Serum Heparan Sulfate Concentration is Correlated with the Failure of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Treatment in Patients with Lung Adenocarcinoma

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Introduction: The epidermal growth factor receptor (EGFR) mutation status is a validated biomarker for the stratification of EGFR-tyrosine kinase inhibitor (EGFR-TKIs) treatment in patients with non-small cell lung cancer (NSCLC); however, its use is limited in patients with wild-type EGFR, and new biomarkers are needed. We hypothesized that the serum concentration of heparan sulfate (HS), which activates oncogenic growth factor receptor signaling through Heparinase and non-EGFR signaling pathways, may be a novel glycobiological biomarker for EGFR-TKIs treatment in NSCLC.

Methods: The pretreatment serum HS concentrations were determined using enzyme-linked immunosorbent assay in 83 patients with stage IV non-small cell lung adenocarcinoma who received EGFR-TKIs treatment. The relationship between the serum HS concentrations and patient characteristics, tumor response, progression-free survival (PFS), and overall survival (OS) were analyzed.

Results: Patient sex, performance status, smoking history, and EGFR mutation status were associated with tumor response. The serum HS concentrations were significantly higher among patients with progressive disease than among those without progressive disease (p = 0.003). Furthermore, the serum HS concentrations were strongly associated with a poor PFS and OS in a univariate Cox analysis (p = 0.0022 and p = 0.0003, respectively). A stratified multivariate Cox model according to the EGFR mutation status showed that higher HS concentrations were significantly associated with a shorter PFS and OS (p = 0.0012 and p = 0.0003).

Conclusion: We concluded that a high-serum HS concentration was strongly related to a poor treatment outcome of EGFR-TKIs and may be a promising noninvasive and repeatable glycobiological biomarker in cancer treatment.

Key Words: Heparan sulfate, Non-small cell lung cancer, EGFR-tyrosine kinase inhibitors.

(J Thorac Oncol. 2011;6: 1889–1894)

Heparan sulfate proteoglycans (HSPGs) are composed of a core protein and one or more heparan sulfate (HS) glycosaminoglycan (GAG) chains. Many studies have demonstrated the importance of these molecules in development and normal physiology including metabolism, transport, information transfer, support, and regulation at the systemic level and the cellular level. Heparin and HS consist of repeating disaccharide units that comprised a hexuronic acid and a D-glucosamine linked to each other and to other disaccharides by 1α4 linkages. The HS component sugars (N-acetylgalactosamine and β-D-glucuronic acid/α-L-iduronic acid) and patterns of sulfating modifications create an extraordinarily large potential for structural diversity. The structural diversity of HS is considered to be important because HS can bind and interact with a wide variety of proteins including thrombin, fibroblast growth factors (FGFs) 1 and 2, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), interleukin-8, MIP-1β, P-selectin, laminin, and fibronectin. Such interactions are thought to mediate the enhancement of growth factor/receptor signaling activity, promote tumor growth, regulate differentiation, induce angiogenesis, modulate host immune cell responses to tumor cells, and promote metastasis in cancer cells. Among the biological activities of HS, a large body of structural data.
has demonstrated that HS enhances FGFs/FGF receptor signaling by acting as a template that bridges FGF and the FGF receptor. A structure-based proposal for an HS sequence able to bind FGF and FGFR showed that the interaction between HS and FGFs or FGFRs seemed to be determined by a specific sequence of 5-10 saccharides with sulfating modifications. Other growth factor/receptor interactions may follow a similar binding and activation process. Thus, HS and HSPG expression may enhance the activity of oncogenic growth factor receptor signaling in cancer cells.

Meanwhile, selective epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) block EGFR signal transduction pathways implicated in the proliferation and survival of cancer cells and have exhibited clinical activity against non-small cell lung carcinoma (NSCLC). Several clinical and molecular biomarkers of EGFR-TKI treatment have been identified such as gender, smoking status, NSCLC histology, East Asian ethnicity, and an active EGFR mutation status that confers constitutively active tyrosine kinase activity and a hyperresponsiveness to gefitinib among patients with NSCLC. These mutations are observed mostly in exons 18 (G719A/C) and 21 (L858R and L861Q) or in-frame deletions in exon 19 located at position 745. Two recent phase III trials targeting adenocarcinoma in patients with NSCLC with EGFR mutations have demonstrated that the gefitinib group had a significantly longer progression-free survival (PFS) than the platinum-doublet therapy group. This data indicated that the EGFR mutation status is a powerful predictor of the tumor response to EGFR-TKIs.

Recently, we have shown that the serum concentrations of heparin binding growth factors including heparin-binding EGF-like growth factor (HB-EGF), HGF, and VEGF are closely related to the treatment response of EGFR-TKIs in patients with NSCLC. Our results have demonstrated that the serum concentrations of these growth factors were strongly related to the outcome of EGFR-TKIs treatment and suggest that these levels could be used to refine the selection of patients expected to respond to EGFR-TKIs treatment. On the basis of these findings, we speculated that the serum concentration of HS, which activates oncogenic growth factor receptor signaling through EGFR and non-EGFR signaling pathways, may be a novel glycobiological biomarker for EGFR-TKIs treatment in NSCLC. Identifying such a marker would contribute to the further individualization of treatment for NSCLC. In this report, we retrospectively studied the pretreatment serum HS concentrations in patients with stage IV non-small cell lung adenocarcinoma who underwent treatment with EGFR-TKIs.

**UNITED STATES**

**JUMP TO PAGE**

**PATIENTS AND METHODS**

**Patients**

Pretreatment serum samples from histologically confirmed adenocarcinoma and patients with stage IV NSCLC ($n = 93$) were evaluated in this study. Six patients were excluded because their tumor response was not evaluated. Three additional patients were excluded because a complete clinical data set was not available, and a sufficient serum sample was not available for one patient. Thus, 83 patients were included in the final analysis. All the patients had been treated with EGFR-TKIs (gefitinib, $n = 78$; erlotinib, $n = 5$) at one of three centers (Kanazawa University, Japan; Cancer Institute Hospital, Japan; and Tokyo Medical University, Japan). The tumor response was evaluated every 2 to 3 months using computerized tomography according to the Response Evaluation Criteria in Solid Tumors; the response was then classified as a complete response, a partial response (PR), stable disease (SD), or progressive disease (PD). Clinicopathological features including age, sex, Eastern Cooperative Oncology Group performance status (PS), TNM stage, smoking status and EGFR mutation status were recorded. To detect active EGFR mutations, direct sequencing of a tumor sample was performed in 37 patients; 18 of these samples were found to harbor an EGFR mutation, whereas the remaining 19 samples exhibited wild-type EGFR. The mutation status of the other 46 patients was not evaluated. The median follow-up period was 8.2 months. This study was approved by the institutional review boards of all the centers involved in the study.

**Preparation of Serum Samples**

Blood samples were collected before the initiation of EGFR-TKI treatment. The separated serum was stocked at −80°C until use.

**Serum HS Concentrations**

Serum HS concentrations were determined using a human heparan sulfate enzyme-linked immunosorbent assay (ELISA) Kit (Code. No. 280564; Seikagaku Biobusiness, Tokyo, Japan). This sandwich-type ELISA kit is composed of two specific monoclonal antibodies recognizing the disaccharide units of HS. It specifically detects HS but does not crossreact with heparin, hyaluronic acid, chondroitin sulfate (CS), or keratin sulfate. In brief, a 50 µl aliquot of serum was treated with 5 µl of actinase E at a concentration of 20 µg/ml at 37°C for 20 hours; the reaction was stopped by heating at 100°C for 5 minutes. The sample was then centrifuged at 10,000 rpm for 10 minutes, and the supernatant (20 µl) was used for the analysis. The sample was diluted with 40 µl of the reaction buffer. Then, 20 µl of the samples were measured in duplicate according to the manufacturer’s instructions. The absorbance of the samples at 450 nm and 630 nm was measured using VERSAmax (Japan Molecular Devices, Tokyo, Japan). The average was used for the subsequent analyses.

**Statistical Analysis**

The primary objective was to investigate novel markers correlated with treatment efficacy independently of EGFR status. If a molecule was very strongly associated with survival after adjustments for the EGFR status and important prognostic factors, then that molecule was deemed as warranting further prospective study to determine whether it was a predictive factor, a prognostic factor, or both. The distributions of the clinical factors were compared between patients with PD and those without PD using the Fisher’s exact test. In terms of the analysis for survival time (PFS and overall survival [OS]), clinical factors including age, sex, Eastern Cooperative Oncology Group PS, and smoking status were
examined using the Cox proportional hazards model. After selecting the important clinical variables, we considered these variables fixedly in a Cox proportional hazards model and then determined whether the molecule was associated with survival independent of the important clinical variables at a two-sided significance level of 0.05. Log-transformed values were used for the molecule in the Cox models. The proportional hazards assumption was assessed graphically and using an individual time-dependent component for each covariate. In the multivariate Cox models, the EGFR status (wild type/mutant/unknown) was treated as a stratified variable. We applied the above analyses to all the cases, to the cases in which the EGFR status was evaluated, and to the cases with wild-type EGFR to check the robustness of the conclusions. The survival curves for PFS and OS were estimated using the Kaplan-Meier method. All the statistical analyses were performed using SAS for Windows (version 9.1.3).

RESULTS

Patient Characteristics and Tumor Response

The patient characteristics are listed in Table 1. All 83 patients were of Asian ethnicity and had been treated with EGFR-TKIs (gefitinib, n = 79; erlotinib, n = 4). Sixty-six (80%) and four (5%) patients had previously received chemotherapy and radiotherapy, respectively. Nineteen patients had wild-type EGFR, 18 had active mutations (exon 19, n = 13 and exon 21, n = 5), and 46 had an unknown status because their samples had been collected before the identification of this biomarker.12,13 Regarding the response to EGFR-TKIs treatment, a PR was observed in 34 (41%) patients, SD was observed in 20 (24%) patients, and PD was observed in 29 (35%) patients; none of the patients exhibited a complete response. Significant differences in the tumor response were observed for patients characteristics such as a sex (p = 0.0002), PS (p = 0.04), smoking history (p = 0.003), and EGFR status (p = 0.00001). These findings were consistent with those of many previous reports.

Serum Concentrations of HS and Tumor Response

The serum concentration of HS ranged from 3.3 to 85.8 µg/ml in all the patients (Table 1) and were over 20 µg/ml in 13 patients, indicating the presence of large individual differences in serum HS concentration. The serum HS concentration is shown for the tumor response groups in Figure 1. Of note, the serum HS concentration was significantly higher among patients with PD (22.2 ± 23.1 µg/ml) than among those without PD (10.9 ± 9.8 µg/ml, p = 0.003). The sensitivity and specificity of HS for discriminating PD from PR + SD were determined using the optimal cutoff value (13.5 µg /ml) obtained from a receiver operating characteristic (ROC) curve according to a previous report.17 The sensitivity and specificity of HS for discriminating PD from PR + SD were 0.448 and 0.851, respectively.

Univariate Analysis of Clinical Molecular Factors for PFS and OS

The median PFS and OS were 4.1 and 10.2 months, respectively. Among the clinical factors that were examined, a male sex, a positive smoking history, and a poor PS were significantly related with a poor PFS and OS (Table 2). A higher serum HS concentration was significantly associated with a shorter PFS (HR, 3.61; p = 0.0003) and OS (HR, 5.57; p = 0.0003; Table 2). Thus, similar to the results for tumor response, a high serum HS concentration was closely associated with a poor EGFR-TKIs treatment outcome. Figures 2A, B shows the Kaplan-Meier estimates for PFS and OS with respect to the concentrations of serum HS. All the patients were divided into two groups according to the cutoff value (13.5 µg /ml) described earlier. The curves indicated that the high serum HS group had a significantly poorer treatment outcome with respect to both PFS (median, 47 versus 161 days; p = 0.002) and OS (median, 105 versus 406 days; p = 0.0002).

Multivariate Analysis of Clinical Molecular Factors for PFS and OS

As the EGFR status of half the patients in this study was unknown, we used a stratified multivariate Cox analysis

<p>| TABLE 1. Patient Characteristics, Serum Concentration of Heparan Sulfate, and Response to EGFR-TKIs |
|-----------------------------------------------|-------------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Total (n = 83) (%)</th>
<th>PR + SD (n = 54)</th>
<th>PD (n = 29)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td>≤65</td>
<td>43 (52)</td>
<td>27</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>&gt;65</td>
<td>40 (48)</td>
<td>27</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.0002</td>
</tr>
<tr>
<td>Male</td>
<td>46 (55)</td>
<td>22</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>37 (45)</td>
<td>32</td>
<td>5</td>
<td></td>
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<tr>
<td>PS</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>0–1</td>
<td>60 (72)</td>
<td>43</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>23 (28)</td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Yes</td>
<td>51 (61)</td>
<td>27</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 (39)</td>
<td>27</td>
<td>5</td>
<td></td>
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<tr>
<td>CTx</td>
<td></td>
<td></td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>Yes</td>
<td>66 (80)</td>
<td>41</td>
<td>25</td>
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<td>No</td>
<td>17 (20)</td>
<td>13</td>
<td>4</td>
<td></td>
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<td>RTx</td>
<td></td>
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<td>0.08</td>
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<td>Yes</td>
<td>4 (5)</td>
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<td>3</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>79 (95)</td>
<td>53</td>
<td>26</td>
<td></td>
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<tr>
<td>EGFR status</td>
<td></td>
<td></td>
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<td>0.00001</td>
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<tr>
<td>Wild</td>
<td>19 (23)</td>
<td>6</td>
<td>13</td>
<td></td>
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<tr>
<td>Mutant</td>
<td>18 (22)</td>
<td>18</td>
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<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>46 (55)</td>
<td>30</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Range</td>
<td>3.3–85.8</td>
<td>3.3–51.0</td>
<td>3.8–85.8</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>14.9 ± 16.5</td>
<td>10.9 ± 9.8</td>
<td>22.2 ± 23.1</td>
<td>0.003</td>
</tr>
</tbody>
</table>

p values are calculated using the t test for serum concentration of heparan sulfate and the Fisher’s exact test for other variables.

* Comparison between wild type and mutant.

HS, serum concentration of heparan sulfate (µg/ml); PR, partial response; SD, stable disease; PD, progressive disease; PS, performance status; EGFR-TKIs, epidermal growth factor receptor tyrosine kinase inhibitors; CTx, prior chemotherapy; RTx, prior radiotherapy; —, not done.
that included the EGFR status as a stratification factor (Table 3). First, sex and PS remained statistically significant at a level of 0.05 in a multivariate model after backward selection. The smoking status was no longer significant (p = 0.46 and p = 0.40 for PFS and OS, respectively) because it was highly correlated with sex (p < 0.0001, Fisher’s exact test). Thus, we used sex and PS as fixed factors in the Cox model. The serum HS concentration was significantly correlated with poor treatment outcomes for PFS (HR = 3.98, p = 0.0012) and OS (HR = 5.42, p = 0.0003) in the final model; a high concentration of HS was correlated with a shorter PFS and OS independently of the EGFR status, sex, and PS (Table 3). In the final model, no interaction was shown between the EGFR status and the serum HS concentration (p > 0.20 for both PFS and OS). The results presented in Table 3 were also stable in analyses of subsets of patients with a known EGFR status as well as patients with wild-type EGFR (data not shown). Regarding EGFR mutations and the HS concentration, we verified the results in additional experiments using an independent set of 48 serum samples from patients whose tumor EGFR status was known. The results showed that a high serum HS level was reproducibly associated with a poor PFS during EGFR-TKI treatment, although the p value was not significant (p = 0.087, Supplementary Figure 1A, http://links.lww.com/JTO/A109). The EGFR status did not seem to be associated with the serum HS concentrations in the 48 additional samples (p = 0.48, Supplementary Figure 1B, http://links.lww.com/JTO/A109).

Taken together, these observations suggested that a high serum HS concentration was significantly associated with the failure of EGFR-TKIs treatment and may be a novel glycochemical biomarker.

### DISCUSSION

The major GAG in the blood is CS, and other serum GAGs include HS, keratin sulfate, and hyaluronan. Many methods are now available to measure the concentration of serum/plasma GAGs; these methods include cellulose acetate membrane electrophoresis, paper, affinity, and gas chromatography, capillary electrophoretic analysis, and HPLC. Nevertheless, no standardized methods exist for serum/plasma GAG isolation and quantification. Our approach using a sandwich ELISA was easy to perform, quantitative, and reproducible. The C.V. value was below 10% in intraplate, intrakit, and intraday analyses (data of Seikagaku-kogyo). Regarding individual differences, the Alcian blue dot blot method showed that the GAG concentration of plasma from hospitalized patients exhibited a variation of plasma GAGs of 0.1 to 17.6 μg/ml and our result for the HS concentration was 3.3 to 85.8 μg/ml. Identifying the cause of these individual differences will require further study. Recent studies have shown that the pleural fluid/serum GAG ratio may be useful for the simultaneous differentiation of exudates from transudates and of malignant exudates from benign exudates. In ovarian cancer, the serum CS level may be useful as a discriminator between benign ovarian disorders and malignant ovarian diseases.

Accumulating evidence has demonstrated that HSPG has oncogenic roles in cancer cells. Perlecan is a potent inducer of bFGF-mediated neovascularization in vivo. In addition, several studies have shown that large deposits of perlecan were observed in the tumor stroma and blood vessel walls in liver tumor and invasive breast cancer in clinical specimens. The strong reactivity for perlecan in tumoral stromal vessels suggests a role for these HSPGs in tumoral angiogenesis, and the angiogenic effect is considered to interact with various proangiogenic ligands. On the other hand, high expression levels of shed/soluble syndecans-1 are found in the serum of patients with myeloma and lung cancer,

![FIGURE 1. Box-whisker plots of serum HS concentration in patients with a partial response (PR, n = 34), stable disease (SD, n = 20), and progressive disease (PD, n = 29). HS, serum concentration of heparan sulfate (μg/ml).](http://links.lww.com/JTO/A109)
and these high expression levels were predictors of a poor prognosis.\textsuperscript{27,28} Compared with the normal form of syndecans-1, the shed form of syndecans-1 gains oncogenic functions leading to hyperinvasiveness and the increased tumor growth of myeloma tumors in vivo.\textsuperscript{29} Thus, shed HSPGs remain highly biologically active and can regulate cell growth and metastasis in cancer.\textsuperscript{30} In line with this observation, our findings that a high HS expression level was correlated with a poor clinical outcome may be associated with the expression of the shed/soluble form of HSPGs. Regarding the correlation between the HS expression levels in tumor and serum samples, we examined the HS expression levels in 10 independent pairs of serum and surgical samples. Representative results of the immunostaining for tumor HS expression are shown in Supplementary Figure 2 (http://links.lww.com/JTO/A110). An anti-HS antibody was used in this experiment with or without heparitinase I digestion. Heparitinase I digestion completely abolished the staining (left panel). Under such conditions, HS was strongly expressed on the membranes of lung cancer cells (lower right panel). Furthermore, HS expression in the tumor tissues was relatively weak in all five cases in the group with a low serum HS level (lower panel, Supplementary Figure 3, http://links.lww.com/JTO/A111), whereas strong HS expression in the tumor tissues was observed in three of the five cases in the group with a high serum HS level (upper panel, Supplementary Figure 3). These results suggested that the tumor and serum HS expression levels may be positively correlated.

The activation of EGFR signaling occurs as a result of mutations affecting the adenosine triphosphate-binding cleft of EGFR, and EGFR mutants exhibit constitutive tyrosine kinase activity independently of any ligand. We found that the serum HS concentration was significantly higher among patients with PD and was strongly associated with a poor PFS and OS in EGFR-TKIs-treated patients. No difference in the serum HS concentration was observed between the PR and SD groups, but a difference was seen between the PD and non-PD groups (Figure 1). Therefore, the serum HS concentration may be involved in drug resistance but not in sensitivity. Regarding resistance to EGFR-TKIs treatment, the amplification of met proto-oncogene (MET) causes gefitinib resistance by driving the v-erb-b2 avian erythroblastic leukemia oncogene homolog 3 (ERBB3)-dependent activation of phosphoinositide-3-kinase, and previous authors have proposed that MET amplification may promote drug resistance in other ERBB-driven cancers.\textsuperscript{31} Yano et al.\textsuperscript{32} showed that HGF-mediated MET activation is involved in gefitinib resistance in lung adenocarcinoma with EGFR-activating mutations. A recent study has clearly demonstrated that HGF accelerates the develop-

![Figure 2](http://links.lww.com/JTO/A110)  

**Figure 2.** Kaplan-Meier curves for progression-free survival (PFS) and OS according to serum concentrations of heparan sulfate (HS). Optimal cutoff point (13.5 μg/ml) of serum HS was determined from a receiver operating characteristic (ROC) curve to discriminate progressive disease (PD) or without PD. Kaplan-Meier curves for PFS (A) and OS (B) are shown. HS high, patients with a serum HS concentration >13.5 μg/ml. HS low, serum HS concentration <13.5 μg/ml.

**Table 3.** Final Multivariate Model for Progression-Free Survival and Overall Survival

<table>
<thead>
<tr>
<th></th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male vs. female)</td>
<td>1.82 (0.0231)</td>
<td>2.43 (0.0031)</td>
</tr>
<tr>
<td>PS (2–4 vs. 0–1)</td>
<td>1.09 (0.7610)</td>
<td>1.95 (0.0235)</td>
</tr>
<tr>
<td>HS\textsuperscript{a}</td>
<td>3.98 (0.0012)</td>
<td>5.42 (0.0003)</td>
</tr>
</tbody>
</table>

Sex and PS were fixed in the model. Molecular markers were then selected using the backward selection procedure with a removal probability of 0.05. In all the steps, a Cox model stratified according to the EGFR status (wild type/mutant/unknown) was applied.

\textsuperscript{a} Log-transformed values are used for HS.

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; EGFR, epidermal growth factor receptor; HS, serum concentration of heparan sulfate (μg/ml); PS, performance status.
opment of MET amplification both in vitro and in vivo, mediating the EGFR kinase inhibitor resistance caused by either MET amplification or autocrine HGF production. These studies indicate that the activation of HGF-MET signaling confers resistance to EGFR-TKIs. Our previous study also showed that a high concentration of serum HGF is a predictive biomarker for EGFR-TKIs treatment. Therefore, the activation of HGF-MET signaling in lung cancer cells is considered to be a cause of drug resistance to EGFR-TKIs. In addition, combined with data on the serum HGF, VEGF, HB-EGF, and PDGF-BB levels from a previous study, the correlation coefficient between the serum HS and the HGF, VEGF, HB-EGF, and PDGF-BB levels were 0.45, 0.46, −0.03, and −0.13, respectively. These results indicated that the expression pattern of the serum HS level was weakly similar to those of the HGF and VEGF levels but was not correlated with the HB-EGF or PDGF-BB levels.

In this study, the serum HS concentration was identified as another candidate biomarker for treatment resistance, and this finding may provide novel glycobiological insight into drug resistance to EGFR-TKIs. The results suggest that a high serum HS concentration may be related to the activation of non-EGFR signaling, such as HGF, FGF, and VEGF signaling in cancer cells. We plan to conduct a prospective study to validate the ability of the serum HS concentration to predict the response to EGFR-TKIs treatment.

ACKNOWLEDGMENTS

Supported by the Third-Term Comprehensive 10-Year Strategy for Cancer Control, a Grant-in-Aid for Scientific Research, and a Grant-in-Aid for Cancer Research (H20-20-9 and H22-2) from the Ministry of Health, Labour and Welfare.

The authors thank Miss Tomoko Kitayama for technical assistance.

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