin in the presence of anti-SCF antibody resulted in a significant reduction in survived cells (Fig. 5B). Thus, the major inhibitory effect of imatinib on the growth of CSCs was mediated via c-kit–SCF signaling. Because imatinib inhibits not only c-kit but also PDGFR signaling (16), we analyzed whether PDGF signaling is involved in the inhibitory effects of imatinib. Both bulk cells and CSCs express PDGFRA and PDGFRβ receptors; however, antibody-neutralizing PDGFAA, PDGFBB, and their receptors did not inhibit the growth of CSCs in tumor spheres (Supplementary Fig. S1).

It was reported that imatinib could reverse drug resistance in osteosarcoma cells (33). To exclude a possibility that imatinib increases tumor cell drug uptake rather than inhibiting

![Figure 4](https://www.aacrjournals.org/CancerRes/article-figures/Figure4.jpg)

**Figure 4.** Effects of combined chemotherapy with SCF–c-kit inhibition on the growth of lung CSCs. A and B, single-cell suspensions prepared from CSC/H and CSC/A spheres were plated and cultured in the stem cell–selective conditions. Cisplatin (cis; 1 μmol/L) or doxorubicin (dox; 0.0085 μg/mL) alone or in combination with imatinib (ima; 100 nmol/L) was added to the wells. The numbers of tumor spheres generated after 2 wk are presented. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.  

**A**. CSC/A spheres.  

- **Control (cont)**: no treatment.  
- **Cisplatin (cis)**: 1 μmol/L.  
- **Cisplatin + Imatinib (cis + ima)**: 1 μmol/L + 100 nmol/L.  
- **Doxorubicin (dox)**: 0.0085 μg/mL.  
- **Doxorubicin + Imatinib (dox + ima)**: 0.0085 μg/mL + 100 nmol/L.

**B**. CSC/H spheres.  

- **Control (cont)**: no treatment.  
- **Cisplatin (cis)**: 1 μmol/L.  
- **Cisplatin + Imatinib (cis + ima)**: 1 μmol/L + 100 nmol/L.  
- **Doxorubicin (dox)**: 0.0085 μg/mL.  
- **Doxorubicin + Imatinib (dox + ima)**: 0.0085 μg/mL + 100 nmol/L.
or eliminating CSCs, we tested if the combination of cisplatin-imatinib was effective if imatinib was applied 1 to 3 days after cisplatin treatment. Bulk cells were treated with cisplatin for 24 hours and the medium was replaced. Imatinib was added to cultures after 24 or 72 hours of cisplatin treatment. Surviving cells were grown in stem cell–selective conditions, and the generation of tumor spheres was evaluated. It was found that imatinib treatment completely prevented sphere formation (Supplementary Fig. S2). Thus, imatinib was able to eliminate CSCs even when it was added 1 to 3 days after cisplatin treatments.

Discussion

In this study, we showed that lung CSCs expressed c-kit and produced high levels of SCF. Coexpression of c-kit and SCF was shown only in a fraction of metastatic NSCLC (34). The functional analysis of SCF–c-kit axis signaling in CSCs revealed that SCF stimulates the proliferation of CSCs, whereas neutralizing SCF with an antibody or inhibition of c-kit with imatinib abrogated growth of CSCs. However, the degree of inhibition with the neutralizing antibody was higher than the level of CSC growth stimulation with exogenous SCF. This discrepancy can be explained by our findings that CSCs produce SCF that, by binding to c-kit, could stimulate CSC proliferation. Adding exogenous SCF only had a limited effect. In contrast, imatinib blocks the ability of endogenous or exogenous SCF to activate c-kit receptors, thus resulting in a profound inhibition of CSC growth. These data show the prime importance of SCF–c-kit signaling for the proliferation and survival of lung CSCs.

Imatinib is used as monotherapy against GIST and leukemia (27, 35). In patients, imatinib plasma concentrations fall in the range of 0.17 to 5.68 μmol/L when 25 to 600 mg of the drug were administered per day (27). Our finding revealed that imatinib blocked growth of CSCs at concentrations that were similar to the plasma levels found in patients who were treated with imatinib. In contrast to CSCs, imatinib had no detectable effect on bulk NSCLC cells. This failure seems to be due to lack of the c-kit expression by the vast majority of these cells. Although c-kit–positive CSCs exist in the bulk cell lines, they represent a very small proportion and the effect on their inhibition through imatinib is very difficult to detect from the cell count.

Imatinib inhibits not only c-kit but also PDGFR signaling (16). PDGF and its receptors are frequently expressed in solid tumors and are associated with the distant metastasis of tumors (36). It has been considered that imatinib inhibits ovarian cancer cell growth through the PDGFR inactivation (37). However, the concentrations of imatinib that inhibited PDGFR signaling were much higher than those that inhibit CSCs and can be achieved in the plasma of GIST patients (27). Our data showed that the major inhibitory effect of imatinib on the growth of CSCs was mediated via c-kit–SCF signaling.

Recent studies have shown that GIST and AML cancers can develop a resistance to imatinib as a result of mutations

Figure 5. Effects of combined treatment with imatinib-cisplatin or anti-SCF-cisplatin on proliferation of NSCLC cells. A, cells were incubated in the control medium or in the presence of imatinib (1 μmol/L) or cisplatin (2 μmol/L) or cisplatin-imatinib for 72 h. The mean numbers of cells per well are shown. B, cells were plated and incubated in the control medium or in the presence of imatinib (1 μmol/L), or cisplatin (2 μmol/L), or cisplatin-imatinib or cisplatin (2 μmol/L) with anti-SCF antibody (100 ng/mL) for 3 to 20 d. The mean numbers of cells per well are shown. C, phase-contrast microscopy photographs of cells treated with cisplatin alone or with combination cisplatin-imatinib for 12 d. D, cells were treated for 12 d as noted in B. They were then harvested, replated in stem cell–selective conditions, and cultivated for 10 d. The numbers of tumor spheres were counted. ***, P < 0.001.
in KIT (38, 39) or overexpression of ABCB1 pump protein (40). Strategies are developing to overcome therapeutic imatinib resistance, including screening for second-generation inhibitors (40, 41).

Here, we showed that a cisplatin-imatinib treatment of NSCLC cells growing in vitro led to potent inhibition or even elimination of both non-CSCs and CSCs. Our previous studies have shown that chemotherapeutic drugs substantially upregulate the production of multiple factors (15). This stimulation of growth factors could be a result of an adaptive stress response through which tumor cells protect themselves from toxicity and provide prosurvival stimuli. This may also stimulate CSC proliferation. Drug treatment induced a multifactorial signaling, with an activation of various transcription factors, such as NF-κB, AP-1-2, ATF-2, cyclic AMP-responsive element binding protein, hypoxia-inducible factor–1, STAT-1, STAT-3, and STAT-5, all of which play an important role in the upregulation of genes that encode various cytokine, chemokine, and growth factors (15, 42, 43). Previously, we showed that lung CSCs had an advanced cytokine network in comparison with bulk NSCLC cells (10). The inhibitory effect imatinib has on CSCs may be associated with the downregulation of prosurvival signaling. Akt, constitutively activated in NSCLC cells, is very important for survival of cells exposed to drugs (44, 45). Imatinib treatment downregulates the PI3K-Akt pathway (46, 47).

Clinically, chemotherapy is administered in cycles that are separated by 3-week intervals. However, during this resting period, tumor cells can aggressively repopulate the tumor (48). It is considered that conventional chemotherapy could lead to elimination of the bulk tumor cells and increase the proportion of drug-resistant CSCs capable of re-storing progressive tumor growth (10). Our data indicate that the SCF–c-kit axis is a key regulator for lung CSC survival and proliferation; therefore, antitumor therapy of patients with advanced lung cancer might be significantly improved by combining conventional chemotherapy with inhibitors such as imatinib that would target the SCF–c-kit axis.

To summarize, our in vitro studies show the importance of SCF–c-kit axis in the survival and proliferation of lung CSCs. We found that blocking the SCF–c-kit autocrine loop resulted in the complete elimination of CSCs. Combined cisplatin-imatinib therapy for lung cancer patients might lead to an inhibition of both non-CSC and CSC populations and thereby substantially improve the efficacy of chemotherapy. Further in vivo studies are necessary to confirm that imatinib in combination with cisplatin could have a potent therapeutic effect against NSCLC xenografted in severe combined immunodeficient mice.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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