C-Met immunoreactivity in operated stage I non-small cell lung cancer patients: Is it a prognostic marker?

Opere edilmiş evre I küçük hücreli dışı akciğer kanserini olgularda c-Met immünreaktivitesi: Prognostik bir belirteç mi?

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Summary

Aim: In this study we evaluated the correlation between c-Met immunoreactivity and survival, thus the effect of hepatocyte growth factor (HGF) on prognosis, in operated early-stage non-small cell lung carcinoma (NSCLC) patients.

Materials and Methods: Seventy patients who underwent resection for stage IA or IB NSCLC were examined retrospectively. Pathological preparations were evaluated to investigate c-Met immunoreactivity. Those with normal bronchial epithelium in the sections were used as positive control group and the sections of those who had not been stained by primary antibody were regarded as the negative control group. C-Met immunoreactivities were determined according to scores of staining.

Results: There were no significant differences between c-Met immunoreactivity and clinicopathological factors. The analysis after exclusion of large cell carcinoma cases showed that c-Met immunoreactivity was higher in adenocarcinomas than in squamous cell carcinomas and the difference was statistically significant. Survival analysis to estimate the prognostic significance of c-Met immunoreactivity showed no significant differences for both disease-free (p=0.499) and long-term survival (p=0.261). The difference between c-Met immunoreactivity and survival for the adenocarcinoma and squamous cell carcinoma groups was not significant.

Conclusion: It seems that HGF expression and serum HGF levels appeared to be stronger prognostic markers than c-Met immunoreactivity for NSCLC, therefore detailed studies were needed regarding these prognostic factors.

Key Words: Lung cancer, c-Met immunoreactivity, prognosis, survival.
Introduction

Although the survival rates after surgery can be low, the best curative treatment of early stage lung cancer is surgical resection. Local recurrence and distant metastasis rates can be decreased by postoperative adjuvant treatments such as chemotherapy, radiotherapy and chemoradiotherapy. To estimate prognosis, patient factors like age, smoking history, nutrition, physical properties, histopathological factors like stage and tumor cell differentiation, and molecular tumor markers can be used. The studies about prognostic factors have shown that tumor stage is the most important predictor of survival and it can be determined only by surgical procedure. For determination of prognosis, molecular studies on factors like oncogenes, growth factors and chromosomal abnormalities have started in recent years (1,2).

Growth factors that are effective on formation of carcinoma are also used in finding new therapeutic agents and methods (3). The studies with the “Western Blotting” technique have shown that levels of immunoreactive hepatocyte growth factor (HGF) are significantly higher in lung cancer tissues. The difference is more specific for non-small cell lung cancer (NSCLC) compared with other types (4). The HGF level is a good indicator for patient survival, and therefore it can be used as a prognostic marker (5). The expression and amplification of hepatocyte growth factor receptor (c-Met) also increased in lung cancer as well as in stomach, colon, liver, prostate and ovarian cancers (6,7). As reported in studies, the relationship between c-Met immunoreactivity and prognosis is stronger than HGF (5).

The aim of this study is to investigate the interaction between c-Met immunoreactivity and prognosis, thus the effect of HGF on prognosis in operated early-stage NSCLC patients.

Materials and Methods

207 patients who underwent pulmonary resection for primary lung cancer in the Department of Thoracic Surgery of Ege University School of Medicine between January 2000 and December 2007 were analyzed retrospectively. Due to the short follow up duration, patients operated after December 2007 were not included, so that the shortest follow-up period was 24 months. Ninety-four patients who underwent resection for stage IA or IB NSCLC were enrolled in the study. Since survival data could not be accessed or a pathological examination could not be completed, 24 patients excluded from the study. Pathological preparations of patients were evaluated again and c-Met immunoreactivity was investigated.

Protocol of Immunohistochemical (IHC) Evaluation

Sections (5 µm thick) which were obtained from parafin blocks with sufficient tumor tissue and normal lung parenchyma were taken on the electrostatistical slides (X-traTM, Surgipath Medical Industries, Richmond, Illinois, USA) and dried at 60 °C for at least two hours. All IHC staining procedures including deparafinization and antigen revealing were fully performed in the BenchMark XT automated immunohistochemistry staining instrument. Multimeric HRP based, without biotin, containing hydrogen peroxide substrate and 3,3'-diaminobenzidine tetrahydrochlorite (DAB) chromogene kit (ultraView™ Universal DAB Detection Kit, Catalog number 760–500, Ventana Medical Systems, Tucson, AZ) and a full automatic immunohistochemistry staining instrument (Ventana BenchMark XT, Ventana Medical Systems, Tucson, AZ) were used as IHC staining system. Only the c-Met primary antibody (clone 8F11, Novoceastra, 1:500 dilution) was administered manually and samples were incubated at 37°C for 32 minutes. After completion of opposite staining with hematoxylin and bluing solution, steps including dehydration, transparenting of sections with xylen, closure of lamels were performed by hand and then the process was terminated.

Normal bronchial epithelium in the sections were used as positive control. The sections which were prepared for all cases and had not been stained by primary antibody were regarded as negative control. c-Met immunoreactivities were evaluated with the Nakamura et al. method (8). Accordingly, the following scores were assigned: staining (-), absence of staining or only focal weak staining, 1+: weak to moderate staining in less than 40% of cancer cells, 2+: weak to moderate staining in at least 40% of cancer cells, 3+: strong staining in at least 10% of cancer cells, among the specimens with weak to moderate staining in at least 40% of cancer cells. Weak to moderate staining was accepted as same staining or weaker staining than the staining of surrounding bronchial epithelium and strong staining was accepted as more intense staining than the staining of surrounding bronchial epithelium. Cases were divided into two groups as low c-Met (1+/2+) and high c-Met (2+/3+).

Staging of cancer was performed according to the International TNM Classification System for Lung Cancer (9). Survival data were obtained from our own Thoracic Oncology Departments’ records where postoperative follow up of patients have been recorded.

Statistical Analysis

Data analysis was performed using SPSS version 15.0 for Windows. Associations between c-Met immunoreactivity and the other factors were determined.
by the χ²-test and Fisher's Exact Test. The Kaplan-Meier method was used for the analysis of long term survival and disease free survival. The Log-Rank Test was used to evaluate statistical significance of the factors affecting long term survival and disease free survival. p values of less than 0.05 were considered to be statistically significant.

**Results**

The patients included 62 (88.6%) males and 8 (11.4%) females and the mean age of cases was 60.19 ± 9.90 years (range, 35-86 years; median: 60.50). Sixty-two (88.6%) cases had a smoking history ranging from 15-120 packages per year (mean: 41.51 ± 25.51 package per year; median: 40). 31 (44.3%) right superior lobectomy, 15 (21.4%) right inferior lobectomy, 12 (17.1%) left superior lobectomy, 9 (12.9%) left inferior lobectomy and 3 (4.3%) left pneumonectomy were performed. The histopathological diagnosis were adenocarcinoma in 46 (65.7%), squamous cell carcinoma in 18 (25.7%) and large cell carcinoma in 6 (8.6%) patients. Thirty-three (47.1%) patients were classified as stage IA and 37 (52.9%) were classified as stage IB. Tumor sizes were greater than 3 cm in 26 (70.27%) of the stage IB cases, the remaining 11 (29.73%) were staged as IB because of other factors. The mean tumor size was 32.06 ± 16.22 mm (range 5-80 mm; median: 30).

In respect of c-Met immunoreactivity, the score of staining was "-" in 18 (25.7%), "1+" in 16 (22.9%), "2+" in 30 (42.9%) and "3+" in 6 (8.6%). Thirty-four (48.6%) were in the "low c-Met" group and 36 (51.4%) were in "high c-Met" group.

After the analysis of survival data, recurrence was seen in 22 (31.4%) patients and 18 (25.7%) patients were exitus. The means of disease-free survival and long-term survival were 42.76 ± 26.79 months (range 4 - 106 months; median: 43.5) and 46.79 ± 26.50 months (range 5 - 106 months; median: 44.5), respectively. The mean of recurrence time after surgery was 20.77 ± 16.48 months (range 4 - 64 months; median: 16). Average life time of exitus was 25.67 ± 18.78 months (range 5-78 months; median: 24) (Table-1).

There were no significant differences between c-Met immunoreactivity and the other factors. The analysis after the exclusion of large cell carcinomas (because the number of large cell carcinomas was low) showed that the c-Met immunoreactivity was higher in adenocarcinomas than squamous cell carcinomas and the difference was significant (p<0.05) (Table-2). There were no significant differences between c-Met immunoreactivity, disease-free survival and long-term survival (Table-3).

**Table-1. Disease-Free and Long Term Survival of Patients.**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence (+)</td>
<td>22</td>
<td>31.4</td>
</tr>
<tr>
<td>Recurrence (−)</td>
<td>48</td>
<td>68.6</td>
</tr>
<tr>
<td>Exitus</td>
<td>18</td>
<td>25.7</td>
</tr>
<tr>
<td>Alive</td>
<td>52</td>
<td>74.3</td>
</tr>
<tr>
<td>Mean of disease-free survival</td>
<td>42.76 ± 26.79 (4–106) months</td>
<td></td>
</tr>
<tr>
<td>Mean of long-term survival</td>
<td>46.79 ± 26.50 (5–106) months</td>
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</tbody>
</table>

**Table-2. Correlation of c-Met Immunoreactivity with Clinical and Pathological Parameters.**

<table>
<thead>
<tr>
<th></th>
<th>c-Met Immunoreactivity</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>&lt; 65</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>≥ 65</td>
<td>11</td>
</tr>
<tr>
<td>Smoking History</td>
<td>(+)</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>(−)</td>
<td>2</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Adenocarcinoma</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Large cell carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Stage</td>
<td>IA</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>16</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>≤ 3</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>&gt; 3</td>
<td>13</td>
</tr>
</tbody>
</table>

*p value after the exclusion of large cell carcinoma cases from analysis.

**Table-3. Correlations of c-Met Immunoreactivity with Disease-Free Survival and Long-Term Survival.**

<table>
<thead>
<tr>
<th></th>
<th>c-Met Immunoreactivity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Disease-Free Survival</td>
<td>Recurrence (+)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Recurrence (−)</td>
<td>10</td>
</tr>
<tr>
<td>Long-Term Survival</td>
<td>Alive</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Exitus</td>
<td>7</td>
</tr>
</tbody>
</table>
Survival analysis using the Kaplan-Meier method was carried out to estimate the prognostic significance of c-Met immunoreactivity and there were no significant differences for both disease-free survival (p=0.499) and long-term survival (p=0.261) (Figure 1 and 2).

The analysis among the groups of adenocarcinoma and squamous cell carcinoma showed no significant differences between c-Met immunoreactivity and survival for both groups.

**Figure 1.** Disease-free survival according to the c-Met immunoreactivity.

**Figure 2.** Long-term survival according to the c-Met immunoreactivity.

**Discussion**

Many studies have been conducted on the prognostic factors and markers of NSCLC. Tumor size, lymph node involvement and distant metastases have also been used in staging. Several studies have been conducted on research markers that could be identified in patient’s serum and used as a prognostic factor. However, D’Amico et al. (10) examined the hepatocyte growth factor, vascular endothelial growth factor and e-selectine as a prognostic factors and they could not find any significant differences. Although many factors like carcinoembryonic antigen, epithelial growth factor and hepatocyte growth factor were found to be used as a prognostic factor, none of them are recognized as a powerful marker for prognosis (11,12).

In this study, we examined the relationship between c-Met immunoreactivity and survival in operated stage I NSCLC patients and also we investigated whether hepatocyte growth factor could be used as a prognostic factor. While most of the studies about hepatocyte growth factor and c-Met included all stages of NSCLC, this report differs from these studies in that only stage I NSCLC cases were included in our study.

Firstly we investigated the correlation between c-Met immunoreactivity and the other clinical variables and we could not find any significant relation. In similar studies it was reported that there had been no significant relationship between c-Met immunoreactivity and clinical variables such as age, sex, smoking history (4,8,13,14).

Chen et al. (15), studied the relationship between smoking history and the production of hepatocyte growth factor and reported increased expression of hepatocyte growth factor in type II pneumocytes both in healthy smokers and smokers with NSCLC. The researchers indicated that the patients in the healthy group with increased expression of growth factor had a higher risk of developing lung cancer.

There were no significant differences between c-Met immunoreactivity and the pathological parameters such as histopathological diagnosis, stage and tumor size. The analysis after the exclusion of large cell carcinoma showed that c-Met immunoreactivity was significantly higher in adenocarcinoma than squamous cell carcinoma. Ichimura et al. (16), reported that c-Met immunoreactivity had been 77% in adenocarcinoma and 37% in squamous cell carcinoma. Harvey et al. (17) reported that almost all squamous cell carcinoma cells have shown c-Met immunoreactivity but the reactivity has been more powerful and higher in adenocarcinoma cells. Tsao et al. (18), reported that adenocarcinoma cells’ c-Met expression had been three times higher than the normal bronchial epithelium and more than 90% of squamous cell carcinomas had shown lower c-Met protein levels than normal epithelium. The high c-Met immunoreactivity in adenocarcinoma can be related to the positive effect of hepatocyte growth factor on glandular differentiation of lung and development of adeocarcinoma from the differentiation of these neoplastic glandular cells.

The high levels of c-Met immunoreactivity in adenocarcinoma brought about the studies on hepatocyte growth factor to be conducted in this tumor group. Takanami et al. (5), suggested there was significant evidence on how this situation caused that the activation of autocrine HGF-c-Met signal pathways and as a result of this the activation of tumorigenesis and...
differentiation in adenocarcinoma. In addition, Yi et al. (19) announced that mechanisms and regulators that make up this autocrine effect should be investigated.

Analysis conducted in order to evaluate hepatocyte growth factor receptor immunoreactivity as a prognostic factor in early stage NSCLC, which was the basic purpose of our study, showed no significant relationship for both disease-free survival and long-term survival. Nakamura et al. (20), stated that no significant difference between c-Met immunoreactivity and disease-free survival and long-term survival in cases with stage I-III adenocarcinoma, which were similar in our study. On the other hand, as a result of their study on adenocarcinoma cases Takanami et al. (5), reported a significant relationship between overexpression of c-Met and short survival. Siegfried et al. (21), investigated c-Met receptor and its ligand hepatocyte growth factor in their study and reported a significant negative correlation between increased HGF expression and survival. They emphasized that the significance was valid for both two groups including early staged cases and all cases. In the study published in 1998 and resumed by the same researchers (22), a significant relationship between c-Met immunoreactivity and survival was reported. A negative correlation between increased serum HGF levels and prognosis in various organ carcinomas such as breast and pancreas was reported in several studies (23,24). As a result of these studies and our study, it is understood that HGF expression and serum HGF level are stronger and more significant prognostic markers compared to c-Met immunoreactivity.

Conclusion

The majority of the studies about HGF and c-Met included groups which were formed from patients of all stages. The difference in survival between stage I and other stages can be determined according to tumor size and lymph node metastasis, but the number of prognostic factors to determine the difference in survival between the patients in the same stage is very low. This is because why we examined the patients only in stage I. We could not find any significant difference between c-Met immunoreactivity and survival, neither in the patient groups of the same stage nor in the patient groups of the same histopathology. We suggest that studies about HGF expression and serum HGF levels be planned because it seems that these factors appeared to be stronger prognostic markers than c-Met immunoreactivity for NSCLC patients.

References