Heterogeneous Nuclear Ribonucleoprotein B1 as Early Cancer Biomarker for Occult Cancer of Human Lungs and Bronchial Dysplasia

Eisaburo Sueoka, Naoko Sueoka, Yuri Goto, Satoru Matsuyama, Hitoshi Nishimura, Masami Sato, Shigefumi Fujimura, Hiroshi Chiba, and Hirotu Fujiki

ABSTRACT

Heterogeneous nuclear ribonucleoprotein (hnRNP) B1 is a RNA-binding protein of M, 37,000. We previously reported that hnRNP B1 was specifically overexpressed in the nuclei of human lung cancer cells, particularly in squamous cell carcinoma (E. Sueoka et al., Cancer Res., 59: 1404–1407, 1999). We extended this study to determine whether hnRNP B1 was overexpressed in roentgenographically occult cancers of the lungs and premalignant lesions of squamous cell carcinomas, such as bronchial dysplasia. The additional object of our study was to examine the usefulness of hnRNP B1 as a potential diagnostic marker for squamous cell carcinoma of various organs, such as the oral cavity and esophagus in humans. Surgically resected specimens of bronchial dysplasia, lung cancers, and various human squamous cell carcinomas, collected at two hospitals in Japan, were subjected to immunohistochemical staining with anti-hnRNP B1 antibody. Overexpression of hnRNP B1 protein was observed in 100% of stage I lung cancer tissues, but it was not found in normal bronchial epithelium. Squamous cell carcinoma of the lungs showed stronger staining than other histological types, and elevation of hnRNP B1 was found in both roentgenographically occult lung cancers and bronchial dysplasia. Furthermore, cytological examination with anti-hnRNP B1 antibody detected cancer cells in sputum, suggesting the potential of hnRNP B1 protein as a new biomarker for the very early stage of lung cancer in humans. Because strong staining of hnRNP B1 was also observed in various squamous cell carcinomas of oral and esophageal tissues as shown in our recent reports, overexpression of hnRNP B1 seems to be a common event in the carcinogenic processes of squamous cell carcinoma. These results suggest that hnRNP B1 protein could be a useful diagnostic biomarker for both the very early stages of lung cancer and various squamous cell carcinomas in humans.

INTRODUCTION

In Japan, lung cancer is the leading cause of cancer-related death among males (21.4%) and the second leading cause among females (12.3%). Frequency of death from the disease has increased remarkably (1), with 50,000 people dying of lung cancer in 1998 (2). In an effort to combat this, technology of roentgenographically occult lung cancers and bronchial dysplasia. Furthermore, cytological examination with anti-hnRNP B1 antibody detected cancer cells in sputum, suggesting the potential of hnRNP B1 protein as a new biomarker for the very early stage of lung cancer in humans. Because strong staining of hnRNP B1 was observed in various squamous cell carcinomas of oral and esophageal tissues as shown in our recent reports, overexpression of hnRNP B1 seems to be a common event in the carcinogenic processes of squamous cell carcinoma. Thus, overexpression of hnRNP B1 protein could be a useful diagnostic biomarker for both the very early stages of lung cancer and various squamous cell carcinomas in humans.

The abbreviation used is: hnRNP, heterogeneous nuclear ribonucleoprotein.
oral tissue and the esophagus (12, 13). Here, we report the usefulness of hnRNP B1 as a potential biomarker for early detection of various human cancers and we discuss the molecular significance of dysplasia in cancer diagnosis.

**RESULTS**

**Overexpression of hnRNP B1 in Human Lung Cancers.** We recently reported that hnRNP B1 was significantly overexpressed in nuclei of human lung cancer cells (11). To investigate the significance of hnRNP B1 as an early detection marker for lung cancer, we next determined expression levels of hnRNP B1 in various clinical features of 43 patients, classified by histological type and clinical stage (Table 1). The 43 lung cancers included 15 squamous cell carcinomas, 24 adenocarcinomas, 3 large cell carcinomas, and 1 small cell carcinoma; the patients were 14 stage I, 11 stage II, 16 stage III, and 2 stage IV. Strength of staining with anti-hnRNP B1 antibody was divided into four groups (negative, weak, moderate, and strong) and compared with the histological and clinical types mentioned above.

As shown in Table 1, all four histological types of lung cancer tissues showed positive staining with anti-hnRNP B1 antibody, ranging from 70.8–100% (Table 1 and Fig. 1). The strength of staining for squamous cell carcinoma was 66.7% moderate and 26.7% strong staining; that of adenocarcinoma was 29.1% negative, 16.7% weak, and 54.2% moderate staining. As for control, normal bronchial epithelial cells and alveolar epithelial cells of adjacent noncancerous tissue did not show any positive staining with anti-hnRNP B1 antibody (Fig. 1C). In data not shown, the lung of bacterial pneumonia and interstitial pneumonia did not express hnRNP B1 protein, indicating that hnRNP B1 expression is not associated with inflammatory reactions.

**Elevated Expression of hnRNP B1 in Roentgenographically Occult Cancer.** Next, we determined the expression level of hnRNP B1 protein in 43 roentgenographically occult cancers that were not detected by roentgenographic diagnosis, such as chest X-ray or computed tomography. The clinical features of the cancer patients, such as invasive grade, mean age, mean smoking index, pathological diagnosis, and positive staining of hnRNP B1 in the tissues, are summarized in Table 2. Invasive grade of the occult cancers was divided into three types: carcinoma in situ (11 cases), intrabronchial invasion (14 cases), and extrabronchial invasion (18 cases). Pathological diagnosis revealed that all 43 occult cancers were squamous cell carcinomas.

Fig. 2A shows a representative occult cancer, with a very small lesion (<5 mm in diameter) of early lung cancer tissue. Of the 43 occult cancer patients, 58.1% showed positive staining of hnRNP B1: intra- and extrabronchial invasions showed 71.4% and 61.1% positivity, respectively, considerably higher than carcinoma in situ, 36.4% (Table 2). The results suggest that overexpression of hnRNP B1

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### Table 1: Positivity of immunohistochemical staining with anti-hnRNP B1 antibody in lung cancer patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No.</th>
<th>Negative</th>
<th>Weak</th>
<th>Moderate</th>
<th>Strong</th>
<th>Total positivity</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>15</td>
<td>0/15 (0)</td>
<td>1/15 (6.6)</td>
<td>10/15 (66.7)</td>
<td>4/15 (26.7)</td>
<td>15/15 (100)</td>
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<tr>
<td>Adenocarcinoma</td>
<td>24</td>
<td>7/24 (29.1)</td>
<td>4/24 (16.7)</td>
<td>13/24 (54.2)</td>
<td>0/24 (0)</td>
<td>17/24 (70.8)</td>
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<tr>
<td>Large cell carcinoma</td>
<td>3</td>
<td>0/3 (0)</td>
<td>0/3 (0)</td>
<td>3/3 (100)</td>
<td>0/3 (0)</td>
<td>3/3 (100)</td>
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<tr>
<td>Small cell carcinoma</td>
<td>1</td>
<td>0/1 (0)</td>
<td>0/1 (0)</td>
<td>1/1 (100)</td>
<td>0/1 (0)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Total no.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical stages</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>14</td>
<td>0/14 (0)</td>
<td>2/14 (14.3)</td>
<td>11/14 (78.6)</td>
<td>2/14 (14.3)</td>
<td>14/14 (100)</td>
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<tr>
<td>II</td>
<td>11</td>
<td>3/11 (27.3)</td>
<td>2/11 (18.2)</td>
<td>4/11 (36.4)</td>
<td>2/11 (18.2)</td>
<td>8/11 (72.3)</td>
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<tr>
<td>III</td>
<td>16</td>
<td>3/16 (18.8)</td>
<td>2/16 (12.5)</td>
<td>9/16 (56.3)</td>
<td>3/16 (18.8)</td>
<td>13/16 (81.3)</td>
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<tr>
<td>IV</td>
<td>2</td>
<td>1/2 (50)</td>
<td>1/2 (50)</td>
<td>0/2 (0)</td>
<td>0/2 (0)</td>
<td>1/2 (50)</td>
</tr>
<tr>
<td>Total no.</td>
<td>43</td>
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</table>

*Number of patients with tumor of given cell type or stage.

*“Positive” was defined as nuclear staining observed in over 50% of >300 cells examined for each case.*
begins in the early stage of the lung cancer and is also associated with cancer progression.

**Elevated Expression of hnRNP B1 in Bronchial Dysplasia.**

Eleven patients with bronchial dysplasia were diagnosed by transbronchial lung biopsy. The clinical features of these patients were then summarized by age, smoking index, pathological diagnosis, and positive staining of hnRNP B1 in the lesions (Table 3). The mean age was 60 years. Pathological diagnosis of all dysplasia showed three mild, three moderate, and five severe dysplasias.

Fig. 2B shows a representative bronchial dysplasia: positive hnRNP B1 staining for dysplasia patients was 63.6% in total [100% (three of three) for mild, 33.3% (one of three) for moderate, and 60.0% (three of five) for severe dysplasia]; adjacent noncancerous tissue did not show any positive staining. Whether positively stained bronchial
Fig. 2. Overexpression of hnRNP B1 in roentgenographically occult cancer and bronchial dysplasia. Immunohistochemical staining of roentgenographically occult cancer (A) and bronchial dysplasia (B) was performed. The hnRNP B1 protein was overexpressed in nuclei of cancer cells and bronchial dysplasia (arrowhead), but not in those of normal bronchial epithelial cell adjacent to dysplasia. C; H&E staining of the same case in B.
dysplasia and nonstained bronchial dysplasia represent different carcinogenic processes remains to be investigated. However, the results suggest the presence of qualitative differences in the carcinogenesis of bronchial dysplasia. For population-based lung cancer screening approaches, anti-hnRNP B1 antibody staining will provide useful diagnostic information for early lesions of human lung cancer.

**Detection of Tumor Cells by Sputum Cytology Using Anti-hnRNP B1 Antibody.** To confirm the usefulness of hnRNP B1 in early diagnosis of lung cancer, we applied anti-hnRNP B1 antibody in sputum cytology, first developing the optimum condition of immunocytochemistry for sputum staining with anti-hnRNP B1 antibody. Fig. 3 shows positive staining of adenocarcinoma cells contained in sputum samples obtained from an adenocarcinoma patient: no positive staining was observed in normal bronchial epithelial cells or oral squamous cells contained in sputum samples. Until now, we have analyzed the sputum samples of 12 lung cancer cases. Three of 12 samples that were diagnosed as class V by cytology showed cancer cells stained positively, whereas the other 9 samples diagnosed class I or II were negative in immunostaining.

**DISCUSSION**

Early detection of lung cancer is the key to long-term survival: the 5-year survival rate of patients diagnosed at the microinvasive stage of cancer is estimated at over 90%. Early diagnosis of lung cancer patients, however, has remained an elusive goal, with <1% of the cases being so diagnosed (1–3). Therefore, the development of a new diagnostic method specifically for the very early stage of lung cancer is urgently required. We recently demonstrated that hnRNP B1 is overexpressed in nuclei of lung cancer cells, but not in normal bronchial cells or alveolar cells (11). In this study, we confirmed that 100% of lung cancer tissue at clinical stage I was positively stained with anti-hnRNP B1 antibody. Here, we found that 58.1% of roentgenographically occult lung squamous cell carcinomas and 63.6% of bronchial dysplasias were positive in staining with anti-hnRNP B1 antibody. These results strongly suggested that overexpression of hnRNP B1 protein is a very early event in lung carcinogenesis, one that may be an early biomarker for detection of premalignant lesions of the lungs as well as the very early stages of various squamous cell carcinomas.

Our results suggested that overexpression of hnRNP B1 representing cell proliferation appears in very early lesions of lung cancers. Still to be determined are the mechanisms of hnRNP B1 overexpression in lung cancer tissue, or its relationship with other molecular markers that are early events in lung carcinogenesis including mutation of p53, loss of p16 expression, expression of telomerase, and loss of heterozygosity of 3p, 9p or 17p (14–18), we assumed that hnRNP B1 overexpression was partly related to cell cycle regulation, based on our previous evidence that hnRNP B1 expression is associated with rapid cell growth of lung cancer cell lines (11). The expression of hnRNP B1 is thought to be up-regulated by both transcriptional and posttranscriptional mechanisms in lung cancer tissue (11), and Michelson et al. (19) recently found that nuclear DEAF-1-related protein NUDR binds to hnRNP A2/B1 promoter and regulates its promoter activity. However, our previous results presented that the elevated expression of the *hnRNP B1* gene and protein in lung cancer cell line and cancer tissue was more apparent than that of the *hnRNP A2* gene.

| Table 3 Characteristics of clinical features of bronchial dysplasia patients |
|-----------------------------|-----------|----------------|----------------|-----------------|
| Case | Age (yr) | Smoking index | Pathological diagnosis | Immunoreactivity of hnRNP B1 |
| 1 | 61 | 1400 | Mild D<sup>a</sup> | Positive |
| 2 | 63 | 600 | Mild D | Positive |
| 3 | 71 | 2000 | Mild D | Positive |
| 4 | 64 | 800 | Moderate D | Negative |
| 5 | 66 | 1500 | Moderate D | Negative |
| 6 | 33 | 740 | Moderate D | Positive |
| 7 | 55 | 900 | Severe D | Negative |
| 8 | 65 | 1000 | Severe D | Positive |
| 9 | 63 | 600 | Severe D | Negative |
| 10 | 62 | 1200 | Severe D | Negative |
| 11 | 56 | 800 | Severe D | Positive |

<sup>a</sup> D, dysplasia.

<sup>b</sup> Sq, squamous cell carcinoma.
(11). Although Montuenga et al. (20) reported that immunohistochemistry of human fetal lung using anti-hnRNP A2/B1 antibody showed positive staining, we found that fetal normal lung and adult lung cancer tissues showed different expression patterns: the differences included intensity of staining and intracellular localization of the hnRNP B1 protein.

From the results of epidemiological studies and some animal experiments, we know that dysplasia is a precancerous state in the sequential process of lung carcinogenesis (21–23). However, not all cases of bronchial dysplasia develop into squamous cell carcinomas (24–26) and, in our experiments, 36.4% of bronchial dysplasias did not show positivity with anti-hnRNP B1 antibody. Because positive hnRNP B1 staining varied among cases of bronchial dysplasia, we think the high-risk group, which progresses to malignancy, might be predicted by this method. Tockman et al. (27) previously reported that positive immunostaining of hnRNP A2/B1 in sputum specimens made it possible to predict clinical onset of primary lung cancer within 12 months. Their initial work was an essential step toward prediction of the high-risk group, but their anti-hnRNP A2/B1 antibody also stained 41% of normal bronchial epithelium (28). However, our anti-hnRNP B1 antibody will provide high specificity for detection of the high-risk group, because it did not stain normal bronchial epithelium in sputum cytology. Like hnRNP B1, cyclins D1 and E are also overexpressed in dysplasia, overexpressions that are strongly associated with the process of progression (29). A prospective cohort study should be conducted to determine whether hnRNP B1 overexpression, rather than that of cyclins D1 and E, is related to development into overt lung cancer.

As we recently reported, hnRNP B1 overexpression was found in 100% of squamous cell carcinomas of the lungs, in oral cancer tissue of stage I patients, in leukoplakia with severe dysplasia, and in those of the esophagus (11–13). All oral squamous cell carcinomas and severe dysplastic leukoplakia, a premalignant lesion, showed strong staining with anti-hnRNP B1 antibody (seven of seven and three of three, respectively; Ref. 12). hnRNP B1 expression in 16 paraffinized sections of esophageal cancer, both cancers and noncancerous regions, was intensively studied, and positive staining of hnRNP B1 was also observed in the cancerous regions (13). These results indicated that squamous cell carcinomas of other organs, such as oral and esophageal cancers, are also useful targets for this method. Furthermore, we found that subcellular localization of hnRNP B1 in a keratinized cell is different from that in basal lesion of cancer tissue; it will be possible that hnRNP B1 regulates specific gene expression in squamous cell carcinomas. The functional role and the target molecule of hnRNP B1 in squamous cell carcinoma are under investigation.

The overall mortality rate of lung cancer has not changed in the past 25 years, but recent developments in the study of molecular carcinogenesis show us that prevention is a rational approach to lung cancer. As we recently reported, hnRNP B1 overexpression was found in primary lung cancer, but also in recurrent and second primary cancers in humans.

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REFERENCES


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4. N. Fujimoto et al., manuscript in preparation.
5. E. Sueoka et al., unpublished results.


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