Changes in Angiogenic Growth Factor Levels After Gefitinib Treatment in Non-small Cell Lung Cancer

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Received March 16, 2005; accepted March 21, 2005; published online May 10, 2005

Background: To investigate the changes in angiogenic growth factor expression before and after gefitinib treatment, and the association between this expression and response to gefitinib treatment, we measured circulating levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), matrix metalloproteinase (MMP) -2 and -9, and tissue inhibitors of metalloproteinase (TIMP) -1 and -2 in patients with non-small cell lung cancer (NSCLC).

Methods: Serum and plasma samples were collected from 52 patients before and after gefitinib treatment. The levels of VEGF, bFGF, MMP-2, MMP-9, TIMP-1 and TIMP-2 were measured using a sandwich enzyme immunoassay kit.

Results: Of the 52 patients, 17 (32.7%) achieved a partial response, 19 (36.5%) had stable disease and 16 (30.8%) had progressive disease. The levels of VEGF, bFGF, MMP-2, MMP-9, TIMP-1 and TIMP-2 did not change significantly after gefitinib treatment, even in responders. The levels of VEGF in volunteers, responders and non-responders were 384 ± 86.4, 404 ± 94.3 and 719 ± 99.8 pg/ml, respectively. The difference between volunteers and responders was not significant (\(P = 0.540\)), while the differences between volunteers and non-responders (\(P = 0.031\)), and responders and non-responders (\(P = 0.028\)) were significant.

Conclusions: Although our results indicate that gefitinib treatment does not affect circulating levels of angiogenic growth factors even in patients who showed a response to gefitinib treatment, low levels of VEGF may predict response to gefitinib treatment in patients with NSCLC.

Key words: angiogenic growth factors – epidermal growth factor receptor – gefitinib – non-small cell lung cancer

INTRODUCTION

Angiogenesis is the formation of new blood vessels from the existing vasculature, and neovascularization is a prerequisite for the growth of solid tumors beyond 1–2 mm in diameter (1). Pro-angiogenic stimuli may be released by tumors, stromal cells or inflammatory cells (2), and may trigger an angiogenic switch to allow the tumor to induce the formation of microvessels from the surrounding host vasculature (2). Angiogenic growth factors are produced not only by tumor cells, but also by normal bronchiolar and differentiated columnar epithelial cells and alveolar macrophages (3).

Vascular endothelial growth factor (VEGF) is the most potent and specific growth factor for endothelial cells, and is associated with tumor vessel density, cancer metastasis and prognosis (3–6); high levels of circulating VEGF have been reported in patients with non-small cell lung cancer (NSCLC) (5–14). Basic fibroblast growth factor (bFGF) is a cytokine that plays pleiotropic roles in the growth of various tissues (15). These functions include angiogenesis, prolongation of survival, promotion of cell migration, and induction of cell differentiation in a variety of developmental processes. In addition to these angiogenic factors, members of the matrix metalloproteinase (MMP) family, such as MMP-2 and MMP-9, have been implicated in the expansion and metastasis of...
cancers (4). MMP-2 and MMP-9 degrade type IV collagen, which is one of the main constituents of basement membranes and is considered to be the first barrier to tumor metastasis (4). MMPs are inhibited by tissue inhibitors of metalloproteinase (TIMPs), which are generally inhibitors of angiogenesis (4).

Epidermal growth factor receptor (EGFR) is a transmembrane receptor protein that is often highly expressed in NSCLC (16,17). After ligand binding, receptor dimerization leads to tyrosine kinase activation and the recruitment and phosphorylation of intracellular substrates, eventually inducing cell proliferation and motility as well as angiogenesis (18). Interest has been focused on EGFR as a target for anticancer therapy with agents such as gefitinib (Iressa™), an orally active EGFR tyrosine kinase inhibitor. Gefitinib blocks EGFR autophosphorylation (19) and the subsequent signal transduction pathways implicated in proliferation, metastasis and inhibition of apoptosis, as well as angiogenesis (20,21). The inhibition of EGFR with gefitinib has been shown to reduce production of angiogenic growth factors in various types of cancer cells (21,22).

However, it remains unclear whether the clinical effects of gefitinib in patients with NSCLC are correlated with reductions in the levels of angiogenic growth factors. Furthermore, it is unclear whether these factors are correlated with response to gefitinib treatment. To clarify these issues, we investigated the changes in the levels of VEGF, bFGF, MMP-2, MMP-9, TIMP-1 and TIMP-2 before and after gefitinib treatment and we investigated the correlation between these factors and response to gefitinib treatment in patients with NSCLC.

SUBJECTS AND METHODS

PATIENTS AND TREATMENT

Fifty-two patients with NSCLC treated from July 2002 to February 2003 were enrolled. Forty-six patients were refractory to cytotoxic chemotherapy and six received gefitinib as first-line chemotherapy. Patients received gefitinib at 250 mg/day until either progression or the development of a severe drug-related adverse event. Pre-treatment evaluation included a complete history and physical examination, evaluation of their Eastern Cooperative Oncology Group performance status (PS), X-ray and computed tomography (CT) scan of the chest. If necessary, a CT scan of the upper abdomen, CT or magnetic resonance imaging (MRI) scan of the brain, and radionuclide bone scan were performed. All patients gave written informed consent to participation in the study and received gefitinib treatment for at least 4 weeks.

RESPONSE

Tumor size was assessed by imaging within 14 days of starting gefitinib, and subsequently at 4-week intervals until withdrawal of treatment, according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (23). Patients with complete response or partial response (PR) underwent disease assessment at least 4 weeks later to confirm the response. In this study, responders were defined as patients with PR, while non-responders were defined as patients with stable disease (SD) or progressive disease (PD). We made a comparison between responders and non-responders.

SERUM AND PLASMA SAMPLES

Serum and plasma samples were collected at baseline (prior to gefitinib treatment) and at 2- or 4-week intervals thereafter and VEGF, bFGF, MMP-2, MMP-9, TIMP-1 and TIMP-2 levels were measured. As a control, serum samples were also collected from 16 healthy volunteers (all men, median age of 44.5 years, range 24–56 years) and VEGF and TIMP-2 levels were measured. All samples were processed into serum or plasma, and stored at −80°C until use.

MEASUREMENT OF ANGIOGENIC FACTORS

The levels of bFGF and VEGF in serum were measured using a sandwich enzyme immunoassay kit with monoclonal antibodies specific for bFGF and for VEGF (R & D Systems, Minneapolis, MN), according to the manufacturer’s instructions. The levels of MMP-2, TIMP-1 and TIMP-2 in serum and of MMP-9 in plasma were also measured using a sandwich enzyme immunoassay kit with monoclonal antibodies specific for MMP-2, MMP-9, TIMP-1 and TIMP-2 (Daiichi Fine Chemical Industries, Toyama).

STATISTICAL ANALYSIS

Data are expressed as the means ± standard error (SE). Comparisons between pre- and post-treatment levels of angiogenic growth factors were performed using Student’s t test. Differences between pairs of groups were analysed by Student’s t test and differences among more than two groups were analyzed by analysis of variance (ANOVA) tests. Differences were considered significant at P < 0.05 in two-tailed analyses.

RESULTS

PATIENT CHARACTERISTICS AND RESPONSE TO GEFITINIB TREATMENT

A total of 52 patients were enrolled into this study. Patient characteristics are shown in Table 1. Twenty five (48.1%) of the 52 patients were female; the median age was 62 years (range 31–79 years). Never smokers made up 53.9% (28 patients) of patients. Forty-six (88.5%) patients had received cytotoxic chemotherapy prior to entry. Thirty-eight patients had good PS (0 or 1) and nine had poor PS (3 or 4). Histological types included 41 (78.8%) adenocarcinomas. Forty-six patients (88.5%) had stage IV disease at the start of gefitinib treatment. Table 1 summarizes objective responses to gefitinib. Of the 52 patients, 17 (32.7%) achieved a PR, 19 (36.5%) had SD,
16 (30.8%) had PD. Various factors such as age, gender, PS, histology, disease stage, smoking history and prior treatment were examined for their relationships with pre-treatment circulating levels of VEGF, bFGF, MMP-2, MMP-9, TIMP-1 and TIMP-2. None of these factors were correlated with circulating levels of angiogenic growth factors.

Pre- and post-treatment levels of angiogenic growth factors in all patients are shown in Table 2. There were no significant differences between pre- and post-treatment levels of any of the angiogenic growth factors. Furthermore, there were no significant differences in the levels of these factors according to the response to gefitinib (Figs 1–6).

Table 1. Patient characteristics and objective response

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients</th>
</tr>
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<tr>
<td>No. of patients</td>
<td>52</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>62 (31–79)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male (51.9%)</td>
<td>27</td>
</tr>
<tr>
<td>Female (48.1%)</td>
<td>25</td>
</tr>
<tr>
<td>Smoking history</td>
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<tr>
<td>Never smoker (53.9%)</td>
<td>28</td>
</tr>
<tr>
<td>Former or current smoker (46.1%)</td>
<td>24</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
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<td>0 (15.4%)</td>
<td>8</td>
</tr>
<tr>
<td>1 (57.7%)</td>
<td>30</td>
</tr>
<tr>
<td>2 (9.6%)</td>
<td>5</td>
</tr>
<tr>
<td>3/4 (13.5%)</td>
<td>9</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Adenocarcinoma (78.8%)</td>
<td>41</td>
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<tr>
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</tr>
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</tr>
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<td>Clinical stage</td>
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</tr>
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<td>IIIA/IIIB (11.5%)</td>
<td>6</td>
</tr>
<tr>
<td>IV (88.5%)</td>
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<td>Chemotherapy (88.5%)</td>
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<td>≥two regimens (59.6%)</td>
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<tr>
<td>Thoracic radiotherapy</td>
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<td>6 (11.5%)</td>
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<td>Best response to gefitinib</td>
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<tr>
<td>Partial response (32.7%)</td>
<td>17</td>
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<tr>
<td>Stable disease (36.5%)</td>
<td>19</td>
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<tr>
<td>Progressive disease (30.8%)</td>
<td>16</td>
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</table>

Pre- and post-treatment levels of angiogenic growth factors according to response to gefitinib

Pre-treatment levels of the angiogenic growth factors were shown in Figs 1–6 according to their response to gefitinib. The difference of VEGF levels between volunteers and responders was not significant (P = 0.540), while the differences between volunteers and non-responders (P = 0.031), and responders and non-responders (P = 0.028) were significant (Fig. 1). On the other hand, there was no difference between responders and non-responders in pre-treatment levels of bFGF, MMP-2, MMP-9, TIMP-1 or TIMP-2 (Figs 2–6). The levels of TIMP-2 in volunteers and patients with NSCLC were 52.5 ± 2.75 and 48.2 ± 3.40 ng/ml, respectively, and there was no difference between healthy volunteers and patients.
DISCUSSION

Activation of EGFR signaling can up-regulate the production of VEGF in human cancer cells (24). Previous pre-clinical studies showed that inhibition of EGFR by gefitinib reduced production of angiogenic growth factors in various cancer cell types (21,22) and that the EGF-induced up-regulation of VEGF was blocked almost completely by gefitinib (22). These studies suggested that the antitumor effects of gefitinib could be mediated in part by the inhibition of tumor angiogenesis through its direct effects on microvascular endothelial cells expressing EGFR, as well as through the reduced production of pro-angiogenic factors by tumor cells both in vivo and in vitro (22). Based on these pre-clinical studies, EGFR activation has been shown to play an important role in up-regulating
the expression of angiogenic growth factors such as VEGF (21,22). Therefore, serum levels of angiogenic growth factors are thought to reflect the in vivo activity of angiogenesis to some extent. In contrast to these pre-clinical results, our clinical study showed no significant differences between pre- and post-treatment levels of angiogenic growth factors. Serum levels of VEGF remained unchanged, even in patients who showed a response to gefitinib. These results disagreed with those of pre-clinical studies; however, the precise reasons for the discrepancy between our results and those of pre-clinical studies remain unclear.

The clinical significance of circulating levels of VEGF in patients with NSCLC is controversial. Some studies have shown that circulating levels of VEGF increase significantly according to disease stage progression (5–8), while others have shown the opposite results (10–14). Low levels of VEGF have been shown to be correlated with a good response to chemotherapy and to decrease after successful chemotherapy (10). Our study also showed that low levels of VEGF were correlated with a good response to gefitinib treatment. Furthermore, levels of VEGF in responders were not significantly different from volunteers, but were different from non-responders (Fig. 1). These results may show that gefitinib treatment is effective for patients with NSCLC who do not produce VEGF either directly or indirectly.

High levels of serum bFGF were found in patients with NSCLC (6,10,14,25,26), but they were not correlated with the response to chemotherapy (27). High circulating levels of MMP-9 were also found in patients with NSCLC (27–30), but the levels of MMP-2 were comparable with those of normal controls (27,29,31), except in one report (32). Circulating levels of TIMP-1 were higher in patients with NSCLC than in controls (27,30,33), whereas the levels of TIMP-2 were lower in patients with NSCLC than in controls (27). In the present study, only VEGF and TIMP-2 levels were measured in control subjects. Our results regarding serum levels of TIMP-2 disagreed with those reported previously. Among these angiogenic growth factors, only the levels of VEGF were measured before and after chemotherapy in previous studies (10). In the present study, serum levels of VEGF did not change even in patients who responded to gefitinib. Furthermore, we first measured circulating levels of bFGF, MMP-2, MMP-9, TIMP-1 and TIMP-2 before and after chemotherapy, and these levels did not change even in patients who responded to gefitinib. Circulating levels of MMP-9 had a tendency to increase after gefitinib treatment (Table 2, Fig. 4). The increase in non-responders was due to tumor progression, but the reason for the increase in responders was unclear in this small study.

EGFR phosphorylation has been shown to up-regulate its downstream (phosphorylated Akt and the ras-mitogen-activated protein kinase) and angiogenic growth factors (VEGF and bFGF). Recently, two study groups reported that EGFR phosphorylation was activated by EGFR mutations in tumor samples from patients who showed a clinical response to gefitinib (34,35). These mutations are all clustered around the ATP-binding site of the tyrosine kinase domain and lead to increased growth factor signaling. Sordella et al. (36) reported that these EGFR mutations activated Akt and signal transduction and activator of transcription signaling pathways that promote cell survival in lung cancer cell lines. However, there have been no previous reports concerning the relationship between these EGFR mutations and the signaling pathway that promotes angiogenesis. If these EGFR mutations activate a signaling pathway that promotes angiogenesis, gefitinib treatment should block angiogenesis in responders to gefitinib. Based on this hypothesis, responders to gefitinib should show a reduction in the increased levels of angiogenic growth factors after gefitinib treatment. However, in the present study, serum levels remained unchanged in most patients following gefitinib treatment, even in those who showed a response to gefitinib. In contrast to pre-clinical studies (21,22), our clinical trial did not show a reduction in angiogenic growth factor levels. Gefitinib may not have the potential to reduce angiogenic growth factor levels and may cause tumor reduction without blocking subsequent signaling pathways that promote angiogenesis in patients with NSCLC treated with gefitinib.

In conclusion, although our results indicate that gefitinib treatment does not affect circulating levels of angiogenic growth factors even in patients who showed a response to gefitinib treatment, low levels of VEGF may predict response to gefitinib treatment in patients with NSCLC.

References


