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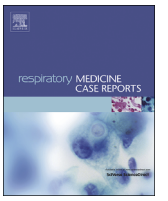

Case Report

A case of pleural mesothelioma with immunohistochemical staining positive for Krebs von den Lungen-6

Yugo Matsumura [a], Seidai Sato [a], Keiko Haji [a], Takeshi Masuda [b], Hiroto Yoneda [a], Hirokazu Ogino [a], Hirohisa Ogawa [c], Masaki Hanibuchi [a, d], Noboru Hattori [b], Yasuhiko Nishioka [a, *]

[a] *Department of Respiratory Medicine and Rheumatology, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan*
[b] *Department of Respiratory Medicine, Hiroshima University Hospital, Hiroshima, Japan*
[c] *Department of Pathology and Laboratory Medicine, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan*
[d] *Department of Community Medicine for Respirology, Hematology and Metabolism, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan*

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ABSTRACT

A 71-year-old male visited a hospital with a chief complaint of exertional dyspnea. A chest CT revealed multiple nodular lesions on the parietal pleura. Thoracoscopic pleural biopsy was performed resulting in a diagnosis of pleural mesothelioma with epithelioid type. When chemotherapy was initially initiated, his serum level of Krebs von den Lungen-6 (KL-6) was high. However, once chemotherapy was started, the serum KL-6 level gradually decreased with tumor shrinkage. Immunohistochemical staining revealed the expression of KL-6 from the tumor cells. This is the first report of KL-6 production directly from tumor cells in epithelial-type pleural mesothelioma.

1. Introduction

Mesothelioma is a neoplasm that can arise in the pleura (80–85 %), peritoneum (10–15 %), pericardium, and tunica vaginalis of the testis [1]. Asbestos exposure has been reported as a major cause of pleural mesothelioma. Pleural mesothelioma typically manifests with non-specific clinical symptoms and lacks definitive clinical tests or immunohistochemical markers, rendering its early diagnosis challenging.

Krebs von den Lungen-6 (KL-6) is expressed moderately in type II lung cells, respiratory bronchial epithelial cells, and gastric foveolar cells [2]. In interstitial lung disease, it is highly produced by regenerating type II lung cells and has been reported as a highly specific marker for disease activity [3,4]. On the other hand, several recent researches have focused on KL-6 as a candidate tumor marker in several malignancies, including pleural mesothelioma [5–12]. However, the underlying mechanism by which serum KL-6 is elevated in pleural mesotheliomas cases has not been elucidated.

In the present case, we present a case of pleural mesothelioma in which serum KL-6 correlated with disease transition and immunohistochemical staining demonstrated KL-6 production from tumor cells.

* Corresponding author. Department of Respiratory Medicine and Rheumatology Graduate School of Biomedical Sciences, Tokushima University, 3Kuramoto-cho, Tokushima, 770-8503, Japan.
E-mail address: yasuhiko@tokushima-u.ac.jp (Y. Nishioka).

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2. Case report

A 71-year-old male with a height of 161 cm and weight of 55 kg visited a nearby hospital with exertional dyspnea. He worked at a construction industry for 50 years without known exposure to asbestos. He had no smoking history. There was no significant medical, surgical, or medication history.

Chest X-ray showed a left massive pleural effusion with mediastinal shift. After drainage of bloody pleural fluid, a nodular lesion on the left upper lung field was revealed by chest X-ray (Fig. 1A). There were multiple nodules on the left pleura, but there was no interstitial pneumonia with chest computed tomography (CT) (Fig. 1B, C, 4A). Although the cytology of pleural effusion was class II, a thoracoscopic pleural biopsy was performed because malignancy was strongly suspected. Hematoxylin and Eosin (H&E) staining of chest wall nodules revealed sheet like epithelioid tumor cells, oval shaped tumor cells with rich-cytoplasm and prominent nuclei with inconspicuous nucleoli (Fig. 2A). In immunohistochemical staining, tumor cells were negative for thyroid transcription factor (TTF)-1

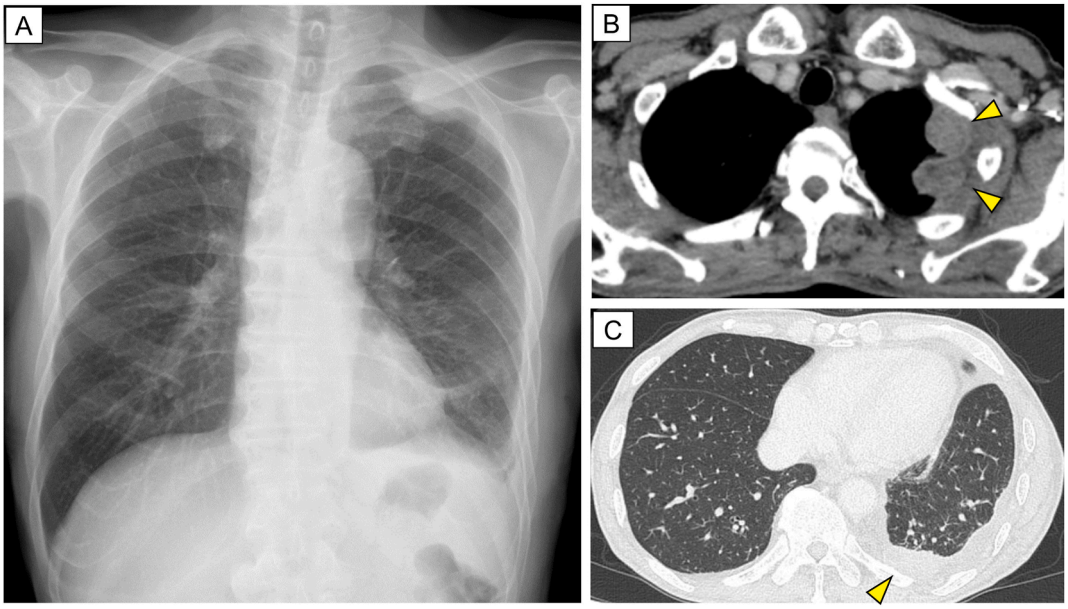

Fig. 1. (A): Chest X-ray image after drainage of bloody pleural fluid at first visit to nearby hospital. (B, C): High-resolution chest tomography (HRCT) images after drainage of bloody pleural fluid at first visit to nearby hospital. HRCT shows multiple nodular lesions on the pleura (arrowhead) in the left apical pulmonary region and base of the lung. HRCT showed no findings suggestive of interstitial pneumonia in the lung.

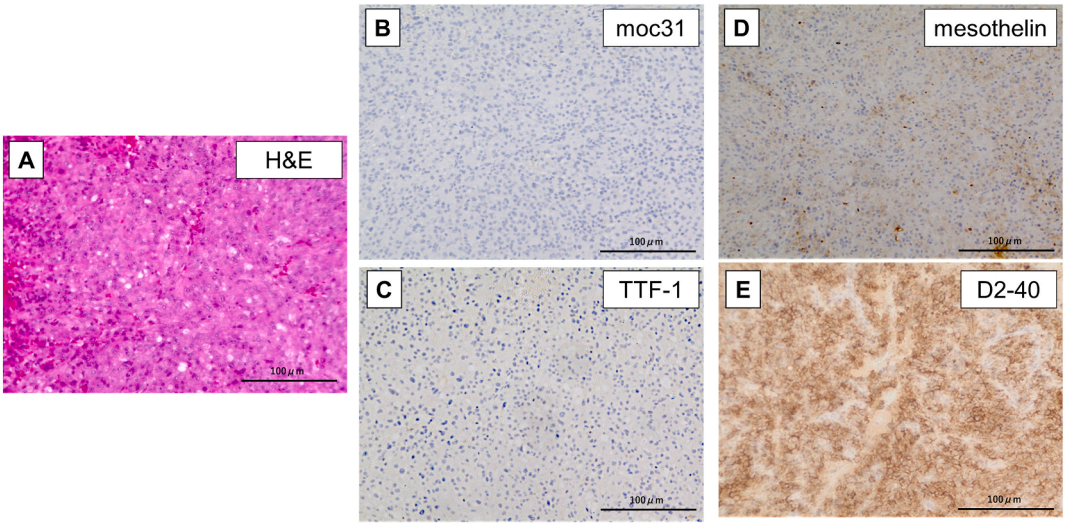

Fig. 2. Histopathological findings of the thoracoscopic pleural biopsy specimen. Pathological specimens obtained from a nodule on the left visceral pleura with Hematoxylin and Eosin showed sheet like epithelioid tumor cells, oval shaped mesothelioma cells with rich-cytoplasm and prominent nuclei with inconspicuous nucleoli (A). Tumor cells showed negativity for moc31 (B) and thyroid transcription factor (TTF)-1 (C). Tumor cells were moderate positive for mesothelin (D) and strong positive for D2-40 (E). All scale bars in figures shows 100 μm.

and MOC-31, but positive for D2-40 and mesothelin, leading to a diagnosis of pleural mesothelioma (Fig. 2B–E). Based on systemic imaging studies, the clinical stage was diagnosed as cT4N0M0 Stage IIIB. Two months after his initial visit to nearby hospital, the patient was referred to our hospital for chemotherapy.

On admission to our hospital, he complained dyspnea on exertion, with Modified British Medical Research Council (mMRC) grade 1. His initial vital signs were; blood pressure,130/66 mmHg; a heart rate, 111 bpm; a body temperature, 37.1 °C; percutaneous oxygen saturation (SpO_2) at room air, 94 %. Physical examination revealed no audible rales on lung auscultation and no palpable peripheral lymph nodes. The laboratory data showed hypoalbuminemia, elevated C-reactive protein levels and high KL-6 levels. Other dates were within normal limits. Serum carcinoembryonic antigen (CEA) was within normal limits (Table 1). Serum KL-6 levels were found to be elevated at 1778 IU/mL.

Cisplatin (500 mg/m^2) plus pemetrexed (75 mg/m^2) was started as first line chemotherapy. After two cycles of chemotherapy, chemotherapy was stopped because of drug-induced renal injury. However, because renal function was not expected to improve with the cessation of chemotherapy, steroid treatment with prednisolone was initiated. On the other hand, during the cessation of chemotherapy, serum KL-6 increased with the progression of pleural mesothelioma (Figs. 3 and 4B). As renal function improved, steroids were gradually tapered off. Second line treatment with nivolumab (240 mg/body) monotherapy was initiated when the prednisolone dose reached 10 mg/day. After the initiation of nivolumab, serum KL-6 levels gradually decreased as the tumors shrank (Figs. 3 and 4C).

Because serum KL-6 trends were apparently in sync with tumor size and response to chemotherapy, we performed immunohistochemical staining for KL-6 in pleural mesothelioma tissue using anti-KL-6 mouse monoclonal antibody (1:100, provided by Hiroshima University). Immunoreactivity was detected using 3,30-diaminobenzidine Tetrahydrochloride (DAB) Liquid System (Nichirei), and

Table 1
Laboratory data on admission.

Complete blood count		Blood chemistry	
WBC	5700/μL	TP	6.8g/dL
Neut	69.9 %	Alb	3.0g/dL
Lymp	20.7 %	AST	25U/L
Mono	7.3 %	ALT	23U/L
Eo	0.3 %	LD	173U/L
Baso	0.6 %	T-Bil	0.7mg/dL
RBC	464 × 104/μL	CK	37U/L
Hb	13.9g/dL	BUN	12mg/dL
Hct	42.8 %	Cre	0.78mg/dL
Plt	39.4 × 104/μL	Na	139mEq/L
Coagulation system		K	4.8mEq/L
APTT	36.7Sec	Cl	101mEq/L
PT	14.7Sec	CRP	4.64mg/dL
PTR	70 %	Tumor marker	
Fib	919mg/dL	CEA	2.3ng/mL
D-dimer	1.4μg/mL	KL-6	1,778U/mL

WBC: white blood cell, Neut: neutrophil, Lymp: lymphocyte, Mono: monocyte, Eo: eosinophil, Baso: basophil, RBC: red blood cell, Hb: hemoglobin, Hct: Hematocrit, Plt: platelet count, APTT: activated partial thromboplastin time, PT: prothrombin time, PTR: prothrombin ratio, Fib: fibrinogen, TP: total protein, Alb: albumin, AST: asparate transaminase, ALT: alanine transaminase, LD: lactate dehydrogenase, T-Bil: total bilirubin, CK: creatine phosphokinase, BUN: blood urea nitrogen, Cre: Creatinine, CRP: C-reactive protein, CEA: carcinoembryonic antigen, KL-6: Krebs von den Lungen-6.

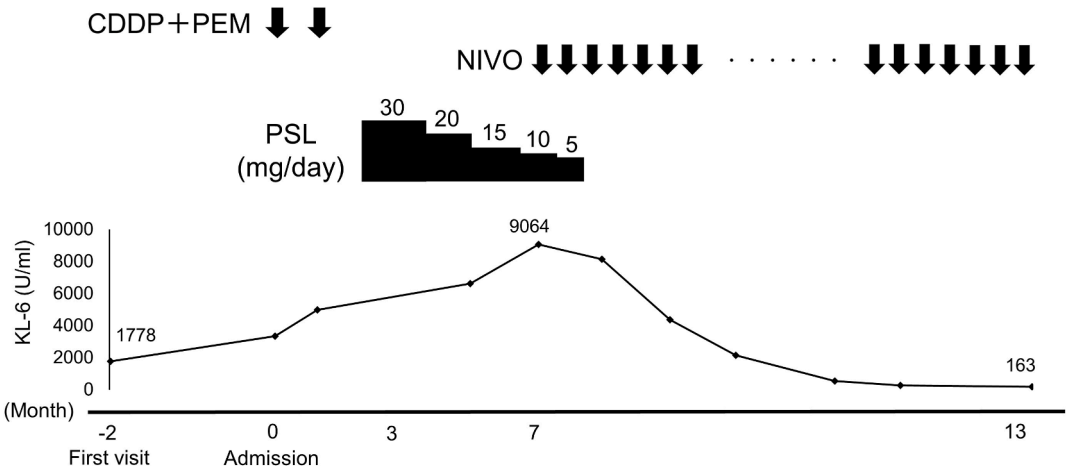

Fig. 3. Clinical course of the patient after first visit to our hospital. CDDP: cisplatine, PEM: pemetrexed, NIVO: nivolumab, PSL: prednisolone, KL-6: Krebs von den Lungen-6.

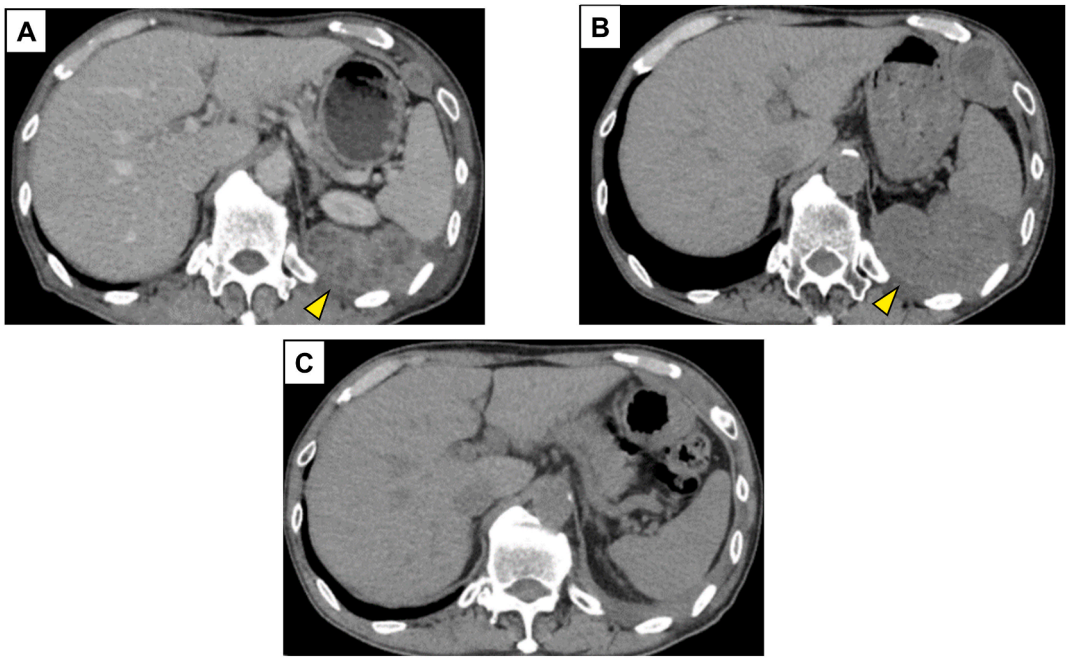

Fig. 4. (A): High-resolution chest tomography (HRCT) image at the start of chemotherapy with cisplatin and pemetrexed. There was a tumor in the pleura of the left lung base (arrowheads). (B): HRCT image at the start of starting nivolumab. Tumor had increased during the period of prednisolone use for drug-induced renal injury (arrowhead). (C): HRCT image at six months after starting nivolumab treatment. Tumor shrinkage was observed after nivolumab administration.

samples were counterstained with hematoxylin. In normal lung tissue, alveolar epithelial cells were KL-6 positive (Fig. 5A and B), as previously reported [13]. Furthermore, in pleural mesothelioma tissue, KL-6 was positive on the cell surface (Fig. 5C and D), suggesting tumor cells themselves produced KL-6.

3. Discussion

Mesothelioma is an aggressive tumor that arises from the mesothelial cells lining the internal cavities of the body, such as the pleura, peritoneum, and pericardium [1]. It is closely associated with asbestos exposure. The definitive diagnosis of pleural mesothelioma relies on pathological examination using various immunohistochemical tests based on enough tumor cells obtained through procedures such as thoracoscopy. However, because early detection of pleural mesothelioma is extremely difficult, most cases are already in an advanced stage when a definitive diagnosis is made. Therefore, in order to achieve early diagnosis, it is essential to advance imaging and endoscopy, as well as to develop useful biomarkers for diagnosis using blood or body cavity fluid.

KL-6 is widely used in clinical practice as a serum and immunohistochemical marker for the diagnosis and assessment of interstitial lung diseases. Initially, KL-6 was discovered in a study to search for cancer-specific and cancer-related antigens in lung cancer [14]. On the other hand, further investigation revealed that high levels of KL-6 were observed in the serum of patients with interstitial pneumonia and correlated with disease activity [15]. Moreover, studies comparing KL-6, surfactant protein A, surfactant protein D, and monocyte chemoattractant protein-1 as serum markers for interstitial lung disease [16], or comparing KL-6 to CA19-9 and SLX [17], both showed that KL-6 is the best diagnostic marker for interstitial lung disease. Thus, KL-6 has since been developed as a serum marker for interstitial pneumonia,

On the other hand, the immunohistochemical evaluation of KL-6 has been attempted to be applied to the diagnosis of various epithelial malignancies other than lung cancer, such as in the digestive tract, liver, pancreas, and ovaries [7,12]. In mesothelial malignancies, Stockhammer et al. evaluated KL-6 levels in the pleural effusion of pleural mesothelioma, and reported that pleural mesothelioma patients had significantly higher KL-6 levels in pleural effusion than non-malignant controls [11]. However, this study did not examine which tissue the KL-6 in the pleural fluid originated from. In the present case, KL-6 was elevated even though there was no evidence of interstitial pneumonia or cancer other than pleural mesothelioma. Besides, immunohistochemical staining revealed that KL-6 was highly expressed on pleural mesothelial cells, suggesting that KL-6 was produced by pleural mesothelioma itself. Therefore, this is the first report examining the source of KL-6 production in a case of pleural mesothelioma. Similarly, in a case of mesothelioma arising in the peritoneum, Nahar et al. performed immunohistochemical staining as in the present case, and confirmed KL-6 expression in mesothelioma cells [10].

In the present study, serum KL-6 levels increased with tumor growth and decreased with tumor shrinkage. There have been no reports about the relationship between the size of pleural mesothelioma and serum KL-6. The clinical course demonstrated in this case is the first indication of the potential of serum KL-6 as a marker for assessing the status of pleural mesothelioma. If a patient with uncomplicated interstitial pneumonia has an elevated KL-6, it might have better to recognize mesothelioma as a tumor that can be listed

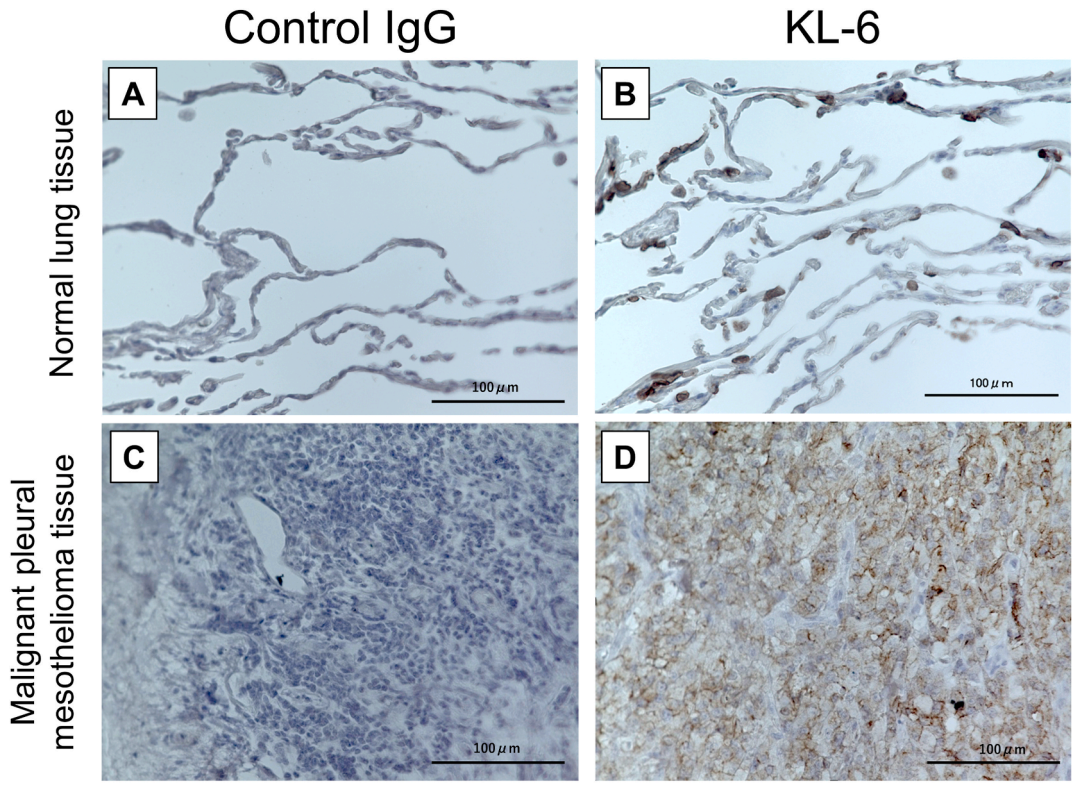

Fig. 5. Immunohistochemical staining of Krebs von den Lungen-6 (KL-6). (A, B): The surfaces of alveolar epithelial cells of normal lung tissue were stained by KL-6 antibody but not with control IgG. (C, D): The surfaces of mesothelioma cells were stained by KL-6 antibody but not with control IgG. All scale bars in figures shows 100 μm.

as a differential diagnosis. On the other hand, no known tumor markers of pleural mesothelioma, such as soluble mesothelin-related peptide or CYFRA-21-1, were measured in the present case except CEA. Therefore, the correlation between KL-6 levels and these tumor markers in pleural mesothelioma with high KL-6 levels requires further investigation.

KL-6 is a large glycoprotein with a molecular weight of more than 200 kDa and belongs to the Human mucin-1 glycoprotein (MUC1) mucin. Anti-KL-6 antibody, a mouse monoclonal antibody, recognizes sialoglycans expressed on the human MUC1 molecule [2](14). In other words, KL-6 is the antigen in MUC1 that has the sialoglycans recognized by the anti-KL-6 antibody. KL-6 expression in malignant tumors has been reported more in epithelial than in non-epithelial tumors [7,12], which may be because MUC1 is expressed on the luminal surface of many epithelial cells. Indeed, in the report by Stockhammer et al., the elevation of KL-6 in pleural effusion was more common in epithelial or biphasic forms of pleural mesothelioma [11]. In both our case and in previously reported case [10], the mesothelioma with elevated serum KL-6 was of the epithelial-type. Thus, pleural mesothelioma with KL-6 expression may be limited to epithelial or biphasic mesothelioma with at least 10 % epithelial component.

In summary, we described a case of pleural mesothelioma in which KL-6 expression was confirmed by immunohistochemical staining, indicating its potential as a biomarker for response to therapy. Further studies are needed to elucidate the molecular characteristics of KL-6 in relation to pleural mesothelioma and to evaluate its clinical significance.

CRediT authorship contribution statement

Yugo Matsumura: Writing – original draft, Methodology. **Seidai Sato:** Writing – original draft, Writing – review & editing, Supervision. **Keiko Haji:** Writing – review & editing, Investigation, Supervision. **Takeshi Masuda:** Resources, Writing – review & editing. **Hiroto Yoneda:** Supervision. **Hirokazu Ogino:** Supervision, Writing – review & editing. **Hirohisa Ogawa:** Methodology, Supervision. **Masaki Hanibuchi:** Supervision, Writing – review & editing. **Noboru Hattori:** Resources, Supervision. **Yasuhiko Nishioka:** Supervision, Writing – review & editing.

Declaration of competing interest

No authors have conflicts of interest to declare.

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