Fluorodeoxyglucose Positron Emission Tomography Can Predict Pathological Tumor Stage and Proliferative Activity Determined by Ki-67 in Clinical Stage IA Lung Adenocarcinomas

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INTRODUCTION

It has been reported that tumor proliferative activity and alterations of tumor suppressor genes could be prognostic factors in non-small cell lung cancer (NSCLC). Some authors have demonstrated that proliferative activities determined by Ki-67 and cyclin D1, and an alteration of p53 were correlated with the prognosis of NSCLC patients. In recent years, F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) has been frequently used for the diagnosis and staging of lung cancer. FDG uptake has been reported to be correlated not only with the prognosis in NSCLC patients but also with the proliferative activity and alteration of p53 of the tumor. However, we previously reported that the FDG uptake in NSCLC tumors was dependent on histological types, that is, FDG-uptake of adenocarcinomas correlated with the pathological tumor stage and tumor invasiveness, but that of the other histological types did not. Therefore, we consider that the correlation between the FDG uptake and pathological tumor stage is stronger than the correlation with the proliferation and alteration of p53.

Objective: To predict a malignant grade of lung cancer by fluorodeoxyglucose positron emission tomography (FDG-PET) scanning, we investigated the correlation between FDG uptake and pathological tumor stage, proliferative activities determined by Ki-67 and cyclin D1, and an alteration of p53, in clinical stage (c-stage) IA lung adenocarcinomas.

Methods: FDG-PET was performed for 71 patients with c-stage IA lung adenocarcinomas. FDG uptake was measured by a contrast ratio (CR) between the tumor and contralateral lung. Ki-67, cyclin D1, and p53 staining scores were examined by immunohistochemistry.

Results: The lesions with ground-glass opacity were found in 26 patients, and solid lesions in 45 by computed tomography. The pathological tumor stages (p-stage) were stage IA in 59 and more advanced stages in 12. The latter had significantly higher CR value than the former (P < 0.001). Patients with CR > 0.55 could be predicted to be at advanced tumor stages, with a sensitivity of 0.83 and a specificity of 0.82. The CR and staining scores of Ki-67 were significantly correlated with each other (P < 0.0001), and both the values were significantly higher in advanced tumor stages than in p-stage IA, and were also significantly higher in tumors with intratumoral lymphatic, vascular, and pleural involvements than in those without such features (P < 0.05–0.0001).

Conclusions: In c-stage IA lung adenocarcinomas, the FDG uptake can predict p-stage and tumor proliferative activity determined by Ki-67. For c-stage IA lung adenocarcinomas showing CR > 0.55, mediastinoscopy or neoadjuvant chemotherapy is indicated.

Key words: positron emission tomography – lung cancer – adenocarcinoma – tumor stage – proliferative activity – tumor suppressor gene
uptake and the proliferative activity or alteration of p53 should be examined in adenocarcinomas, but not in NSCLC including all histological types. Furthermore, FDG uptake is known to be higher in larger tumors (17,18). Therefore, in the present study, we measured the FDG uptake in clinical stage IA adenocarcinomas and investigated its relationships with the pathological tumor stage, intratumoral invasiveness, proliferative activity determined by Ki-67 and cyclin D1 and the alteration of p53.

PATIENTS AND METHODS

PATIENTS

Between December 2001 and February 2005, FDG-PET was performed for 386 patients with pulmonary nodules. Of these, 301 patients had malignant tumors and 178 of the 301 patients had lung adenocarcinoma. Of the 178 adenocarcinoma patients, 77 were diagnosed as clinical stage IA by PET and thin section computed tomography (TSCT: <2 mm in thickness). They were treated by segmentectomy or lobectomy and lymph node dissection. Of these, we excluded six adenocarcinomas <1 cm, because the spatial resolution of the PET scanner is 0.7–0.8 cm, making it difficult to image the pulmonary nodules that are <1 cm (16). Therefore, we studied 71 patients with clinical stage IA adenocarcinoma with a size range of 1–3 cm.

FDG-PET SCANNING

Patients were instructed to fast for at least 4 h before intravenous (i.v.) administration of F-18 FDG. The dosage of F-18 FDG administered was 125 μCi/kg (4.6 MBq/kg) for non-diabetic patients and 150 μCi/kg (5.6 MBq/kg) for diabetic patients. PET imaging was performed ~60 min after administration of FDG with a POSICAM.HZL-POWER (Positron Co., Houston, TX, USA). No attenuation-corrected emission scans were initially obtained in two-dimensional, high-sensitivity mode for 4 min per bed position, and taken from the vertical skull through the mid-thighs. Immediately thereafter, a two-bed-position attenuation-corrected examination was performed with 6 min for the emission sequence and 6 min for the transmission sequence at each bed position.

PET IMAGE PROCESSING AND DATA ANALYSIS

The images were usually reconstructed in a 256 × 256 matrix by using ordered subset expectation maximization corresponding to a pixel size of 4 × 4 mm, with section spacing of 2.66 mm. FDG uptake was evaluated by contrast ratio (CR) with contralateral lung, as previously reported (15,16,19). Briefly, the regions of interest (ROI) were placed in the nodules and contralateral normal lung. Highest standardized uptake ratios in the tumor ROI (T) and in the contralateral lung ROI (N) were measured. The CR value was calculated by using the formula [(T − N)/(T + N)] in each nodule as an index of FDG uptake.

PATHOLOGICAL ANALYSIS

Hematoxylin and eosin and Elastica-van Gieson stainings were performed in all sections to investigate the intratumoral lymphatic and vascular invasions and pleural involvement. Pleural involvement was classified as p0, p1, p2 and p3; that is, a p0 tumor did not extend beyond the pleural elastic layer; a p1 tumor invaded the visceral pleural elastic layer, but did not reach the pleural surface; a p2 tumor included tumor exposure on the pleural surface; and a p3 tumor invaded the parietal pleura or the chest wall. The tumor stages were based on the TNM classification of the International Union Against Cancer (20): p2 tumors were classified as T2, p3 tumors were classified as T3 and tumors with intrapulmonary metastases within the same lobe were classified as T4.

PREDICTING ADVANCED TUMOR STAGES FROM FDG UPTAKE

Receiver operating characteristics (ROC) curve (21) was constructed according to the CR value, and the cut-off value was determined for predicting the pathological stages that were more advanced than stage IB.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunostaining was performed by using the Dako envision system (Dako Cytomation, Glostrup, Denmark). The antibodies for Ki-67 (monoclonal mouse antibody MIB-1, 1 : 100 dilution), cyclin D1 (monoclonal mouse antibody DCS-1, 1 : 50 dilution) and p53 (monoclonal mouse antibody, DO7, 1 : 400 dilution) were purchased from Dako Co. Sections of 4 μm were cut from the paraffin blocks. Immunostaining was performed with antigen-retrieval techniques.

EVALUATION OF IMMUNOHISTOCHEMICAL STAINING

Ki-67

According to the method of Martin et al. (1), the labeling index of Ki-67 was measured by determining the percentage of cells with positive nuclei in >1000 tumor cells in >4 fields.

Cyclin D1 and p53

The Allred score used to examine the staining scores of cyclin D1 as well as p53 was obtained by determining the percentage of positive tumor cells and staining intensity (22). Briefly, a percentage score was measured by determining the percentage of positive tumor cells in >1000 tumor cells in >4 fields (0, none; 1, <1/100; 2, 1/100–1/10; 3, 1/10–1/3; 4, 1/3–2/3; 5, >2/3). An intensity score was measured by the average of staining intensity (0, none; 1, weak; 2, intermediate; and 3, strong). The sum of the percentage score and the intensity score was used as the Allred score.

STATISTICAL ANALYSIS

The values of CR and staining scores of Ki-67, cyclin D1 and p53 were compared between the pathological stage IA and the
more advanced stages and between the two groups with or without intratumoral lymphatic, vascular invasion and pleural involvement, by the non-parametric Mann–Whitney’s U-test. The correlations between the CR values and the staining scores of Ki-67, cyclin D1 and p53 were analyzed by using the non-parametric Spearman’s rank test. All values in the text and tables are given as mean ± standard deviation.

RESULTS

Table 1 shows patients’ characteristics. The lesion showing ground-glass opacity (GGO) image were found in 26 patients, and the solid lesions in 45 patients at visual TSCT findings. The pathological tumor stages were T1N0M0 in 59 patients and more advanced in 12 patients (i.e. T2N0M0 in 3, T1N1M0 in 4, T2N1M0 in 1, T1N2M0 in 1, T4N0M0 in 2 and T4N2M1 in 1).

Figure 1 shows the distribution of CR values in 59 patients with pathological stage IA and 12 patients with stages advanced more than IA. The mean CR value of the 12 patients with advanced stages was 0.66 ± 0.12, which was significantly higher than 0.32 ± 0.18 of the 59 patients with pathological stage IA (P < 0.001).

Figure 2 depicts the ROC curve for predicting tumor stages more advanced than IA. The ROC curve is 0.83, 0.81 and 0.82, respectively. While 10 of the 12 patients (83%) with advanced stage showed CR values > 0.55, 48 of the 59 patients (81%) with pathological stage IA showed CR values < 0.55 (Table 2).

Figure 3–5 show the distribution of staining scores of Ki-67, cyclin D1 and p53 in the 59 patients with pathological stage IA and 12 patients with more advanced stages. The mean score for Ki-67 in the 12 patients with advanced stages was 18.0 ± 11.0, which was significantly higher than 9.4 ± 12.0 for the 59 patients with pathological stage IA (P = 0.012). However, the mean scores for cyclin D1 in the advanced tumor stages and pathological stage IA were 3.9 ± 1.7 and 4.1 ± 1.8, respectively, of which difference was not significant. The mean scores for p53 in the advanced tumor stages and pathological stage IA were 3.3 ± 3.6 and 2.6 ± 2.6, respectively, of which difference was also not significant.

Table 1. Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 ± 10 (33–83)*</td>
</tr>
<tr>
<td>Size (mm)</td>
<td>19 ± 7 (10–30)*</td>
</tr>
<tr>
<td>CT findings</td>
<td></td>
</tr>
<tr>
<td>GGO</td>
<td>26</td>
</tr>
<tr>
<td>Solid</td>
<td>45</td>
</tr>
<tr>
<td>Pathological stage</td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>59</td>
</tr>
<tr>
<td>&gt;IB</td>
<td>12</td>
</tr>
<tr>
<td>T2N0M0</td>
<td>3</td>
</tr>
<tr>
<td>T1N1M0</td>
<td>4</td>
</tr>
<tr>
<td>T2N1M0</td>
<td>1</td>
</tr>
<tr>
<td>T1N2M0</td>
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<tr>
<td>T4N0M0</td>
<td>2</td>
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<tr>
<td>T4N2M0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation (range), GGO: ground-glass opacity.
Table 3 shows the mean values of CR, Ki-67, cyclin D1 and p53 staining scores in tumors with or without intratumoral lymphatic, vascular and pleural involvements. Tumors with intratumoral lymphatic, vascular and pleural involvements had significantly higher values of both CR and Ki-67 staining scores ($P < 0.05$–$0.001$). However, there were no significant differences of tumor invasiveness in the cyclin D1 and p53 staining scores.

DISCUSSION

Although the standard uptake value (SUV) has been frequently used for evaluation of FDG-PET, it has been reported that several factors can affect the SUV, such as body size (23), blood glucose level (24), time after injection (25) and lesion size (17,18). In fact, the mean SUV of malignant pulmonary nodules has been reported to be various, ranging from 5.5 to 10.1 (26–29). We previously compared the results of SUV, CR with contralateral lung and CR with cerebellum for pulmonary nodules, and reported that the CR with contralateral lung or...
CR, contrast ratio; ly, lymphatic invasion; v, vascular invasion; p, pleural involvement.

The differences of CR or Ki-67 staining scores between the GGO and solid images were significant, with CR values of 0.63 and 0.34 for stage IA tumors with high FDG uptake (CR > 0.55) and stage IB tumors with low stage IB tumors, respectively. A recent study reported that adenocarcinoma with GGO image showed higher Ki-67 staining scores than solid one. However, one adenocarcinoma with GGO image was stage IB, which showed lower Ki-67 scores than those with solid one. Therefore, the FDG uptake on PET could be an independent factor for predicting pathological stage in clinical stage IA adenocarcinoma.

Earlier reports have indicated that adenocarcinomas with GGO image are usually NO stage and show low FDG uptake on PET (15,16,30). The present study is basically in agreement with these results, that is, adenocarcinomas with GGO image showed lower FDG uptake than those with solid one. However, one adenocarcinoma with GGO image was stage IB, which showed rather high CR than 25 stage IA tumors. In tumors with solid image, the 11 tumors with advanced stages showed significantly higher CR values than the 34 tumors with solid image. However, it has been reported that 25% of clinical stage IA adenocarcinomas are pathologically advanced tumor stage (31). Recently, neoadjuvant chemotherapy has been reported to improve survival in clinical stages IB, II and IIIA of NSCLC (32). The present study showed that the CR value of 0.55 could be the cut-off value for predicting advanced tumor stages more than IB. Therefore, we propose that to improve patients’ prognosis in clinical stage IA adenocarcinoma, mediastinoscopy or neoadjuvant chemotherapy should be performed for clinical stage IA adenocarcinoma with CR ≥ 0.55.

The Ki-67 antigen exists in the nucleus of proliferating cells and has been reported to be a prognostic factor in lung cancer (1). Vesselle et al. (14) reported that the FDG uptake correlated with Ki-67 staining scores in 39 patients with NSCLC. They also described that the correlation between the FDG-uptake and Ki-67 staining scores was a predictor of poor prognosis of NSCLC (4,5), others have questioned its role as a predictor (3).

**Table 3.** Correlation between intratumoral invasiveness and CR values and staining scores of Ki-67, cyclin D1 and p53

<table>
<thead>
<tr>
<th>Invasiveness</th>
<th>Number of patients</th>
<th>CR</th>
<th>Ki-67</th>
<th>p53</th>
<th>Cyclin D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ly (–)</td>
<td>38</td>
<td>0.29 ± 0.21</td>
<td>5.6 ± 8.1</td>
<td>2.2 ± 2.3</td>
<td>3.9 ± 1.8</td>
</tr>
<tr>
<td>(+)</td>
<td>33</td>
<td>0.48 ± 0.21*</td>
<td>17 ± 13*</td>
<td>3.4 ± 3.1</td>
<td>4.3 ± 1.8</td>
</tr>
<tr>
<td>v (–)</td>
<td>58</td>
<td>0.34 ± 0.23</td>
<td>9.9 ± 12</td>
<td>2.9 ± 2.7</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>(+)</td>
<td>13</td>
<td>0.55 ± 0.20**</td>
<td>17 ± 12**</td>
<td>2.1 ± 3.0</td>
<td>3.8 ± 1.7</td>
</tr>
<tr>
<td>p 0</td>
<td>63</td>
<td>0.34 ± 0.20</td>
<td>9.7 ± 12</td>
<td>2.5 ± 2.6</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>1–2</td>
<td>8</td>
<td>0.63 ± 0.13*</td>
<td>21 ± 8.3**</td>
<td>4.5 ± 3.6</td>
<td>3.8 ± 2.0</td>
</tr>
</tbody>
</table>

Table 4. The values of CR and Ki-67 of tumors with stage IA and advanced stages in tumors with solid and GGO images

<table>
<thead>
<tr>
<th>Tumor findings and pathological stage</th>
<th>CR</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GGO</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cases (n = 26)</td>
<td>0.18 ± 0.14</td>
<td>4.5 ± 6.8</td>
</tr>
<tr>
<td>Stage IA (n = 25)</td>
<td>0.17 ± 0.14</td>
<td>4.7 ± 6.9</td>
</tr>
<tr>
<td>Stage IB (n = 1)</td>
<td>0.48</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Solid</strong></td>
<td></td>
<td></td>
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<tr>
<td>All cases (n = 45)</td>
<td>0.48 ± 0.2*</td>
<td>15 ± 13*</td>
</tr>
<tr>
<td>Stage IA (n = 34)</td>
<td>0.43 ± 0.20</td>
<td>13 ± 13</td>
</tr>
<tr>
<td>≥Stage IB (n = 11)</td>
<td>0.67 ± 0.11</td>
<td>21 ± 10</td>
</tr>
</tbody>
</table>

Figure 6. The correlation between CR values and Ki-67 staining scores in the 71 patients with clinical stage IA: r = 0.42; P = 0.00043. Although cyclin D1 and p53 did not show such correlation; and (iii) FDG uptake and Ki-67 scores were significantly correlated with each other.
On the other hand, another report described that the negative staining for cyclin D1 correlated with poor prognosis (2). While these previous studies examined the expression of cyclin D1 in NSCLC including all histological types, the present study investigated the clinical stage IA adenocarcinomas, showing that cyclin D1 scores did not correlate with the FDG uptake, pathological tumor stage or tumor invasiveness. Our results indicate that Ki-67 is a better marker for the proliferative activity and malignant grade of adenocarcinomas than cyclin D1.

An alteration of p53 is reported to be a poor prognostic factor in many types of tumors including lung cancer (6), although one recent report has denied it (2). While Sasaki et al. (7) reported that the FDG uptake in tumors with the alteration of p53 tended to be higher than in those without, the present study did not find any correlation of p53 staining scores with the FDG uptake, tumor stage and intratumoral invasiveness in clinical stage IA lung adenocarcinomas. The differences between the report by Sasaki et al. and ours were (i) the number of the patients (28 versus 71 patients); (ii) histological types (NSCLC including all histological types versus adenocarcinoma); (iii) size of tumors (17,18) (no details about specific sizes versus 1–3 cm); (iv) measurement of FDG uptake (SUV versus CR). As described in our previous reports, the FDG uptake should be measured by CR and not by SUV, and should also be evaluated in adenocarcinomas with limited tumor size (16,19). Therefore, we believe that the alteration of p53 does not correlate with FDG uptake and therefore cannot be a dictator for the malignant grade in clinical stage IA lung adenocarcinomas.

We conclude that the FDG uptake can predict pathological tumor stage and tumor proliferative activity determined by Ki-67, but cannot predict the alteration of p53 in clinical stage IA lung adenocarcinomas. These results should be useful in determining the indication for mediastinoscopy or neoadjuvant chemotherapy in patients with clinical stage IA lung adenocarcinoma.

Acknowledgments

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References