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Pathological and Biological Assessment of Lung Tumors Showing Ground-glass Opacity

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Abstract

Background: We evaluated the pathological and biological aspects of lung tumors 3.0 cm or less in diameter with the appearance of ground-glass opacity (GGO).

Patients and Methods: Of 988 patients with non-small cell lung cancer who underwent operations at our institute between January 1994 and December 2004, 87 resected lung tumor specimens that showed GGO appearance on helical CT were obtained from 81 patients. Forty-four lesions were pure GGO with no solid component in the tumor and 43 lesions were mixed GGO consisting of areas of attenuation with a solid component. Together with histological features, MIB1 and nm23 expression within tumors were examined immunohistochemically.

Results: The mean tumor size in the pure GGO group was significantly smaller than that in the mixed GGO group. The composition of pathological subtypes and biological characteristics were clearly different between the two groups. Although atypical adenomatous hyperplasia and localized bronchioloalveolar cell carcinoma of Noguchi's A and B were the predominant pathological subtypes and Nm23 negativity was rare in the pure GGO group, a high score for expression of MIB1 was often found in pure GGO tumors even though the tumors were less than 10 mm in diameter.

Conclusions: If the tumor is 2 cm or less in diameter, the ability of invasion and metastasis appears to be low in pure GGO tumors. However, the proliferation ability of these tumors suggests the necessity of a careful follow-up schedule if the tumor is greater than 5 mm in diameter. For mixed

GGO tumors, surgical resection instead of observation is justified.

Key words: lung cancer; ground-glass opacity; bronchioloalveolar carcinoma, nm23, MIB1

Abbreviation: GGO, ground-glass opacity

Introduction

With the advent of radiology, *i.e.*, helical computed tomography (CT) mass-screening systems, our thoracic surgeons have often encountered tiny or small lung nodules with the appearance of ground-glass opacity (GGO). Interestingly, some recent investigators have begun to address the possibility of lung parenchymal sublobar limited resection for this specific subgroup of small lung cancers with GGO appearance [1-7]. Although operative procedures are generally dependent on size, number, and location of lesions, limited resection procedures, such as wedge resection, are a well-recognized form of operative procedure for small-sized pure GGO. The basis of this surgical tactic is the observation that non-invasive localized bronchioloalveolar carcinoma (LBAC) and atypical adenomatous hyperplasia (AAH) are the dominant pathological types in tumors with pure GGO appearance and the risk of regional nodal metastasis is very low [8,9]. On the other hand, in the management of pure GGO, the timing of the operation is also controversial. In the management of small-sized pure GGO, while some authors advocate a positive stance for VATS biopsy, others

recommend a careful follow-up schedule in Japan. Here, although surgery remains the main form of treatment for localized non-small cell lung cancer, indications for surgical treatment for pure GGO remain obscure. To address this issue, more information is required, including determination of the biological aspects of this specific subgroup of lung tumors. The present study was performed to evaluate both pathological and biological aspects of small lung tumors with GGO appearance.

Patients and methods

Between January 1994 and December 2004, a total of 988 patients with non-small cell lung cancer underwent operations at Kanazawa University Hospital. Among these cases, 87 resected lung tumor specimens measuring 3.0 cm or less in diameter that showed GGO appearance were obtained from 81 patients (33 men and 47 women; mean age, 63.6±1.4; range, 36–86 years). GGO appearance which showed a diffuse increase in attenuation without obscuring the underlying vascular markings was reviewed on helical CT by 2-3 independent observers including a radiologist who are diagnostic experts in chest radiology. Pure GGO was defined as a homogeneous GGO with no solid components, and mixed GGO was defined as a GGO consisting of areas of attenuation with a solid component. Forty-four lesions obtained from 39 patients were pure GGO and 43 lesions from 42 patients were mixed GGO. For pure GGO, we performed thoracoscopic wedge resection after CT-guided marking if the tumors were not diminished after several months of follow up. For mixed

GGO, we performed VATS biopsy *via* wedge resection instead of follow-up by CT. Generally, the final operative procedures for lung parenchymal resection were determined by the location of the tumor and intraoperative frozen section diagnosis. For patients with definite diagnosis of LBAC of Noguchi's type A/B [10] or AAH 1.0 cm or less in diameter, we completed the operation by wedge resection with a clear surgical margin of more than 1 cm. For patients with invasive carcinoma or uncertain intraoperative pathological diagnosis with regard to Noguchi's classification, we performed standard resection, *i.e.*, lobectomy plus systemic lymphadenectomy. If the area of pure GGA was 1.0–2.0 cm in diameter and the location was definitely restricted to the left upper lobe or S6 segment, we generally performed segmentectomy instead of standard lobectomy. We performed lobectomy in patients with pure GGO measuring more than 2.0 cm in diameter. Written informed consent was obtained from all of patients included in the present study.

Immunohistochemical assessment of nm23 and MIB1

In this study, we performed immunohistochemical assessment of proliferative activity using the monoclonal antibody MIB1, which detects the proliferation-associated antigen Ki-67. In addition to this marker of proliferative activity, we also explored the metastatic ability by assessment of nm23 expression. This metastasis-associated marker because we selected because we previously confirmed its association with nodal micrometastasis in non-small cell lung cancer patients in the early stages

of disease [11].

The primary antibodies used in the present study were an anti-nm23 monoclonal antibody (Dako Corporation, Carpinteria, CA) diluted 50-fold and an anti-MIB1 monoclonal antibody (Dako) diluted 50-fold. After reviewing the hematoxylin and eosin-stained slides of the tumor specimens, we selected blocks of the edge of the tumor area. Paraffin-embedded tumor tissues were cut into sections 4 μm thick, deparaffinized, and immunohistochemical staining was performed using the labeled streptavidin-biotin method, as described previously [11].

For assessment of nm23 protein expression, tumors were considered positive if all the epithelial cells in the lesion showed cytoplasmic staining. If any of the epithelial cells were unstained, they were considered negative [12]. Evaluation of MIB1 staining was carried out within areas with a high degree of cellularity [13,14]. After all fields of the sections were scanned at low (×40) and high (×400) power, we selected the three most strongly stained areas and color photographs were taken in high power fields. More than 1000 tumor cells were counted on the photographs, and proliferative activity was scored as the percentage of MIB1-positive tumor cells [13,14].

Statistics

Associations between variables were analyzed with the χ^2 test. The Mann-Whitney U test for differences in mean values was used for comparison of nominal data. Mean values are shown ± the

standard error.

Results

The basic clinicopathological background characteristics are shown in Table 1. There were no significant differences in gender, age, or tumor location (right *vs*. left, upper lobe *vs*. lower lobe) between pure GGO and mixed GGO groups (Table 1). The mean tumor size in the pure GGO group was significantly smaller than that in the mixed GGO group (9.2±0.5 mm *vs*. 15.5±0.8 mm, $P<0.0001$). In the pure GGO group, 5 patients with GGO more than 10 mm in diameter selected initial operation and 39 patients underwent follow-up work before operation. Of 40 lesions in these patients, 6 lesions increased in size in the mean period of 8.3±3.0 months (range, 2–22 months) and the remaining 33 lesions showed no change in size in the mean period of 7.2±1.5 months (range, 2–45 months). The operative procedures used for pure GGO tumors were wedge resection in 30 cases, segmentectomy in 2 cases, and lobectomy in 6 cases, while those for mixed GGO tumors were wedge resection in 8 cases, segmentectomy in 2 cases, and lobectomy in 32 cases. Pathological subtypes of the tumors of 2.0 cm or less in diameter with pure GGO appearance were atypical adenomatous hyperplasia (AAH) in 7 cases, localized bronchioloalveolar carcinoma (LBAC) of Noguchi's type A in 25 cases, LBAC of Nuguchi's type B in 9 cases, and invasive adenocarcinoma of greater than Noguchi's type C in 2 cases. The pathological type of one pure GGO measuring 2.2

cm in diameter was LBAC. Tumors with mixed GGO appearance were AAH in 0 cases, LBAC of type A in 5 cases, type B in 12 cases, and invasive adenocarcinoma in 26 cases. The composition of pathological subtypes was clearly different between the two groups.

With respect to the two biological markers, nm23 staining was found in the epithelial component and was mainly cytoplasmic in tumor cells, while MIB1 protein showed nuclear staining. There were significant differences in both nm23 and MIB1 expression between the pure GGA group and the mixed GGA group (Table 2). That is, nm23 expression was greater and MIB1 expression score was lower in tumors with pure GGO appearance as compared to tumors with mixed GGO appearance. The pathological distribution and biological characteristics of tumors 2.0 cm or less in diameter with pure GGO appearance are summarized in Table 3. While AAH and non-invasive LBAC were predominant pathological types of pure GGO tumors, invasive adenocarcinoma of Noguchi's C type was found in only 2 lesions (4.5%) among 44 pure GGO tumors. There were no significant differences in nm23 or MIB1 expression between pure GGO tumors 10 mm or less in diameter and those 10–20 mm in diameter. Among these three pathological and biological factors, *i.e.*, invasive adenocarcinoma, nm23negativity, and high MIB1 score, none of the tumors had multiple factors simultaneously if the tumor size was 1.0 cm or less in diameter.

At less than the median follow-up period after surgery of 18 months (2–127 months), 2 patients died of diseases other than lung cancer and one patient in the mixed GGO group who underwent

partial resections for multiple lesions developed bone metastasis 18 months after the operation. The pathological type of this patient with recurrent disease was Noguchi's type C adenocarcinoma 10 mm in diameter. This type C lesion showed negativity for nm23 and MIB1 expression rate of 10%.

Comment

Although clinical roentgenographic data on the natural history of small lung tumors with pure GGO appearance are sparse, a previous study showed that lung cancer nodules with pure GGO appearance do not only increase in size or density, but also decrease in size with the appearance of a solid component [15]. Therefore, while an increase in size and/or density suggests the absolute necessity of surgical removal, a decrease in size does not exclude the requirement of surgery. In our series, excluding 5 patients with initial operation, 34 patients with 39 pure GGO lesions went through observation with a mean follow-up period of 7 months. Six lesions increased in size, 33 lesions showed no change in size or density, and no lesions were found to have decreased or diminished in size. With respect to the operative indications in this study, as described in the Patients and methods section, we performed VATS operation for mixed GGO. Cases of pure GGO 2 cm or less in diameter were observed for several months to exclude inflammatory changes. As the result of pathological examination of resected specimens, 87 lesions with GGO appearance were all found to be tumors.

Pathologically, the Noguchi's classification has prevailed in Japan as a useful indicator of

postoperative outcomes that would serve as a pathological basis for the selection of patients who would benefit from limited surgery. Interestingly, several cases of non-invasive LBAC of so-called Noguchi's A and B types revealed pure GGO appearance. In our series, consistent with previous studies, a large number of pure GGO tumors were included in Noguchi's A or B adenocarcinoma or AAH despite the tumor size. As lung cancers of Noguchi's type A and B are free from nodal metastasis, including micrometastasis [16], this observation appears to support the validity of limited operation for pure GGO measuring 2.0 cm or less in diameter.

In this study, we further assessed the expression of two biological markers by immunohistochemical analysis. MIB1 is a marker of tumor proliferation and nm23 is a putative anti-metastatic gene representing a metastasis-associated marker. Previously, we confirmed that nm23 expression in early-stage non-small cell lung cancers is inversely correlated with nodal micrometastasis. In the present study, using these two novel markers that mirror biological aspects of the tumors, we found significant differences in their expression between pure and mixed GGO groups. These findings support the hypothesis that mixed GGO tumors represent relatively high-grade malignancy with faster growth and greater metastatic ability in comparison with pure GGO tumors. These results also compare well with the observation that the mean tumor size in the mixed GGO group was significantly greater than that in the pure GGO group. In pure GGO tumors, a low MIB1 expression score and negativity of nm23 expression were found regardless of the size of

the tumors. If we look at the critical diameter of pure GGO tumor less than that at which any factors among 1) pathologically invasive type (Noguchi's C≤), 2) high score of MIB1 expression (>5%), and 3) negativity of nm23 expression were not identified, pure GGO less than 5 mm in diameter satisfied the criteria (data not shown). Although further studies in larger numbers of clinical cases should be performed, we concluded that pure GGO 5 mm or less in diameter does not require treatment and observation over a long period by CT is the best option. Based on our observation that several pure GGO tumors showed high MIB1 scores even though pathological examination revealed non-invasive Noguchi's A/B type, we concluded that a careful follow-up schedule would be needed for tumors in this category measuring more than 5 mm in diameter.

In conclusion, based on the pathological features and expression of nm23, the invasive and metastatic potential appears to be low in pure GGO tumors. In addition, this tendency was retained irrespective of tumor size in tumors less than 2 cm in diameter. On the other hand, the tumor proliferative ability assessed by MIB1 expression seems not to be necessarily low, and careful observation is needed in cases in which the tumor is more than 5 mm in diameter. However, surgical resection is justified instead of observation for mixed GGO tumors.

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Table 1. Clinicopathological background characteristics of 73 patients with ground-glass opacity (GGO)

Variables	Pure GGO	Mixed GGO	*P*-value
Total number of patients (Total number of lesions)	39 (44)	42 (43)	
Sex			0.9482
Male	17	17	
Female	22	25	
Mean age	63.5±1.8	64.0±1.6	0.9096
Mean tumor size (mm)	9.2±0.5	15.5±0.8	<0.0001
Location (1)			0.7380
Right	23	25	
Left	21	18	
Location (2)			0.7693
Upper lobe	25	30	
Middle lobe	7	2	
Lower lobe	12	11	
Operative procedure			<0.0001
Partial resection	30	8	
Segmentectomy	2	2	
Lobectomy	7	32	
Pathology			<0.0001
AAH	7	0	
LBAC	35	17	
Invasive adenocarcinoma	2	26	

Table 2. Biological appearance between pure and mixed ground-glass opacity (GGO) tumors 3.0 cm or less in diameter

GGO pattern	Nm23 expression Positive stain (%)	MIB1 expression (%)
Pure GGO (n=44)	97.7	2.7±1.0
Mixed GGO (n=43)	79.1	8.4±1.5
P-value	0.0064	<0.0001

Table 3. Pathological results of pure ground-glass opacity (GGO) tumors 2.0 cm or less in diameter

Tumor size	Pathological type			Biological characteristics	
(mm)	AAH % (n)	LBAC % (n)	Invasive Adenocarcinoma % (n)	Nm23 expression Positive stain (%)	MIB1 expression (%)
≤10	20 (6)	73(21)	7 (2)	93.3	2.2±0.6
10< ≤20	7 (1)	93 (13)	0 (0)	100	4.7±2.9
P-value	0.2595	0.1226	0.3143	0.486	0.2514