Plasma Hepatocyte Growth Factor Elevation May Be Associated with Early Metastatic Disease in Primary Lung Cancer Patients

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Purpose: Hepatocyte growth factor (HGF), a ligand of the c-met proto-oncogene, exhibits activating effects on human lung cancer both in vitro and in vivo. However, few studies have reported the correlations between concentration changes of blood HGF and postsurgical prognosis.

Methods: We evaluated whether surgery-related blood HGF elevation has prognostic significance in patients with surgically resected non-small cell lung cancer. We examined blood HGF concentration, c-met expression, and postoperative prognosis of 25 cases of primary resected, non-small cell lung cancer.

Results: We divided the patients into 2 groups according to receiver operating characteristics curve analysis using 7.2 ng/mL as the cut-off value of blood HGF concentration. Survival curve analysis revealed that patients with a high level of HGF (over the cutoff value) exhibited a poor prognosis of metastatic disease, compared to those in the low-level group after curative surgery (log rank test, \( P = 0.020 \); Wilcoxon test, \( P = 0.016 \)).

Conclusion: Elevation of HGF in plasma may be an important prognostic factor for early metastatic disease in patients with primary lung cancer. Moreover, inhibition of HGF elevation may have therapeutic effects on early distant metastasis of lung cancer.

Keywords: hepatocyte growth factor, c-met, non-small cell lung cancer

Introduction

Hepatocyte growth factor (HGF), a ligand of the c-met proto-oncogene, has been reported to have tumorigenic, vascularizing, and motogenic effects on human lung cancer both in vitro and in vivo. It has also been reported that tumor HGF content has prognostic implications in patients with non-small cell lung carcinoma.1-6

Open thoracotomy and pulmonary resection are invasive procedures used for treating lung cancer. Major surgical operations inevitably stimulate the blood clotting system. HGF is converted at injured organs from pro-HGF protein by an HGF activator circulating in the blood plasma, assisted by thrombin and plasminogen activators. Several investigators have reported that high HGF content in tumors may be an important prognostic factor.
after curative surgical resection. However, there are few reports regarding the correlation between an increase in the concentration of surgery-related blood HGF and post-surgical prognosis in patients with non-small cell lung cancer.

Some patients have recurrent cancers that rapidly begin growing soon after surgery. The growth speed of these recurrent tumors is sometimes more rapid than that before surgery. Surgical procedures induce many types of cytokine production as well as growth factor production due to organ injury. We hypothesized that elevated HGF levels in the blood plasma after surgery stimulates the potential for tumor invasion or distant metastasis. Generally, HGF produced in specific cells influences target cells in a paracrine manner as confirmed by in vivo experiments. Additionally, some investigators have reported that HGF production is caused by several types of tissue damage.

Surgical injury itself may be a trigger for cytokine production or HGF conversion by HGF activator. Several in vitro studies have revealed that HGF activation results from elevated IL-6 expression. Molecular biology experiments indicate that HGF is closely related to a blood protease precursor. HGF is activated by thrombin present in the circulating blood. Furthermore, HGF, which is induced by some types of injury, has wide target cell specificity, affecting several organs and tissues. HGF is known to play an important role as a disseminating factor in in vitro cell culture solutions.

These characteristics suggest that HGF plays an important role in inducing recurrent disease, including distant organ metastasis, which is not detectable by radiographic or serologic examination; HGF may also influence cancer cells circulating in blood vessels or affect small implanted diseases.

We aimed to elucidate the relationship between circulating blood HGF concentration increased by surgical injury after operations and clinical prognosis in the context of distant metastasis of lung tumors by using biochemical assessments in primary lung cancer patients.

Materials and Methods

Tumor specimens from 25 patients with primary lung cancer were examined. All patients included in the study underwent curative lung resection at the Thoracic Surgery Center of Hokushin General Hospital, Nagano, Japan between October 1999 and October 2000. All patients underwent standard anatomical resection by lobectomy or pneumonectomy with complete mediastinal and hilar lymph node dissection. No patient received adjuvant chemotherapy or radiotherapy after surgery until recurrence was detected. Pathological stages were established according to TNM classification.

Statistical analysis was applied to all tissues and blood samples examined. Tumors were histologically categorized according to standard World Health Organization (WHO) criteria. Examined carcinomas included 12 adenocarcinomas, 10 squamous carcinomas, and 3 large cell carcinomas. All patients showed pathological stages from IA to IIB. Recurrences were documented at 2-week intervals by physical examination, chest radiography, and sampling of serum tumor markers, at 3-month intervals, by whole-body computed tomography and brain magnetic resonance imaging (MRI), and at 6-month intervals, by bone RI-gram. Other parameters evaluated for their relationship to prognosis included stage, age, gender, histological type, smoking history, operation time, and volume of blood loss during operation.

The mean follow-up period was 48.2 ± 19.4 months. Venous blood samples were obtained from each patient and placed in 2Na-EDTA-coated tubes 1 hour before the operation and at 24 and 72 hours after surgery. Blood samples were centrifuged at 4°C immediately after blood sampling, and sample plasmas were preserved in a freezer at -70°C. The concentration of circulatory HGF in the blood was measured using an ELISA kit (AN’ALYZA® Immunoassay System; Tecan Corporation, Minneapolis, MN, USA). This assay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for HGF had been pre-coated onto the microplate. The optical density of each sample was determined using a microplate reader (Tecan classic) set at 450 nm. Wavelength correction was set to 540 nm. Data were analyzed using LS-Plate Manager 2000 for Windows™ (Wako Pure Chemical Industries, Ltd., Japan).

All patients were strictly followed for 2 years. Additionally, patients who developed distant metastatic lesions within 2 years were defined as being in the early relapse group. On the basis of this definition, we divided the patients into 2 groups: the early relapse group and the non-early relapse group. Subsequent receiver operating characteristic (ROC) curve analysis using GraphPad Prism software 5.0a for Macintosh (GraphPad Software, Inc.) revealed a cut-off value of 7.2 ng/mL HGF concentration 24 hours after the operation. This value correlated with data from in vitro experiments investigating invasive or metastatic activity by HGF in several human lung cancer cell lines.
Patients with a plasma concentration of HGF exceeding 7.2 ng/mL were defined as being in the high-level HGF group, and those under 7.2 ng/mL were in the low-level HGF group. Patients within our observation period who showed no further evidence of disease were categorized as disease-free, and patients who had a first distant metastasis or another type of recurrence were categorized as being non-disease-free. Comparisons of disease-free survival and overall survival curves between the 2 HGF concentration groups (high-level HGF group vs. low-level HGF group) were carried out using the Kaplan-Meier method, as well as the log rank test and the generalized Wilcoxon test.

Statistical analyses were performed using GraphPad Prism software 5.0a for Macintosh (GraphPad Software, Inc.) and Stat View 5.5J software for Windows (SAS Institute, Cary, NC, USA).

Multivariate analyses of variances were performed to confirm between-group correlations for operation time, blood loss, removed volume of the lung (number of segment), gender, and age. Moreover, multivariate analyses (logistic regression analyses) were performed to evaluate the risk between variables: pathological stages, pathological types, HGF concentration as a dependent value, and prognosis for early distant metastasis as an independent value.

We attempted to evaluate the correlation between changes in HGF concentration and c-met expression in lung cancer cells. Each specimen was obtained from surgically resected lung tumors. Specimens were fixed with 20% of formalin and embedded into paraffin sections. Next, microwave irradiation was applied to each specimen for antigen activation. Anti-c-met monoclonal antibody (Novocastra Laboratories LTD, Newcastle, UK) was added to each specimen. We evaluated c-met expression by measuring the positive cell rate per 2000 cells at 5 high power fields. A mean c-met positive cell rate (percent) of greater than 50% was defined as over-expression, 10 to 49% was defined as middle degree expression, less than 10% was considered minimal expression, and less than 1% was considered negative.

**Results**

**Changes in blood plasma HGF concentration before and after surgery**

Mean preoperative HGF concentration in the patients’ plasma was 0.69 ± 0.25 ng/mL in our assay. These values were nearly identical to the values from normal sample data provided by an independent laboratory. The 24-hour value for mean HGF level was 5.59 ± 0.38 ng/mL, with a median value of 5.30 ng/mL. In nearly all cases, HGF peaked at 24 hours postoperatively (the peaks of HGF levels appear at 24 hours in the data from 7 patients based on hourly laboratory tests; data now shown), and then gradually fell to the normal level (1.73 ± 1.34 ng/mL) at 72 hours after surgery (Fig. 1).

Subsequent receiver operating characteristics (ROC) curve analysis revealed a cut-off value of 7.2 ng/mL ($P = 0.018$) for HGF concentration at 24-hours after operation between the early relapse group and the other patients (Fig. 2).
Table 1  Clinical profile of the patients for analysis

<table>
<thead>
<tr>
<th></th>
<th>HGF &lt;7.2 ng/ml</th>
<th>HGF &gt;7.2 ng/ml</th>
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</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>age</td>
<td>66.6 ± 7.7</td>
<td>76.1 ± 7.0 P = 0.069</td>
</tr>
<tr>
<td>gender</td>
<td>male 14</td>
<td>male 6</td>
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<tr>
<td></td>
<td>female 3</td>
<td>female 2</td>
</tr>
<tr>
<td>smoking history</td>
<td>yes 11</td>
<td>yes 6</td>
</tr>
<tr>
<td></td>
<td>no 6</td>
<td>no 2</td>
</tr>
<tr>
<td>histology</td>
<td>adeno 7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>others 10</td>
<td>3</td>
</tr>
<tr>
<td>pathological-stage</td>
<td>la 6</td>
<td>la 2</td>
</tr>
<tr>
<td></td>
<td>lb 8</td>
<td>lb 5</td>
</tr>
<tr>
<td></td>
<td>2a 1</td>
<td>2a 0</td>
</tr>
<tr>
<td></td>
<td>2b 2</td>
<td>2b 1</td>
</tr>
<tr>
<td>c-met over-expression</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>removed volume (seg.)</td>
<td>4.1 ± 1.0 seg.</td>
<td>5.3 ± 2.7 seg.</td>
</tr>
<tr>
<td>Blood loss</td>
<td>311 ± 189 ml</td>
<td>488 ± 328 ml</td>
</tr>
<tr>
<td>operation time</td>
<td>333 ± 119 min</td>
<td>360 ± 118 min</td>
</tr>
<tr>
<td>recurrence within 24mths</td>
<td>1</td>
<td>4</td>
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<tr>
<td>overall 5year survival</td>
<td>88.2%</td>
<td>50%</td>
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HGF: hepatocyte growth factor

Table 2  Multivariate regression analysis between early metastasis and clinical parameters

<table>
<thead>
<tr>
<th>parameters</th>
<th>Odds ratio</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>HGF value more than cut off value</td>
<td>16.7</td>
<td>0.04</td>
</tr>
<tr>
<td>pathological stage (1a–2b)</td>
<td>NE</td>
<td>0.90</td>
</tr>
<tr>
<td>histological type</td>
<td>NE</td>
<td>0.03</td>
</tr>
<tr>
<td>smoking history</td>
<td>1.31</td>
<td>0.86</td>
</tr>
</tbody>
</table>

HGF: hepatocyte growth factor; NE: not evaluated; NS: no significant

There was no significant relationship between postoperative HGF elevation and clinical data regarding operation time, blood loss, removed volume of the lung (number of segment), pathological stage, pathology, c-met over-expression, and gender between groups (Table 1). However, multivariate analyses of variance (MANOVA) revealed that postoperative blood HGF concentration was related to patient age (P = 0.0069); postoperative HGF concentration is correlated to age (R = 0.4865, P = 0.013) (Fig. 3).

Multivariate analysis (logistic regression analyses) revealed that pathological types and high HGF concentration over the cutoff value were significantly related to prognosis of 24-month recurrence (P = 0.04, P = 0.03 respectively). However, pathological stage (1a–2b) had no significant relationship with 24-month recurrence (P = 0.90) in our study (Table 2).

During the follow-up period, 19 patients survived without recurrent disease, 4 cases (high-level HGF group) developed relapse lesion within 24 months, and 2 cases (low-level HGF group). The mean follow-up period for first relapse of disease and overall survival was 48.2 ± 19.4 months (11 to 72 months).

Survival analysis

In this study, we defined the high-level HGF group as that with HGF values exceeding 7.2 ng/mL at 24 hours after surgery. In this group (n = 8), 4 patients relapsed (all distant metastasis) and 4 patients showed no further evidence of disease. In contrast, in the low-level HGF group (n = 17), only one patient relapsed, and 15 patients showed no further evidence of recurrent disease. Survival

Fig. 3  Correlation between post-operative blood HGF concentration (after 24 hours) and age.

HGF: hepatocyte growth factor
curve analysis revealed a significant difference between the 2 groups with respect to tumor relapse (log rank test: $P = 0.032$, generalized Wilcoxon test: $P = 0.028$) (Fig. 4).

In this study, the Kaplan-Meier method indicated an overall 5-year survival rate of 72%.

Subsequently, analysis of survival revealed that the high-level HGF group had a poor prognosis compared to the low-level HGF group. The 5-year survival rate of the low-level HGF group was 87.8%; in contrast, this rate for the high-level HGF group was only 50%. There was a significant difference in overall survival between the high- and low-level groups (log rank test; $P = 0.020$, Wilcoxon test; $P = 0.015$) (Fig. 5).

**Discussion**

HGF has been described as having tumorigenetic, vascularizing, and motogenic effects on human lung cancer, both in vitro and in vivo, and to have prognostic implications in patients with non-small cell lung carcinoma.\(^{1-11}\) Although many reports suggest that HGF is produced by cancer cells or by synthesis of mRNA for HGF and may affect patient prognosis,\(^{7,10}\) serum or plasma HGF content has not been regarded as a prognostic factor. Indeed, tumor HGF content or production and its autocrine\(^{16}\) or paracrine-like effects\(^{7}\) may be strong prognostic factors, but do not explain clinical findings in patients showing tumor relapses after surgery with rapid growth detected in annual radiological examinations.

Some cancer cell lines show tumorigenicity and invasive activity over a threshold concentration of HGF in culture solution (more than 5 to 10 ng/mL)\(^{7,10}\). The cut-off value of blood HGF for the early relapse group (7.2 ng/mL at 24 hours after surgery) was essentially coincidental with the value of in vitro examination. HGF concentration values for our patients were examined at several time points during the perioperative period.

We observed that for some cases lung cancer relapses very soon after curative surgery despite its relative early stage according to the TNM classification. Many of these recurrences consist of distant metastasis.\(^{17}\) Nearly all patients who developed distant metastasis may also have micro-metastases or small implantation before surgery.\(^{18,19}\) Some reports suggest that cancer cells may drain into the blood and remain in circulation in some advanced cancer patients.\(^{17,20-24}\) It is likely that this phenomenon also occurs in lung cancer patients, particularly in adenocarcinoma patients, since undetectable cancer lesions or circulating cancer cells are necessary to develop postoperative metastatic disease. Multivariate regression analysis revealed that pathological type and high HGF concentration were the most distinctive risk factors for early distant metastasis of lung cancer patients.

Generally, HGF assists with injured organ reconstruction.\(^{8,11}\) There is evidence of the presence of thrombin-triggered activation of an HGF activator (a protease) already circulating in the blood.\(^{15}\) Activating the HGF activator results in proteolytic cleavage of pro-HGF (single chain HGF) in injured organs.\(^{8}\) Proteolytic cleavage is essential for HGF activation of c-met.\(^{8}\) Many biochemical pathways strictly control this mechanism, but there is no organ specificity in the reaction of HGF to organ reconstruction.\(^{8}\)

HGF elevation occurs postoperatively due to surgical injury near the cancer lesion or in normal tissues.\(^{11-13}\) Blood HGF concentration increases in patients who have
undergone major surgery or inflammation or patients suffering from Systemic Inflammatory Reaction Syndrome or pancreatitis. According to in vivo models, tumor metastasis to the lung may be enhanced by normal tissue injury.

Tumor cells circulating in the peripheral blood were detected using several techniques: immunohistochemistry, RT-PCR, and flow cytometry. Notably, using RT-PCR, the m-RNA of several markers of tumor cells has been frequently detected in the blood of patients with solid tumors. Additionally, the presence of circulating cancer cells does not necessarily result in the development of tumor metastatic lesions with clinical significance. Most cancer cells entering blood circulation are killed by mechanical, immunological, or unknown mechanisms. However, there is evidence that metastatic diseases are the major cause of death in patients with cancer. These findings support the hypothesis that early tumor cell migration or dissemination from the primary lesion occurs in solid malignant tumors as a systemic disease component.

High blood plasma concentrations of HGF may affect these circulating tumor cells. According to pathological examination, the presence of circulating cancer cells or micro-metastatic lesions is a prerequisite for the development of fatal metastasis at a relatively early stage after curative surgery. Many studies confirm HGF activity in cancer cells both in vitro and in vivo.

In vitro experiments suggest that only 5 to 10 ng/mL of HGF is sufficient to activate tumor cell activity, leading to invasion or metastasis. Moreover, circulating HGF may have direct effects on micro-metastatic lesions. HGF promotes tumor growth in vivo models of a human lung cancer cell line in which cells are seeded into the lumen of airways. This study suggests that HGF may result in tumor growth after one administration.

Although our study is not conclusive, the results suggest that there are 2 types of patients: those with high-level HGF and those with relatively low-level HGF after surgery. The concentration of HGF in blood may affect recurrent disease, including distant metastasis, within a relatively short period following surgery. Increased blood HGF is correlated with age in our study. Therefore, it is inappropriate to avoid elevation of post-surgical plasma HGF for a better outcome after surgery.

There are some extrinsic factors of HGF down regulation. Interleukin-4, interleukin-12, glucocorticoids, and some protease inhibitors may inhibit HGF elevation during surgery. Alternatively, the administration of HGF antagonists may have the same effect as prevention of the elevation of HGF in blood. NK4 is a known HGF antagonist, and novel NK4 (HGF antagonist/angiogenesis inhibitor) administration may inhibit tumorigenetic action of lung cancer.

In our study, we could not conclude the main cause of HGF elevation during surgical periods. Further investigation is necessary to verify the pathway of injury inducing HGF elevation, particularly in thoracic surgery.

Conclusion

We conclude that surgical procedures for lung cancer results in significant elevation of levels of HGF in blood, and in some cases, an excessive HGF increase results an increased probability of recurrent disease, including distant metastasis following surgery. Since this is a retrospective study examining a limited number of cases, a prospective validation study is necessary to confirm that serum-HGF is a useful prognostic marker after surgery.

Reference

Blood HGF Associates with Poor Prognosis in NSCLC


