Dokkyo Journal of Medical Sciences
40(1) : 1～7, 2013

Original

Clinical Significance of *Propionibacterium acnes* in the Formation of Noncaseating Epithelioid-Cell Granulomas of the Mediastinal Lymph Nodes and Lung in Patients with Lung Cancer : Differential Diagnosis Between Sarcoid Reactions and Sarcoidosis

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SUMMARY

Objectives : Sarcoidosis is a systemic noncaseating epithelioid-cell granulomatous disease of unknown origin. Granulomas occurring around malignant tumors and regional lymph nodes can be caused by sarcoid reactions. The mechanisms underlying sarcoidosis and sarcoid reactions remain unclear. Whether increased uptake of fluorodeoxyglucose (FDG) in lymph nodes on positron emission tomography (PET) is caused by tumor metastasis, the concurrent presence of sarcoidosis, or sarcoid reactions must be determined to ensure proper disease staging and selection of treatment policy. We studied patients who underwent surgery for lung cancer and had no histopathological evidence of lymph-node metastasis in whom concurrent sarcoidosis or sarcoid reactions were diagnosed.

Methods : In six patients who underwent surgery for primary lung cancer, granulomatous lesions were histopathologically studied in dissected lymph nodes and lung. Tissue sections were stained with monoclonal antibodies against *Propionibacterium acnes* (PAB antibodies).

Results : The six patients had noncaseating epithelioid-cell granulomas in mediastinal lymph nodes and lung. Clinically, concurrent sarcoidosis was suspected, but the results of staining the tissue specimens with PAB antibodies (in granulomas, alveolar macrophages, Hamazaki-Wesenberg bodies, and lymphatic sinuses) suggested sarcoid reactions in 5 patients. In one patient in whom granulomas stained positive with PAB antibodies, concurrent sarcoidosis was diagnosed.

Conclusions : In patients with lung cancer who have no distinct systemic evidence of sarcoidosis, the presence of noncaseating epithelioid-cell granulomas in the lung hilum or mediastinum is usually caused by sarcoid reactions.

Key Words : lung cancer, *Propionibacterium acnes*, sarcoid reactions, sarcoidosis

Received July 24, 2012 ; accepted August 20, 2012
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Abbreviations :
FDG : fluorodeoxyglucose
FDG-PET : 18F-Fluorodeoxyglucose positron emission tomography
HW : Hamazaki-Wesenberg
P. acnes : *Propionibacterium acnes*
SUV : standardized uptake value

INTRODUCTION

Among various types of malignant tumors, noncaseating epithelioid-cell granulomas are known to rarely develop around tumors or in regional lymph nodes. Such granulomas are attributed to sarcoid reactions, which are considered a distinct entity from sarcoidosis, a systemic disease[1~4]. In patients with lung cancer, whether enlarged lymph nodes on imaging studies or increased uptake of fluorodeoxyglucose (FDG) in lymph nodes on positron emission tomography (PET) is caused by tumor metastasis, sarcoid reactions, or concurrent sarcoidosis must be determined to stage disease and formulate the treatment strategy. Several potential causes of sarcoid reactions in patients with lung cancer have been speculated, but remain to be established.

Sarcoidosis granulomas are specifically stained with monoclonal antibodies against *Propionibacterium acnes* (*P. acnes*) (PAB antibodies). *P. acnes* is considered to play an important role in the development of sarcoidosis[5,6]. We immunostained specimens of noncaseating epithelioid-cell granulomas arising in the lung and regional or mediastinal lymph nodes with PAB antibodies in patients with primary lung cancer who underwent surgery and had no histopathological evidence of lymph-node metastasis. We then assessed the involvement of *P. acnes*, sarcoid reactions, and concurrent sarcoidosis in the formation of granulomas in individual patients with lung cancer.

PATIENTS AND METHODS

During the 5 years from January 2006 through December 2010, 443 patients with primary lung cancer underwent surgical resection at the Department of General Thoracic Surgery in our hospital. In six patients, histopathological examination of the resected lung and dissected lymph nodes revealed noncaseating epithelioid-cell granulomas in the resected lung and dissected lymph nodes. And there were no deposits of anthracotic pigment in the epithelioid-cell granulomas. Noncaseating epithelioid-cell granulomas found only in regional lymph nodes were attributed to sarcoid reactions affecting only 1 organ. Patients with such granulomas were excluded from the study. Preoperative examinations showed no evidence of systemic changes suggestive of sarcoidosis or of infectious diseases associated with granulomatous lesions, such as tuberculosis, nontuberculous mycobacteriosis, or mycosis. There was also no occupational or environmental exposure to chemical substances such as beryllium, aluminium, or zirconium.

T and N categories in this report were assessed according to the TNM staging system of the Japan Lung Cancer Society[7]. Computed tomographic (CT) scanning was performed with Asteion or Aquillion (Toshiba, Japan). The patients were always supine, and scans were obtained with the following parameters : slice width, 3.0-mm collimation and 3.0-mm slice interval. 18F-Fluorodeoxyglucose positron emission tomography (FDG-PET) was performed as follows. FDG was intravenously administered at 4.5 MBq/kg following fasting for at least 5 hours, and early and delayed images were taken 50 minutes and 2 hours after FDG administration, respectively. FDG-PET/CT images were captured by a Biograph 16 (Siemens, Germany) or GEMINI 16 (Philips, Netherlands) system. The maximum standardized uptake value (SUV max) was evaluated for each lesion.

Monoclonal Antibodies against P. acnes

Antibodies were generated according to the protocol described in a laboratory manual with modifications[8]. The details of establishing monoclonal antibodies specific against *P. acnes* (named PAB antibodies, IgM) were described previously[9]. Kindly the PAB antibodies were provided from Professor Y. Eishi of the Department of Human Pathology, School of Medicine, Tokyo Medical and Dental University.

Immunohistochemistry

The formalin-fixed, paraffin-embedded, lung tissue specimens were stained with hematoxylin and eosin. Serial sections of confirmed sites of noncaseating epithelioid-cell granulomas were mounted on silane-coated slides. The specimens were immunohistochemically stained with PAB antibodies according to the protocol methods described previously and viewed under a light microscope[9]. PAB-antibody-positive staining in granulomas, alveolar macrophages, and Hamazaki-Wesenberg (HW) bodies, and lymphatic sinuses was semi-quantitatively evaluated according to the follow-

Table 1 Clinical Characteristics of Patients

Patient Age/gender	Tumor location	Lung cancer cTNM Stage	Histlogy	Complications	Evaluation of sarcoidosis				
					SUV value of mediastinal lymph nodes (E/D)	ACE	Eye lesions	Cardiac lesions	Activity
① 77 yo F	Rt-S10	T1N0M0 stage IA	Ad	None	6.2/6.6	10.4	None	None	None
② 75 yo M	Rt-S6	T1N0M0 stage IA	Sq	IP	4.3/4.8	11.2	None	Atrial extrasystole	None
③ 63 yo M	Rt-S1	T3N1M0 stage IIIA	Sq	Sigmoid colon cancer	6.9/7.4	18.1	None	Left ventricular hypertrophy	None
④ 79 yo F	Lt-S8	T1N0M0 stage IA	Ad	RA	3.9/4.0	7.6	None	None	None
⑤ 78 yo M	Rt-S6	T2N3M0 stage IIIB	Ad	None	7.7/10.1	17.0	None	None	None
⑥ 69 yo M	Rt-S2	T3N0M0 stage IIB	Ad	None	4.9/5.0	ND	None	None	None

SUV denotes standardized uptake value ; E/D, early and delayed ; yo, years old ; Ad, adenocarcinoma ; Sq, squamous cell carcinoma ; IP, interstitial pneumonia ; RA, rheumatoid arthritis ; ND, not done and ACE, angiotensin converting enzyme.

ing 3-grade scale : −, +, and + +.

This study was approved by the Bioethics Committee Dokkyo Medical University and a signed consent form was obtained from each patient.

RESULTS

Among 443 patients with primary lung cancer who underwent surgical resection at the Department of Respiratory Surgery in our hospital during the 5 years from January 2006 through December 2010, a total of 6 patients had noncaseating epithelioid-cell granulomas in the resected lung and dissected lymph nodes. Table 1 shows the clinical characteristics of the patients and the results of examinations to assess systemic sarcoidosis. There were 4 men and 2 women, with a mean age of 73.5 years (range, 63 to 79). The histologic type of lung cancer was adenocarcinoma in 4 patients and squamous-cell carcinoma in 2. The preoperative clinical stage of lung cancer as evaluated by computed tomography, FDG-PET, and endobronchial ultrasonography-guided transbronchial needle aspiration was stage IA in 3 patients, stage IIB in 1, stage IIIA in 1, and stage IIIB in 1. The postoperative histopathological stage of lung cancer was stage IA in 3 patients and stage IIB in 3. The postoperative tumor stage in the 2 patients with a preoperative diagnosis of stage IIIA or IIIB disease was revised to stage IIB because no lymph-node metastasis was found on operation. One patient was given a preoperative diagnosis of stage IIIA disease because the hilar lymph nodes showed increase uptake of FDG (SUV, 6.9/7.4) on FDG-PET, although no malignant cells were detected on endobronchial ultrasound-guided lymph-node biopsy. The other patient was given a preoperative diagnosis of stage IIIB disease because FDG-PET revealed increased uptake of FDG (SUV, 7.6/10.1) in the mediastinal and contralateral hilar lymph nodes, although no malignant cells were detected on endobronchial ultrasound-guided transbronchial needle aspiration. To our knowledge, clinically active sarcoidosis associated with lesions of the lung, eyes, heart, or skin has not been reported previously.

Table 2 shows the results of immunostaining with PAB antibodies. In one of the six patients, PAB-antibody staining was negative in granulomas in the lung, but positive in granulomas in dissected lymph nodes.

Table 2 Results of Immunostaining with PAB Antibodies

Patient	Lung cancer	LN granuloma	Lung granuloma	Decision
		HW	macrophage	
① 77 yo F	Ad	+	−	Sarcoidosis
		+	+	
② 75 yo M	Sq	−	−	Sarcoid reaction
		+	+	
③ 63 yo M	Sq	−	−	Sarcoid reaction
		+	++	
④ 79 yo F	Ad	−	−	Sarcoid reaction
		+	−	
⑤ 78 yo M	Ad	−	−	Sarcoid reaction
		−	−	
⑥ 69 yo M	Ad	−	−	Sarcoid reaction
		+	+	

Ad denotes adenocarcinoma ; Sq, squamous cell carcinoma and HW, Hamazaki-Wesenberg bodies.

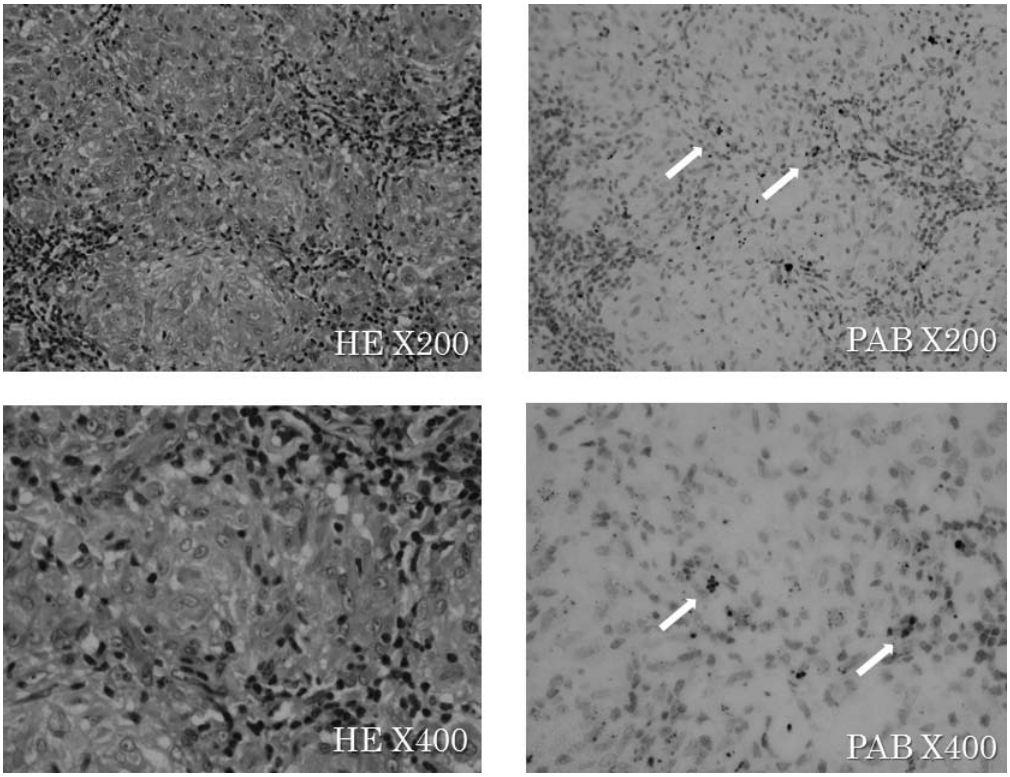

Figure 1 *P. acnes* within noncaseating epithelioid-cell granulomas of the lymph nodes. Hematoxylin and eosin staining (left) and immunostaining with PAB antibodies (right) are shown pairwise (original magnification, X200 and X400). Small brown round bodies were detected by PAB antibodies within granulomas (arrows).

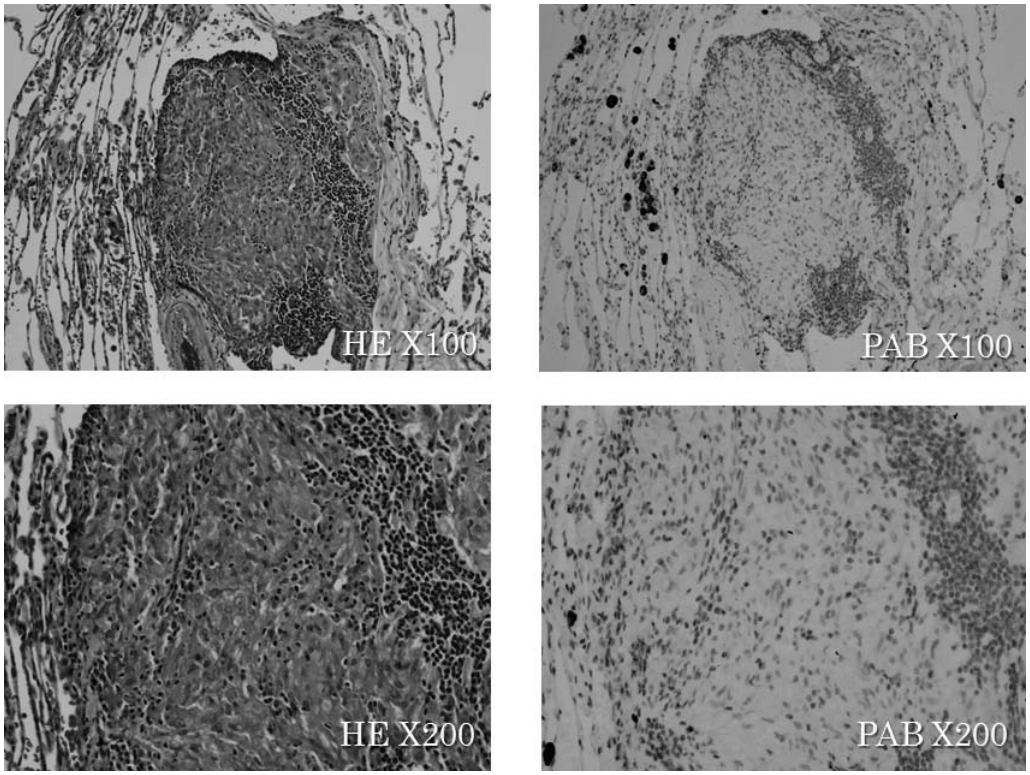

Figure 2 *P. acnes* within noncaseating epithelioid-cell granulomas of the lungs. Hematoxylin and eosin staining (left) and immunostaining with PAB antibodies (right) are shown pairwise (original magnification, X100 and X200). Immunostaining with PAB antibodies were negative within granulomas, while small brown round bodies were detected by PAB antibodies within alveolar macrophages.

Histopathologically, concurrent sarcoidosis was suspected on the basis of these findings (Fig. 1). In the other five patients, PAB-antibody staining was negative in granulomas in the lung and dissected lymph nodes. These granulomas were therefore attributed to sarcoid reactions associated with lung cancer (Fig. 2). In some patients, PAB-antibody staining was weakly positive in HW bodies, alveolar macrophages, and lymphatic sinuses.

DISCUSSION

Sarcoid reactions are found in only about 4.4% of malignant tumors. In lung cancer, the incidence of sarcoid reactions around tumors and in regional lymph nodes has been reported to range between 2.2% and 3.2%[10~12]. Sarcoidosis associated with lung cancer has been reported previously[13~17]. In many patients, sarcoidosis was diagnosed several years before the onset of lung cancer, but both diseases were simultaneously confirmed in some patients[14,18]. It is difficult to determine whether such patients concurrently have sarcoidosis or sarcoid reactions. Histopathologically, granulomas induced by sarcoid reactions tend to be poorly demarcated and scattered, as compared with granulomas induced by sarcoidosis[19,20]. However it was difficult to differentiate between sarcoidosis and sarcoid reactions solely on the basis of histopathological findings in these six cases.

The subjects of the present study were six patients who underwent surgery for primary lung cancer and had noncaseating epithelioid-cell granulomas in the resected lung and dissected lymph nodes, with no evidence of regional lymph-node metastasis. To confirm whether the granulomas were induced by sarcoidosis or sarcoid reactions associated with lung cancer, the involvement of *P. acnes* was examined on immunostaining with PAB antibodies. In five of the six patients, PAB-antibody staining was negative in noncaseating epithelioid-cell granulomas, ruling out an association with *P. acnes*.

The mechanism of tumor-associated sarcoid reactions in regional lymph nodes has not yet been elucidated. There have been several speculations concerning the causal relationship between malignant tumors and sarcoid reactions[21,22]. Sarcoid reactions have been considered 1) a localized defense reaction to tumor cells themselves, 2) a simple tissue reaction to tumor embolism in the lymphatic system or capillaries, and 3) an immunologic reaction to substances released from the tumors and transported along the lymphatic system.

In one patient, PAB-antibody staining was positive in granulomas situated in dissected lymph nodes, indicating an association with *P. acnes*, i.e., the concurrent presence of sarcoidosis could not be completely ruled out. However, this patient had no evidence suggesting active sarcoidosis on the preoperative workup.

Previous studies have suggested three possibilities for the relation between sarcoidosis and lung cancer[23~25] : 1) the two diseases incidentally coexist, 2) the cell-mediated immune abnormalities induced by sarcoidosis are involved in the onset of lung cancer, and 3) lung cancer originates in fibrous tissue because of sarcoidosis. Unanswered questions remain concerning the role of *P. acnes* in the formation of sarcoid granulomas and the sensitivity and specificity of imunostaining with PAB antibodies against *P. acnes*. *P. acnes* is the only microorganism isolated from affected lymph nodes in patients with sarcoidosis and can be effectively isolated from sarcoidosis lesions and cultured in large quantities. However, because *P. acnes* is part of the normal flora of the skin and is isolated from about 25% of biopsy specimens of lymph nodes in patients without sarcoidosis, the causal relation between *P. acnes* and sarcoidosis was difficult to define. In Japan, however, comprehensive studies conducted by Eishi et al. established the theory that "*P. acnes* is a cause of sarcoidosis," and the presence of *P. acnes* in granulomas in affected organs was shown to be particularly useful for the diagnosis of sarcoidosis[9,26~29]. PAB antibodies, a monoclonal antibody specifically against *P. acnes*, specifically reacts with lipoteichoic acid and glycolipid antigens distributed from the bacterial cell membrane through the cell wall. The rates of PAB-antibody positivity against granulomas in the lung and lymph nodes in patients with sarcoidosis was 48% for transbronchial lung-biopsy samples, 74% for video-assisted thoracic surgery lung samples, and 88% for lymph node samples. This antibody never reacts with *Mycobacterium tuberculosis*, other bacteria, or granulomas induced by sarcoid reactions[9]. Therefore, positive immunostaining with this antibody provides indirect evidence of the local presence of *P. acnes*, suggesting that tissue changes are induced by sarcoidosis. Because the results of PAB-antibody testing were negative in noncaseating epithelioid-cell granulomas in five of our six patients, the associated tissue changes were attributed to sarcoid reactions caused by lung cancer.

The developmental mechanism of sarcoid reactions associated with lung cancer and other malignant tumors remains unclear. Our results suggest that the participation of *P. acnes* in sarcoid reactions accompanying lung cancer is rare, even when granulomas are present in the lung as well as regional lymph nodes. Sarcoid reactions are found in only 2.2% to 3.2% of all cases of lung cancer. In patients who have enlarged lymph nodes showing increased uptake of FDG on PET, the absence of malignant cells on histopathological examination strongly suggests the possibility of sarcoid reactions, and this may have important consequences for tumor staging. However, because we studied only six patients, further studies of larger numbers of patients are necessary to confirm our results.

Acknowledgments We thank Professor Y. Eishi of the Department of Human Pathology, School of Medicine, Tokyo Medical and Dental University, for excellent advice for the immunohistochemical analysis.

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