Table 1. Expression of apoptosis related proteins and apoptotic index in mesothelioma tissues and cell lines

Cases	Sex	Age	Histology	AI	Bax[a]	Bcl-2[a]	Survivin[a]	Caspase- 3[a]	Cleaved caspase-3 index[a]
MM-A26	F	52	EM	0	++	-	+/W	+	10.6
MM-S01	M	26	EM	2.0	++	-	++/S	++	15.7
MM-A04	M	23	EM	5.0	++	+	+/S	-	35.6
MM-S46	F	48	EM	6.9	++	-	++/W	++	4.0
MM-S17	M	77	EM	7.0	++	-	++/S	++	14.8
MM-A35	M	57	EM	8.0	++	-	++/S	++	17.4
MM-S16	M	61	EM	9.2	++	-	+/W	++	21.0
MM-S42	M	56	EM	9.8	++	-	++/S	++	2.0
MM-A41	F	42	EM	12.5	++	-	+/S	-	12.3
MM-S39	F	39	EM	12.7	++	-	++/W	++	8.8
MM-S31	M	72	EM	13.9	++	-	++/S	++	19.4
MM-A06	F	74	EM	15.0	++	+	NI	++	2.0
MM-S35	M	46	EM	17.4	++	-	++/S	++	1.0
MM-S41	M	81	EM	17.7	++	-	+/W	++	1.9
MM-S36	M	46	EM	19.8	++	-	+/W	++	14.4
MM-A40	M	75	EM	21.7	++	-	++/W	-	25.8
MM-A19	M	49	EM	22.1	++	-	++/W	+	18.8
MM-S50	F	48	EM	23.1	++	-	++/W	++	0
MM-S52	M	60	EM	32.5	++	-	+/S	++	8.0
MM-S48	M	69	EM	32.9	++	-	+/S	++	18.4
MM-S20	M	67	EM	34.9	++	-	++/W	++	24.4
MM-A10	F	76	EM	NI	++	-	++/W	+	35.1
MM-A30	M	73	EM	NI	++	-	+/W	++	2.0
MM-S15	M	54	SM	5.0	++	-	NI	++	16.0
MM-A02	M	64	SM	7.0	++	-	-	+	1.0
MM-A21	M	49	SM	15.0	++	-	++/W	+	2.0
MM-A31	M	65	SM	22.0	++	-	+/W	+	25.0
MM-A09	M	75	SM	23.0	++	-	+/W	+	3.0
MM-A18	M	65	SM	32.7	++	-	+/W	+	12.3
MM-A38	M	79	SM	32.7	++	-	+/W	+	21.7
MM-S26	M	61	SM	34.5	++	-	NI	++	22.9
MM-S02	M	68	SM	41.9	++	-	++/W	++	36.5
MM-S33	M	51	BM	11.1	++	-	++/S	++	12.8
MM-A39	F	66	BM	23.0	++	-	+/W	++	24.5
ACC MESO 4			EM	58.3	++	-	++/S	++	92.8
ACC MESO 1			SM	57.2	++	-	++/S	+	74.6
NCI-H2452			SM	76.3	++	-	++/S	++	109.2

AI, apoptotic index; BM, biphasic mesothelioma; EM, epithelioid mesothelioma; F, female; M, male; NI, not informative; S, strong; SM, sarcomatoid mesothelioma; W, weak

[a]Details of immunohitochemical scoring in mesothelioma described in methods

Table 2. Apoptotic index in mesothelioma, lung carcinoma, gastric adenocarcinoma and colon adenocarcinoma

		Apoptotic index			
	No. of cases	Range	Mean	SD	p-value[a]
Mesothelioma	32	0 - 41.9	17.6	11.1	
Epitheliod mesothelioma	21	0 - 34.9	15.2	9.9	0.063[b]
Sarcomatoid mesotehlioma	9	5.0 - 41.9	23.5	12.9	
Biphasic mesothelioma	2	11.1 - 23.0	17.1	8.4	
Lung carcinoma	10	10.0 - 57.8	28.6	15.8	0.018[c]
Gastric adenocarcinoma	10	6.5 - 57.0	30.1	16.4	0.009[c]
Colon adenocarcinoma	10	12.8 - 71.3	31.4	17.3	0.005[c]

[a] Student t- test

[b] Epithelioid mesothelioma versus sarcomatoid mesothelioma

[c] Versus mesothelioma

Table 3. Apoptotic index and cleaved caspase-3 index in mesothelioma

	No. of cases	Range	Mean	SD	r	p-value*
Apoptotic index	32	0 - 41.9	17.6	11.3	0.373	0.036
Cleaved caspase-3 index	34	0 - 36.5	14.7	10.6		

* Pearson's correlation used 32 cases, except for 2 cases as not informative of apoptosis.

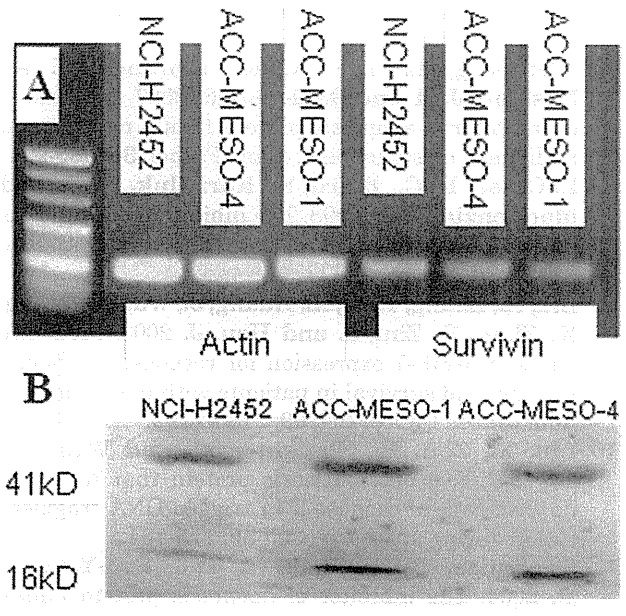

Fig. 2. (A) Electrophoresis of RT-PCR product of survivin mRNA and actin mRNA amplification shows 121 bp and 120 bp fragments. (B) Western blot analysis shows expression of survivin protein (16 kD) and actin (41 kD) in all three of the mesothelioma cell lines.

DISCUSSION

In the present study, the mean apoptotic index (AI) in mesothelioma tissue was 17.6, which is similar to those reported previously in mesothelioma[6, 16]. With similar techniques, mean AI in various other human malignancies of lung, stomach and colon were 28.6, 30.1 and 31.4, respectively (Table 2). The mean AI in non-small cell lung carcinoma has been previously reported to be as low as 13.9[9] or 20.7[12]. This discrepancy of AI in our result may be due to the difference in technique or the kit used for the TUNEL method. Therefore, the mean AI in mesothelioma is significantly lower than that in other malignancies, suggesting the more aggressive nature of mesothelioma.

Caspase-3 is an inactive proenzyme and its cleavage by other upstream proteases such as active caspase-8 and caspase-9 leads to its active form, called cleaved caspase-3[13, 18]. IAPs inhibit the proteolytic cleavage of caspase proforms caspase-3 and caspase-9[8, 11, 17] and/or directly inhibits activated caspases[4]. In the present study, we used two different anti-caspase-3 and anti-cleaved caspase-3 antibodies. The former can detect full length caspase-3 (35 kD) and the large fragment of caspase-3 resulting from cleavage (17 kD), and the latter can specifically detect the large fragment (17/19 kD). Mesothelioma

tissue and cell lines showed high cytoplasmic expression of caspase-3 and very low expression of cleaved caspase-3. We only found a positive correlation between AI and cleaved caspase-3 expression in mesothelioma tissue. Soini et al reported high expression of caspase-3 but no association was found between apoptotic index and caspase-3 immunoreactivity in mesothelioma[15]. They used an anti-caspase-3 antibody that detects both inactive 32 kD pro-enzyme and the active 17 kD fragment[15]. Our result using cleaved caspase-3 is more specific and, therefore, we found a positive correlation of AI to cleaved caspase-3 expression.

Mesothelioma tissue and cell lines showed high cytoplasmic expression of bax and low cytoplasmic expression of bcl-2. This result is similar to a previously published study of mesothelioma[16]. The previous report found no significant difference in bcl-2 mRNA expression between mesothelioma and normal pleural tissue[3], and suggested that apoptosis in mesothelioma has no direct relation to bax or bcl-2 expression. Survivin is the smallest protein among the IAPs[8], and it directly inhibits activation of caspase-3[8, 11]. In the present study, mesothelioma tissue and cell lines showed high cytoplasmic expression of survivin. The immunohistochemical expression of survivin is reinforced by mRNA expression by real time RT-PCR and protein expression by western blot. Falleni et al. have reported high expression of survivin mRNA in mesothelioma tissue compared to corresponding normal tissue[3].

In conclusion, apoptosis is an uncommon event in mesotheliomas. Although apoptosis-inducing proteins such as bax and caspase-3 were highly expressed in mesothelioma tissue and cell line, the expression of cleaved caspase-3 indicated that low activation of caspase-3 was responsible for the inhibition of apoptosis. Furthermore, high expression of survivin, a known inhibitor of caspases, may also play a role in the inhibition of apoptosis in mesothelioma.

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Value of immunohistochemistry in the differential diagnosis of pleural sarcomatoid mesothelioma from lung sarcomatoid carcinoma

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Value of immunohistochemistry in the differential diagnosis of pleural sarcomatoid mesothelioma from lung sarcomatoid carcinoma

Aims: The differential diagnosis of pleural sarcomatoid mesothelioma (SM) from lung sarcomatoid carcinoma (LSC) invading parietal pleura and chest wall is a challenging issue. The aim of this study was to identify useful antibodies that can be used for the differential diagnosis of pleural SM from LSC.
Methods and results: Forty-five cases of pleural SM and 27 cases of LSC were immunohistochemically analysed by using 15 commercially available antibodies, including D2-40 and antibodies to calretinin, thrombomodulin, Wilms' Tumour 1, carcinoembryonic antigen (CEA), Napsin A, thyroid transcription factor (TTF)-1, pan-cytokeratin, CAM5.2, epithelial membrane antigen, Ber-EP4, MOC-31, α-smooth muscle actin, h-caldesmon and desmin. The results revealed that D2-40 positivity was significantly higher in pleural SM (86.7%) than in LSC (25.9%). The positivity of the adenocarcinoma markers, including CEA, Napsin A, and TTF-1, was low even in LSC.
Conclusions: Evaluating the positivity and degree of staining of the well-known mesothelial marker D2-40 could be applied to differentiate pleural SM from the sarcomatoid component of LSC, in addition to assessing clinical and radiological information.

Keywords: asbestos, calretinin, CEA, D2-40, differential diagnosis, immunohistochemistry, sarcomatoid carcinoma, sarcomatoid mesothelioma

Abbreviations: α-SMA, α-smooth muscle actin; CEA, carcinoembryonic antigen; CGH, comparative genomic hybridization; CK, cytokeratin; EMA, epithelial membrane antigen; H&E, haematoxylin and eosin; LSC, lung sarcomatoid carcinoma; SM, sarcomatoid mesothelioma; TM, thrombomodulin; TTF, thyroid transcription factor; WT1, Wilms' Tumour 1

Introduction

Mesothelioma is a rare, aggressive tumour involving the mesothelial cells of the pleura, peritoneum, pericardium, and testicular tunica vaginalis. It is very well correlated with occupational and environmental asbestos exposure.[1–4] In many countries, the incidence of mesothelioma has been increasing, so that pathologists increasingly encounter and diagnose it.

The pathological diagnosis of mesothelioma is occasionally difficult because of its rarity and its heterogeneous morphological and immunohistochemical findings.[5–7] The predominant histological subtypes of mesothelioma include epithelioid, sarcomatoid, biphasic and desmoplastic types.[5,8–10] There are many comprehensive immunohistochemical studies concerning the differentiation between epithelioid mesothelioma and pulmonary adenocarcinoma.[9–15]

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However, there are few reports on the differential immunohistochemical diagnosis of pleural sarcomatoid mesothelioma (SM) and tumours with spindle cell morphology, such as 'true' sarcoma, pulmonary sarcomatoid carcinoma (LSC) and fibrous and/or fibrosing pleuritis.[15–20] Usually, pleural SM grows along the parietal and visceral pleurae;[6] however, occasional cases of localized mesothelioma have also been observed.[21] On the other hand, LSC types, including pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma, grow more aggressively in pulmonary parenchyma than other non-small cell carcinomas, sometimes involving the parietal pleura and the chest wall.[5] In the event that epithelial components in pleomorphic carcinoma, such as adenocarcinoma, squamous cell carcinoma and large cell carcinoma, cannot be detected, and tissue obtained by needle biopsy is submitted for pathological diagnosis, the differential diagnosis between pleural SM and LSC is difficult even if immunohistochemistry is used. Therefore, clinical and radiological information is necessary to diagnose pleural SM definitively.[6,7,22]

Immunohistochemical analyses were using 15 performed, commercially available antibodies to evaluate the usefulness of immunohistochemistry in the differential diagnosis of pleural SM from LSC. In particular, we discussed the usefulness of D2-40 (anti-podoplanin antibody) was assessed in such differential diagnosis.

Materials and methods

Fourty five pleural SM cases and 27 LSC cases were selected, including 22 pleomorphic carcinomas and five spindle cell carcinomas, diagnosed between 1987 and 2008, from the surgical archives of the Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University. Nine pleural SM and nine LSC cases had been included in a previous study.[22] The diagnosis of each case was confirmed by reviewing clinical information (especially chest computed tomography findings), main clinical and pathological tumour location and morphological slides including haematoxylin and eosin (H&E) and immunohistochemically stained slides. All LSC cases in this study were located in the pulmonary parenchyma. Questionable cases were omitted from the study. The average age of the patients with pleural SM was 63.2 years (range 35–85) and that of the patients with LSC was 65.3 years (range 56–89). All the patients were Japanese. The diagnosis in each case was based on the recommended criteria listed in the 2004 World Health Organization Classification.[5,23] In brief, pleural SMs consist of spindle cells arranged in fascicles or having a haphazard distribution, often resembling fibrosarcoma and malignant fibrous histiocytoma,[5] and LSC is a poorly differentiated non-small cell lung carcinoma that contains a component of sarcoma or sarcoma-like (spindle and/or giant cell) differentiation.[23] The subclassification of LSC is listed in Table 1. Biphasic and desmoplastic mesothelioma and pulmonary carcinosarcoma, pulmonary blastoma and giant cell carcinoma were excluded from the study.

Immunohistochemistry of sections from formalin-fixed paraffin-embedded tissues was performed using the Histofine Simple Stain MAX PO (MULTI) kit (Nichirei, Tokyo, Japan) with or without antigen retrieval. The list of primary antibodies, including the 15 antibodies, clone, source, dilution rate and antigen retrieval, is shown in Table 2. Based on the number of tumour cells observed following immmunohistochemistry, the tumours were scored using the following semiquantitative system: 0, no or trace reactivity; score 1+, <10% tumour cells; score 2+, 11–50% tumour cells; score 3+, >51% tumour cells. The definition of a 'positive case' in this study is a case with a score of 1+, 2+ or 3+.

When the expression of D2-40 was investigated, a high level of background staining was occasionally observed in the desmoplastic tissue surrounding infiltrating tumour cells, as pointed out by Chu *et al.*[24]

Table 1. Clinicopathological profile of pleural sarcomatoid mesothelioma and lung sarcomatoid carcinoma

	Sarcomatoid mesothelioma	Sarcomatoid carcinoma
Number of cases	45	27
Age (mean)	35–85 (63.2)	56–89 (65.3)
Male:female	40:5	24:3
Subtypes (WHO, 2004)		
Pleomorphic carcinoma		22
Epithelial components:		
Adenocarcinoma		19
Squamous cell ca.*		2
Large cell ca.*		1
Spindle cell carcinoma		5

*ca., carcinoma.

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Table 2. Antibodies used in this study

Antibody to	Source	Clone	Pretreatment	Dilution
Podoplanin	Nichirei BioScience, Tokyo, Japan	D2-40	Autoclave	Pre-diluted
Calretinin	Zymed, San Francisco, CA, USA	Poly	Autoclave	1:50
WT1	Dako, Glostrup, Denmark	6F-H2	Autoclave	1:400
Thrombomodulin	Dako	1009	None	1:500
CEA	Nichirei BioScience	COL-1	Autoclave	Pre-diluted
Napsin A	IBL, Gumma, Japan	TMU-Ad02	Autoclave	1:200
TTF-1	Dako	8G7G3/1	Autoclave	1:50
CAM5.2	Becton Dickinson, Franklin Lakes, NJ, US	2A4	Autoclave	Pre-diluted
Pancytokeratin	Novocastra, Newcastle upon Tyne, UK	AE1/AE3	Autoclave	1:500
EMA	Dako	E29	Autoclave	1:100
Epithelial antigen	Dako	Ber-EP4	None	1:100
Epithelial related antigen	Dako	MOC-31	None	1:50
α-Smooth muscle actin	Dako	1A4	Autoclave	1:100
h-Caldesmon	Dako	H-CD	Autoclave	1:50
Desmin	Dako	D33	Autoclave	1:100

WT, wilms' tumour; CEA, carcinoembryonic antigen; TTF, thyroid transcription factor; EMA, epithelial membrane antigen.

Therefore, D2-40 was assessed in relatively cellular areas to prevent the background staining from interfering with the results. LSC was evaluated in the 'sarcomatoid' component, i.e. areas where the tumour cells exhibited spindle and/or pleomorphic morphology without clear epithelial tumour cell nest formations. Immunohistochemical evaluation for the 'epithelial' component of LSC was excluded from this study.

Statistical analyses were performed using Fisher's exact test and Mann–Whitney *U*-test. Sensitivity and specificity were calculated for each marker using a simple 2 × 2 table. A *P*-value of <0.05 was considered to be significant.

Results

POSITIVITIES OF ANTIBODIES FOR SARCOMATOID MESOTHELIOMA AND LUNG SARCOMATOID CARCINOMA

The positivities of each antigen for pleural SM and LSC are indicated in Table 3. Representative immunohistochemical reactivity for pleural SM and LSC is shown in Figures 1 and 2. The pattern of reactivity for each of the antibodies for the two tumour types is briefly described below.

D2-40

Of the 45 pleural SM cases, 39 (86.7%) were positive for D2-40. Of the 27 LSC cases, seven (25.9%) were positive for D2-40. Both the tumours exhibited a predominantly cytoplasmic pattern of immunoreactivity; however, both membranous and cytoplasmic patterns of reactivity were also noted in some cases. A high grade of reactivity (i.e. 2+ or 3+) was observed in 21 pleural SM cases (46.7% of the positive cases); however, most positive LSC cases had a low reactivity grade (i.e. 1+). The difference in reactivity grade was statistically significant between pleural SM and LSC. The intensity of reactivity in pleural SM and LSC cells in most cases was less than that in the normal lymphatic endothelium of the same specimens; however, some pleural SM cells were immunoreactive with the strong intensity similar to that of lymphatic endothelium. If a cut-off value of 10% was applied, the sensitivity of D2-40 would be 46.7% in pleural SM and 3.7% in LSC.

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Table 3. Immunohistochemical findings for pleural sarcomatoid mesothelioma and lung sarcomatoid carcinoma

	Sarcomatoid mesothelioma					Sarcomatoid carcinoma						
		Reactivity grade					Reactivity grade					
Markers	*n* (%)	0	1+	2+	3+	*n* (%)	0	1+	2+	3+	*P*-value*	*P*-value†
D2-40	39/45 (86.7)	6	18	11	10	7/27 (25.9)	20	6	1	0	<0.001	<0.001
Calretinin	35/45 (77.8)	10	23	10	2	16/27 (59.3)	11	11	3	2	0.081	0.154
WT1	10/24 (41.7)	14	6	3	1	7/19 (36.8)	12	5	1	1	0.806	0.695
Thrombomodulin	9/23 (39.1)	14	8	0	1	10/23 (43.5)	13	9	1	0	0.500	0.790
CEA	0/27 (0)	27	0	0	0	4/27 (14.8)	23	4	0	0	0.055	0.039
Napsin A	0/15 (0)	15	0	0	0	4/20 (20.0)	16	4	0	0	0.093	0.965
TTF-1	0/15 (0)	15	0	0	0	3/20 (15.0)	17	3	0	0	0.174	0.122
CAM5.2	45/45 (100)	0	9	17	19	25/26 (96.2)	1	10	1	14	0.366	0.743
AE1/AE3	24/27 (88.9)	3	4	11	9	21/23 (91.3)	2	8	5	8	0.578	0.611
EMA	13/24 (54.2)	11	12	1	0	10/15 (66.7)	5	7	2	1	0.240	0.634
Ber-Ep4	0/15 (0)	15	0	0	0	2/21 (9.5)	19	2	0	0	0.333	0.225
MOC-31	0/15 (0)	15	0	0	0	0/19 (0)	19	0	0	0	NA‡	NA‡
α-SMA	14/24 (58.3)	10	6	6	2	9/21 (42.9)	12	6	2	1	0.231	0.193
h-Caldesmon	0/15 (0)	15	0	0	0	0/18 (0)	18	0	0	0	NA‡	NA‡
Desmin	4/36 (11.1)	32	4	0	0	2/25 (8)	23	1	1	0	0.523	0.172

WT, wilms' tumour; CEA, carcinoembryonic antigen; TTF, thyroid transcription factor; EMA, epithelial membrane antigen; α-SMA, α-smooth muscle actin.
**P*-value (the difference in the positive rate between pleural SM and LSC for each antibody calculated by Fisher's exact test).
†*P*-value (the difference in the distribution of reactivity scores between pleural SM and LSC for each antibody calculated by Mann–Whitney *U*-test).
‡Not available.

CALRETININ

Of the 45 cases with pleural SM (77.8%), 35 were positive for calretinin. Most of the positive cases fell in the 1+ reactivity grade. The pattern of immunoreactivity in the pleural SM as well as LSC cases was observed as reactivity in the nucleus and cytoplasm. Most of the positive cases had a lower reactivity score (i.e. 1+). There was no difference in reactivity grades between pleural SM and LSC.

WILMS' TUMOUR 1 AND THROMBOMODULIN

Approximately 40% of the cases of pleural SM (41.7%) and LSC (36.8%) were positive for Wilms' Tumour (WT) 1. Immunoreactivity was located in the cytoplasm in each tumour. Trace nuclear reactivity was observed in certain positive cases.

More than 30% of the pleural SM (39.1%) and LSC (43.5%) cases exhibited a primarily membranous pattern of reactivity for thrombomodulin (TM). However, most of the positive cases had a staining score of 1+.

CARCINOEMBRYONIC ANTIGEN, NAPSIN A AND THYROID TRANSCRIPTION FACTOR-1

None of the pleural SM cases was positive for antibodies to carcinoembryonic antigen (CEA), Napsin A and thyroid transcription factor (TTF)-1. A few LSC cases exhibited a weak cytoplasmic pattern of reactivity for CEA (cytoplasm), Napsin A (cytoplasm) and TTF-1 (nucleus).

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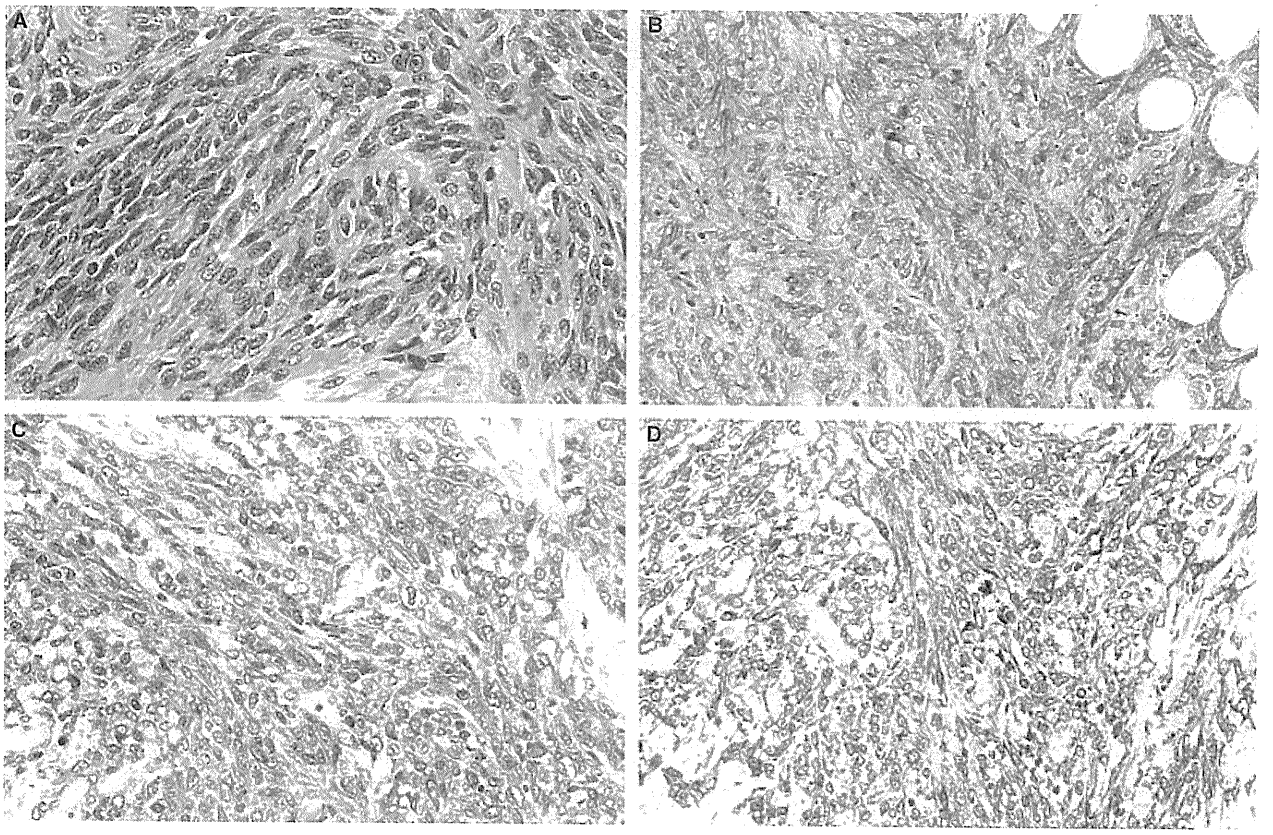

Figure 1. Histological and immunohistochemical results of a case of pleural sarcomatoid mesothelioma (59-year-old male patient). Atypical spindle cells arranged in fascicular pattern (**A**) (H&E). Tumour cells show cytoplasmic positivity for D2-40 (**B**) and nucleus and cytoplasmic positivity for calretinin (**C**). More than half of the tumour cells are immunoreactive for cytoplasmic CAM5.2 (**D**) (Streptavidin–biotin–peroxidase).

CYTOKERATIN (CAM5.2), CYTOKERATIN-MULTI (AE1/AE3), EPITHELIAL MEMBRANE ANTIGEN, BER-EP4 AND MOC-31

All the pleural SM cases and 96.1% of the LSC cases exhibited cytoplasmic patterns of reactivity for CAM5.2. Pan-cytokeratin (AE1/AE3) was also expressed in approximately 90% of the pleural SM and LSC cases. More than 50% of the pleural SM and LSC cases showed a primarily cytoplasmic and/or membranous pattern of reactivity for EMA. However, most of the positive cases had a 1+ reactivity score.

None of the cases of pleural SM was positive for Ber-EP4, and only two LSC cases exhibited a membranous pattern of reactivity. MOC-31 was not detected in either tumour.

α-SMOOTH MUSCLE ACTIN, H-CALDESMON AND DESMIN

Approximately half of the pleural SM and LSC cases exhibited cytoplasmic patterns of reactivity for α-smooth muscle actin (α-SMA). The distribution of expression score was variable. None of the pleural SM and LSC cases exhibited any immunopositivity for h-caldesmon. Four of the 36 pleural SM cases and one of the 25 LSC cases exhibited a weak cytoplasmic pattern of reactivity for desmin.

Table 3 lists the *P*-values for the difference in positivity rates (cases with staining grade >1+) between pleural SM and LSC determined by Fisher's exact test. Only D2-40 showed a significantly higher expression rate in pleural SM than in LSC.

The *P*-values according to the Mann–Whitney *U*-test for the difference in distributions of reactivity grades between pleural SM and LSC for each antibody are also shown in Table 3. Only D2-40 and CEA exhibited a significant difference in the proportions of reactivity grade.

SENSITIVITY AND SPECIFICITY OF EACH ANTIBODY FOR DIFFERENTIAL DIAGNOSIS BETWEEN MESOTHELIOMA AND LUNG SARCOMATOID MESOTHELIOMA

The sensitivity and specificity of each antibody for the differential diagnosis between pleural SM and LSC are

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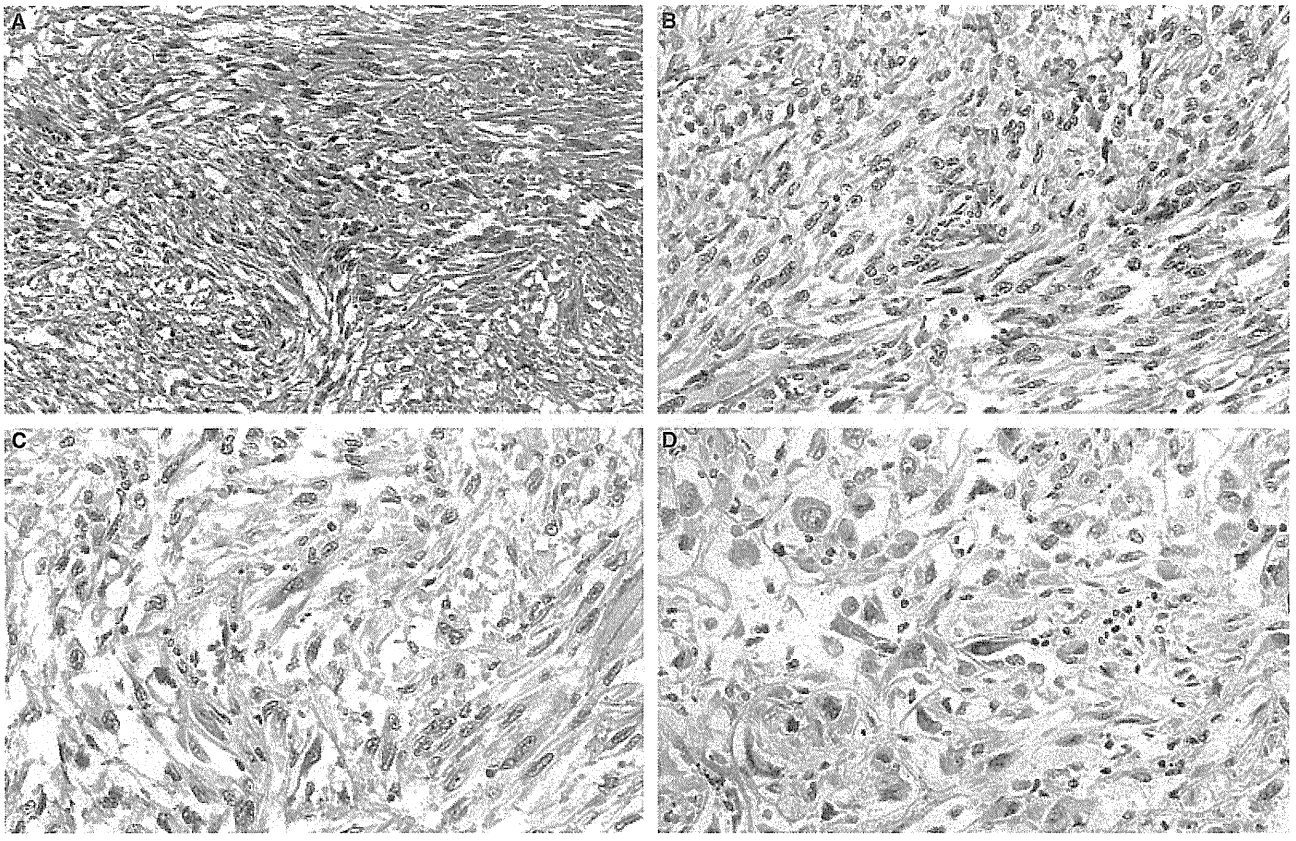

Figure 2. The histological immunohistochemical features of a lung pleomorphic carcinoma with foci of adenocarcinoma (78-year-old male patient). Atypical spindle and/or polygonal cells proliferating in the lung parenchyma (**A**) (H&E). No tumour cells show focal positivity for D2-40. Lymphatic endothelium in the tumour tissue positive for D2-40 (**B**). Some tumour cells exhibit nuclear/cytoplasmic positivity for calretinin (**C**). The focal carcinoembryonic antigen positivity is also illustrated (**D**) (Streptavidin–biotin–peroxidase).

indicated in Table 4. Only D2-40 exhibited a relatively high sensitivity and specificity for differential diagnosis. The other antibodies did not show a good combination of both high sensitivity and specificity.

Discussion

The accurate diagnosis of pleural SM and exclusion of other tumours with spindle cell morphology from the diagnosis is difficult to achieve based on the limited observation of histological features using techniques such as H&E immunohistochemistry, even though associated with clinical information.[6,7] It is especially difficult to differentiate between pleural SM and LSC invading pleural tissue and the chest wall due to their morphological similarities.

Recently, many 'mesothelial markers' including calretinin, D2-40, WT1, TM, mesothelin and cytokeratin (CK) 5/6 have been developed.[10,12,25] It is recommended that a set of antibodies should be used for the differential diagnosis of mesothelioma from other tumours and tumour-like lesions.[5,7,10] However, there are limited reports on the immunohistochemical differentiation of pleural SM and LSC.[15,18–20,26,27] Attanoos *et al.*[18] have described how the combination of a broad-spectrum CK with calretinin increases both the sensitivity and specificity for the diagnosis of pleural SM. Lucas *et al.*[19] conducted a comparative immunohistochemical study of mesothelioma and true sarcoma, including malignant fibrous histiocytoma, leiomyosarcoma, synovial sarcoma and LSC. They concluded that CK and calretinin had the greatest diagnostic value in the differential diagnosis of pleural SM from true sarcoma. However, immunohistochemical analyses with antibodies such as pan-cytokeratin, CK5/6, calretinin, WT1, TM and α-SMA play a limited role in differentiating between pleural SM and LSC. Our previous study indicated that a combination of CAM5.2, WT1 and pan-cytokeratin is useful for differentiating between SM and true sarcomas, but of the 10

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Table 4. Sensitivity and specificity of each antibody for differential diagnosis between pleural sarcomatoid mesothelioma and lung sarcomatoid carcinoma

Markers	Sensitivity	Specificity
D2-40	86.7	74.1
Calretinin	77.8	40.7
WT1	41.7	63.2
Thrombomodulin	39.1	56.5
CEA	0	85.2
Napsin A	0	80
TTF-1	0	85
CAM5.2	100	3.8
AE1/AE3	88.9	8.7
EMA	54.2	33.3
Ber-Ep4	0	90.5
MOC-31	0	100
α-SMA	58.3	58.1
h-Caldesmon	0	100
Desmin	11	92

WT, wilms' tumour; CEA, carcinoembryonic antigen; TTF, thyroid transcription factor; EMA, epithelial membrane antigen; α-SMA, α-smooth muscle actin.

commercially available antibodies used, no useful antibodies were identified for differential diagnosis between pleural SM and LSC.[22] However, our previous study did not include D2-40 immunohistochemistry, and the sample size of our study as well as that of the previous reports comprised relatively few LSC cases (only nine and <10 cases, respectively).[18–20,26,27] Therefore, we tried to elucidate the useful antibodies to differentiate between pleural SM and LSC by increasing the number of cases analysed and the number of antibodies used. Our results indicate that significant efficacy of D2-40 was observed in the differential diagnosis between pleural SM and LSC. In other words, D2-40 showed a higher positivity (86.7%) in pleural SM than in LSC (26.1%) cells and a higher proportion of positivity among pleural SM cells.

Commercially available D2-40 has recently been used as an antibody that reacts with the 40-kDa antigen of the M2A oncofetal membrane antigen originally detected in germ cell neoplasia.[28] A recent study has demonstrated that this mucin-type glycoprotein is podoplanin.[29] This molecule is also expressed in the lymphatic endothelium; therefore, it is routinely used in the investigation of lymphatic invasion in tumours.[30,31] This antibody also reacts with normal mesothelial cells, and its use in the diagnosis of mesothelioma, especially the epithelioid type, has been emphasized.[24,32,33]

However, there are limited reports on D2-40 immunohistochemistry of SM. Ordonez[32] reported that D2-40 staining was positive in 86% of epithelioid mesothelioma cells, whereas no immunoreactivity was detected in six cases of SM and in five cases of biphasic mesothelioma (sarcomatoid component), and concluded that unlike calretinin, D2-40 is not effective in the diagnosis of these tumours. Muller *et al.* [33] have also reported that no D2-40+ cases were observed among 18 SM cases. Chu *et al.*[24] reported that D2-40 reactivity in sarcomatoid differentiation (either biphasic mesothelioma or pure sarcomatoid) was less frequent than in epithelioid mesothelioma [i.e. 33 of 33 epithelioid (100%), with reactivity observed in 10 of 16 biphasic (62.5%) and three of four sarcomatoid (75%) cases]. They further reported that the D2-40 pattern of reactivity in SM is less intense and is observed in the cytoplasm, as opposed to the intense and membranous pattern of reactivity observed in cases of epithelioid mesothelioma. They concluded that D2-40 was limited by its performance in areas of sarcomatoid differentiation and also mentioned that the interpretation of D2-40 reactivity in areas of sarcomatoid growth was complicated by the loss of the characteristic pattern of reactivity observed in epithelioid mesotheliomas and the high level of background staining present in both benign reactive pleural fibrous tissue and/or desmoplastic tissue surrounding infiltrating tumour cells.[24] Our present findings are consistent with the results reported by Chu *et al.*[24]; we selected a relatively cellular area for the immunohistochemical evaluation of D2-40, and excluded the desmoplastic type as a variant of SM, to prevent the above-mentioned problems in analysis.

On the other hand, Hinterberger *et al.*[34] described that 30% of SM or sarcomatoid components of biphasic mesothelioma, assessed using a tissue microarray technique from 341 mesotheliomas, were D2-40+, and the combination with calretinin increased the sensitivity in SM. However, they did not mention the specificity of D2-40 for other spindle cell tumours including LSC. Padgett *et al.*[27] also reported that D2-40 and anti-podoplanin antibody immunohistochemistry was more efficacious than calretinin for diagnosis of SM, i.e. 11 of 14 SM cases (79%) and one

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of 13 sarcomatoid carcinomas from various organs (8%) were positive for D2-40. In the present study, we have demonstrated a sensitivity and specificity of more than 80% and 70%, respectively, for the differential diagnosis of pleural SM and LSC. The discrepancy between the results of these two reports[27,34] and our data may be due to the different interpretation of positivity. A different scoring system was applied in these studies, in which a case was defined as positive if >10% tumour cells were immunopositive. We applied this scoring system to our data and the results revealed D2-40 sensitivity of 46.7% (21/45) in pleural SM and 4% (1/23) in LSC. Therefore, D2-40 specificity is higher in the differential diagnosis of pleural SM and LSC.

Calretinin is strongly expressed particularly in epithelioid mesothelioma of the pleura or peritoneum.[35] On the other hand, reports on the positivity of calretinin in pleural SM and LSC have been limited and controversial. Attanoos *et al.*[18] reported that 12 of 31 SM (39%) and no LSC (0%) cases were positive for calretinin. Lucas *et al.*[19] reported calretinin reactivity of 60% of the sarcomatoid component of biphasic mesothelioma, 70% of SM and 60% of LSC. Kushitani[22] reported that 34 of 39 SM (87.2%) and six of nine LSC (66.7%) cases were positive for calretinin. In this study, the data indicate that the value of calretinin is limited due to its low specificity (41.7%) in the differentiation between pleural SM and LSC and a similar distribution of reactivity grades.

WT1 is expressed in rat and human mesotheliomas[36] and has been reported to be a very useful marker for differentiating between epithelioid mesothelioma and pulmonary adenocarcinoma.[9,15] In the present study, WT1 positivity in pleural SM and LSC was almost identical. Therefore, we consider that WT1 cannot be used for the differential diagnosis of pleural SM and LSC as opposed to its use in the diagnosis of pleural SM and true sarcoma.[22]

TM is a relatively old marker for epithelioid mesothelioma.[37] Reported TM positivity is 29% and [18] 70% [19] in SM, and 0%[18] and 40%[19] in SC. In this study, there was no significant difference in TM expression between pleural SM and LSC.

In this study, we included three so-called 'adenocarcinoma markers' including CEA,[9] TTF-1[9,38] and Napsin A.[39] As expected, none of these markers was positive for all pleural SM cases, although they were positive in some LSC cases. However, their degree of positivity was low. Nakashima *et al.*[40] reported that five of 37 LSC (13.5%) were positive for CEA, which is similar to our data. On the other hand, Rossi *et al.*[41] described a relatively high rate of positivity for TTF-1 in the sarcomatoid component of pleomorphic carcinoma and spindle cell carcinoma of the lung (43.1% and 55%, respectively). This high rate of positivity was not observed in the LSC cases in our study. However, in the event that no other specific carcinoma components are detected in LSC, these 'adenocarcinoma markers' may assist in accurate diagnosis.

Other epithelial cell markers such as CAM5.2 (CK7/8), pan-cytokeratin (AE1/AE3), EMA, Ber-EP4, MOC-31 and myogenic markers (α-SMA, h-caldesmon, and desmin) showed no significant differences between the two tumour types. Pathologists must be aware of this fact when diagnosing spindle cell lesions as intrathoracic neoplasms.

Desmoplastic mesothelioma was excluded from this study because it may have increased the difficulty in immunohistochemical evaluation with D2-40. However, immunohistochemical study of this tumour, which is difficult to diagnose is required, especially the differentiation of fibrous/fibrosing pleuritis.[6]

In conclusion, the well-known mesothelial marker D2-40 can be applied to differentiate between pleural SM and the sarcomatoid component of LSC. However, some pleural SM cases exhibit no reactivity for this antibody. Recently, Knuuttila *et al.*[26] have reported that specific loss of 4q11-p13/p15 and 4q and gain of 5p are detected by comparative genomic hybridization (CGH) analysis. Gordon *et al.*[42,43] have reported gene expression profile differences between malignant pleural mesotheliomas and lung carcinomas using microarray expression and GeneChip technique. These CGH and genome-wide gene expression profiling analyses are likely to provide new potential markers for the differential diagnosis of pleural SM and LSC in the near future.

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中皮腫の鑑別診断

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中皮腫の鑑別診断

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（平成21年4月2日受付）

要旨：アスベスト曝露に関連する中皮腫は近年増加が著しいが，この傾向は今後2030年頃まで続くと予想される．中皮腫患者は労災補償制度あるいは石綿健康被害救済法（2006年施行）によって補償・救済されているが，その認定の状況をみると，申請者の10%以上が中皮腫として認められていない．これは主として中皮腫の病理診断の不適切さによると思われ，中皮腫の病理学的診断における鑑別診断の重要性が指摘される．

中皮腫の発生部位は正常で漿膜の存在部位すなわち，胸膜，腹膜，心膜，精巣鞘膜に限られるが，胸膜発生例が圧倒的に多い．肉眼的には限局型に比べびまん型が圧倒的に多いが，肺癌の胸膜進展例が偽中皮腫様所見を示すこともある．中皮腫の組織型は上皮型，肉腫型，線維形成型，二相型に分けられるが，その他にも多くの特殊型がある．こうした組織像の多彩さゆえにしばしば鑑別が困難な例に遭遇するが，これらの鑑別に際しては，それぞれの組織像に対応した抗体を適切に選択した免疫組織化学的染色が有用である．とくに中皮細胞の増殖病変の良悪性の鑑別が，患者に過大な治療的侵襲を無用に与えないためにも重要である．

（日職災医誌，57：183—189，2009）

—キーワード—

免疫組織化学的染色，抗体

1. はじめに

アスベスト（石綿）への曝露によって生じる悪性腫瘍の代表である中皮腫は，近年本邦においてその増加が著しい．これは本邦のアスベストの輸入量（本邦での生産量はごく少ないので，輸入量が使用量に等しい）をみると，1960年代から1970年代にかけて約5万トンから約35万トンにまで急増したことと関連する．本邦における輸入量は1990年代半ばまで20万トン以上を維持しており，本邦でのこれまでの輸入総量は1,000万トンを超える[1]．この事実は，中皮腫の多くは，最初にアスベストへの曝露を受けてから30年から40年後に発生することを考えると，今後も2030年頃までは，現在の発生数が継続することを示唆している．

従来，アスベストの曝露による疾患は，主としてアスベストを扱う労働者における職業病として扱われ，中皮腫についても，労災補償制度のもとで患者への補償が行われてきたが，その数は2003年までは年間100例を超えることはなかった．しかし，2004年には128例，2005年には503例，2006年には1,006例が補償の対象となり，その急増が指摘される．一方，2005年夏に生じたいわゆる“クボタショック”（尼崎市の旧クボタ神崎工場周辺での中皮腫患者の発生）によって，アスベストを扱う工場周辺でも中皮腫が発生することが知られ，これを契機にアスベスト製品の使用者を含めた一般生活環境のもとでも中皮腫の発生があることが疑われるに至り，アスベスト曝露は公害病の様相を帯びてきた．この事実から2006年3月，新たに“石綿健康被害救済法”が制定・施行され，従来の労災補償制度では補償対象とならない人々の救済が広く行われることとなった．

現時点での被害者の補償・救済の認定の状況をまとめると表1のようになるが，これでみると申請例の10%以上が中皮腫として認められていないことがわかる．これらの不認定の原因の主なものは，中皮腫の病理診断が不完全・不適切である可能性が考えられる[2]．

そこで本稿では，中皮腫の病理学的診断のプロセスについて述べ，とくに他疾患との鑑別について，どのような点に注意を払うべきかを述べてみたい．

2. 中皮腫の発生部位と肉眼所見

中皮腫は正常で中皮細胞の存在する部位から発生する悪性腫瘍であり，胸膜，腹膜，心膜，精巣鞘膜に発生は

表1　アスベスト曝露による中皮腫患者の補償・救済の認定の状況

1. “労災保険制度”による補償	給付請求の決定数	1,145件
	（平成18年4月～平成19年3月）	
	支給	1,006件（88%）
	不支給	139件（12%）
2. “石綿健康被害救済法”による救済	受付件数	1,926件
	（平成18年3月～平成20年3月）	
	中皮腫と判定できる	1,152件（66%）
	中皮腫と判定できない	181件（10%）
	その他（審査中）	404件

表2　中皮腫の発生部位

部位	割合
胸膜	80～90%
腹膜	10%
心膜	2～3%
精巣鞘膜	＜1%

図1　びまん型中皮腫の肉眼像（ホルマリン固定後）

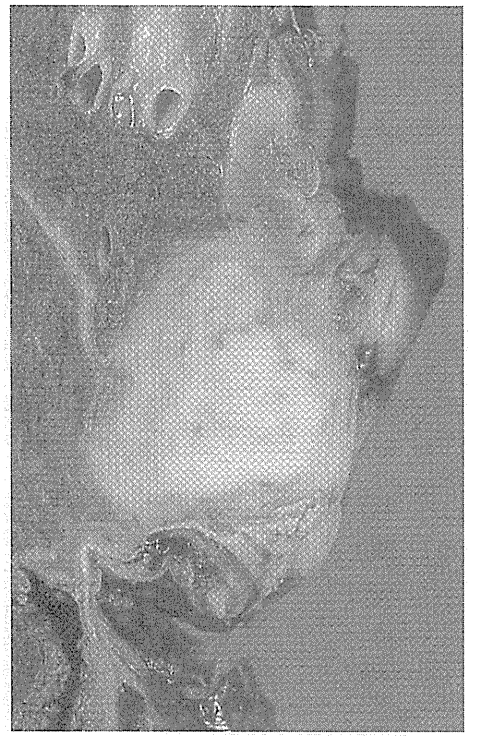

図2　限局型中皮腫の肉眼像（ホルマリン固定後）

限られる．その他の部位，例えば縦隔，肝，卵巣などに生じたとする報告もあるが，これらは腫瘍の広範な進展のために発生部位が不明確となったための局在診断である可能性があり，中皮腫の局所進展が早期におこることを考慮した上で発生部位を推測する必要があると考える．

部位別の割合は表2に示す通り，胸膜が圧倒的に多い．性別と組み合わせると，胸膜例の90%が男性であるのに対して，腹膜例はその約25%が女性である．本邦では諸外国に比べて女性における腹膜発生例が多い傾向にあるが[3]，これら女性の腹膜中皮腫がアスベストへの曝露によるものかについて今後の検討が必要である．

その肉眼所見は部位によってやや異なる．胸膜ではびまん型（図1）と限局型（図2）に分けられるが，前者が圧倒的に多い．中皮腫の大半は壁側胸膜に小結節として発生し，直ちに胸膜面に播種性に拡がり，胸膜表面を被うような拡がりを示すと考えられる．この時点で臓側胸膜との癒着が生じ，肺を囲繞する形態をとる．これに対して限局型は，発生した部位に形成された結節が胸壁方向あるいは肺実質方向に増殖・進展して腫瘤を形成し臨床的に気づかれる．いずれはびまん型と同様の進展を示すと考えられるので，進行期になると，限局型とよぶことのできる例は少ない[4]．

定型的な中皮腫の肉眼像はびまん型であるが，肺癌とくに末梢肺に生じた肺腺癌が同様の拡がりを示す場合があり，こうした例を偽中皮腫様肺癌（肺腺癌）pseudomesotheliomatous carcinoma (adenocarcinoma) とよぶ[5]（図3）．すなわち肉眼像がびまん性であっても肺腺癌との鑑別は必要である．一方，限局型の場合は肺癌（とく

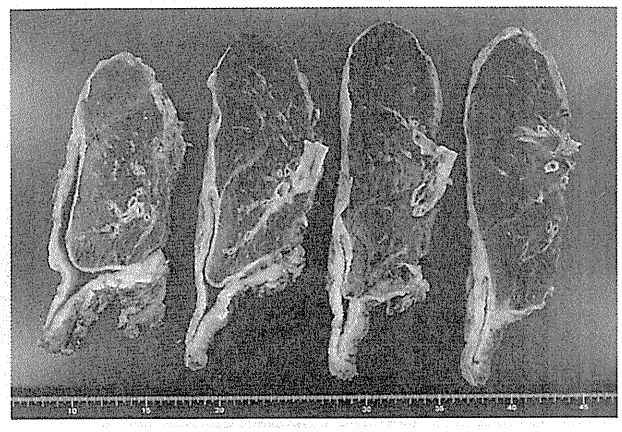

図3　偽中皮腫様肺癌の肉眼像（ホルマリン固定後）

表3　中皮腫の組織型分類

組織型		割合
上皮型　epithelioid type		60%
肉腫型　sarcomatoid type		20%
線維形成型　desmoplastic type		
二相型　biphasic type		20%
特殊型		＜1%
脱落膜様型	deciduoid type	
リンパ組織球様型	lymphohistiocytoid type	
高分化乳頭型	well differentiated papillary type	
その他	others	

（WHO 分類，2004，日本肺癌学会分類，2003 による）

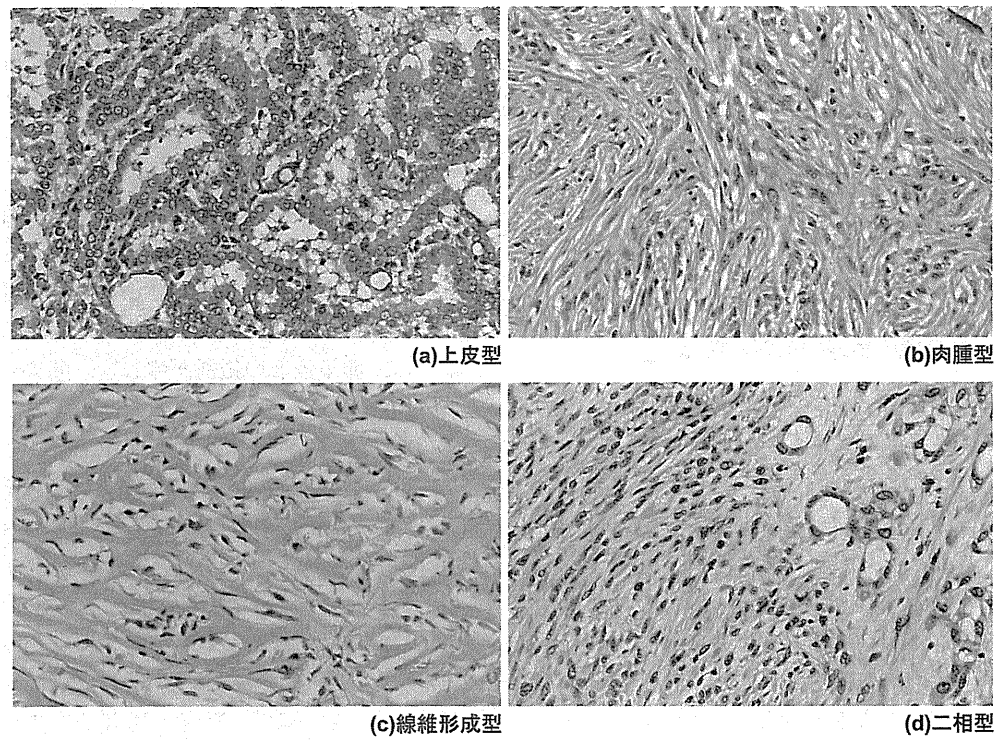

図4　中皮腫の組織像（HE 染色，強拡大）

に肉腫様癌），肺原発の肉腫，胸膜や胸壁に生じた肉腫など他の多くの悪性腫瘍との鑑別が難しいことが多く，限局型中皮腫と診断する場合は，他の悪性腫瘍を否定することが必須である．

腹膜中皮腫については，臨床的には腹水貯留型と結節形成型に分けられることが多いが，病理学的にみると，前者はびまん型，後者は限局型に相当すると考えられる．頻度としては前者が圧倒的に多い．大きな結節は大網や腸間膜に形成されることが多いが，よくみると結節周囲を中心に腹膜への広範囲な進展がすでにあることが多い．

心膜中皮腫は数少ない．殆どがびまん型であり，自験例では，心膜腔への心嚢水の貯留による心不全で発症し，CT 検査などで心膜のびまん性肥厚と癒着をみる．まれに心膜を中心に大きな結節をつくることもある．精巣鞘膜に生じる中皮腫については，自験例では全て限局性に

表4　中皮腫の鑑別診断―他の悪性腫瘍との鑑別

胸膜：上皮型 ⟷	肺腺癌の進展
	他臓器腺癌の胸膜転移
肉腫型 ⟷	肺肉腫様癌の進展
	肺・胸膜の肉腫
	胸壁の肉腫
二相型 ⟷	肺原発の癌肉腫の進展
	胸膜の二相型滑膜肉腫
腹膜：上皮型 ⟷	卵巣漿液性腺癌の進展
	腹膜原発漿液性腺癌
	他臓器腺癌の腹膜転移
肉腫型 ⟷	腹腔内臓器や後腹膜組織の肉腫
二相型 ⟷	腹腔内臓器原発の癌肉腫の進展

表5　中皮腫の鑑別診断に用いる抗体

	陽性となる抗体	陰性となる抗体
上皮型中皮腫	Calretinin	−Lung adenocarcinoma−
	WT1	CEA
	Thrombomodulin	TTF-1
	D2-40	Napsin A
		Surfactant apoprotein
		−Ovarian serous adenocarcinoma−
		Ber EP-4
		MOC-31
		Estrogen receptor（ER）
肉腫型中皮腫	CAM5.2（cytokeratin）	Desmin, h-caldesmon
	AE1/AE3（cytokeratin）	MyoDI, Myoglobin
		CD34, KP1 など

表6　中皮細胞増殖病変—良悪性の鑑別

胸膜：線維形成型中皮腫	⟷ 線維性胸膜炎
上皮型中皮腫（非浸潤）	⟷ 中皮細胞の反応性過形成（ブラの周囲など）
腹膜：上皮型中皮腫（非浸潤）	⟷ 中皮細胞の反応性過形成（腹膜炎後など）

結節をつくる．これは精巣鞘膜腔がきわめて狭いことによると考えられる．

3. 中皮腫の組織型と鑑別疾患

中皮腫の組織型分類[6]を表3に示す．上皮型では高分化な場合は乳頭・腺管状構造をとり，ヒアルロン酸優位の粘液産生を示すが(図4a)，低分化になると，充実性胞巣が主体の組織像をとる．腫瘍細胞も，正常中皮細胞に似て類円形で均一な核をもち細胞質に乏しい小型細胞からなる高分化な例から，核異型が目立ち，細胞質の豊かな大型細胞からなる低分化な例まで多様である．

肉腫型（図4b）は紡錘形細胞の束状配列や花むしろ状配列 storiform pattern などからなるが，間質にヒアルロン酸は乏しい．核の多形性がつよい例もある．腫瘍細胞間の膠原線維量が増し（いわゆる desmoplasia），かつ細胞密度が低下し，細胞異型性も乏しくなる例を線維形成型 desmoplastic type という[7]（図4c）．いわゆる desmoplasia の強い例は肉腫型に限らず上皮型でもありうる．

二相型（図4d）は上記の上皮型と肉腫型が混在してみられる場合であるが，WHO 分類では，いずれかが10%以上混在するという量的な判断基準を加えている．

この他にも中皮腫は様々な組織像をとることが知られており，それらが特殊型として示されているが，いずれも頻度は低い．細胞質が淡明で豊かとなり，核が類円形で中心に位置すると脱落膜細胞に類似し，脱落膜型 deciduoid type と称される．リンパ球の混在がつよく，かつ腫瘍細胞が組織球様にみえる例はリンパ組織球様型 lymphohistiocytoid type とよばれる．線維血管性のコアをもち，よく分化した細胞の乳頭状配列からなる例は高分化乳頭型中皮腫 well differentiated papillary mesothelioma（WDPM）とよばれ，良好な予後を示すとされる．その他には骨・軟骨基質を産生する例，肺小細胞癌のように小型細胞からなる例，多胞性嚢胞を形成する例などがある．

上記のように組織像が多彩であるので，中皮腫と鑑別すべき他の悪性腫瘍をあげると表4となる[8]．胸膜の上皮型では，肺癌とくに肺腺癌との鑑別が難しい．生検の小さな材料で上皮型中皮腫か肺腺癌の胸膜進展かを区別するには，免疫組織化学的染色が必須である．腹膜の上皮型では，卵巣癌との鑑別が難しい．卵巣の上皮性悪性腫瘍は卵巣の表層上皮由来とされ，腹膜の中皮細胞とは近縁である．また，まれながら腹膜癌と称される腫瘍もあり，これらの鑑別のためには免疫組織化学的染色が必須である．胸膜の肉腫型では肺の肉腫様癌（多形癌）との鑑別に苦慮することが多く，免疫組織化学的染色でも決め手に乏しい場合は，肺内に腫瘤があるか否かで決めざるをえない．胸膜でも腹膜でも，肉腫型中皮腫と真の肉腫との鑑別も必要であり，中皮腫と各肉腫のそれぞれが診断できる特異的なマーカーを用いた免疫組織化学的染色による鑑別が行われる．二相型では，二相性を示す他の悪性腫瘍が鑑別にあがる．胸膜ならば肺の癌肉腫や胸膜の二相型滑膜肉腫，腹膜ならば卵巣や子宮の癌肉腫との鑑別が必要である．

以上述べてきた鑑別診断においては，免疫組織化学的染色がしばしば有用である．上皮型，肉腫型に大別して，中皮腫として陽性となる抗体，陰性となる抗体をあげると表5のようになる．正常の中皮細胞で陽性となる中皮細胞マーカーとしては calretinin，WT1，thrombomodulin，D2-40 が用いられるが，前2者は核に，後2者は細胞膜に陽性となる．Cytokeratin（CAM5.2，AE1/AE3）は，上皮型では細胞質に強陽性となるが，肺腺癌や卵巣癌でも陽性であるので鑑別診断には用いることができない．肺腺癌では CEA，TTF-1，Napsin A，surfactant apoprotein などが陽性となり，これらを中皮腫としては陰性マーカーとして用いる[9]．卵巣の漿液性腺癌では，BerEP-4，MOC-31，estrogen receptor（ER）の陽性

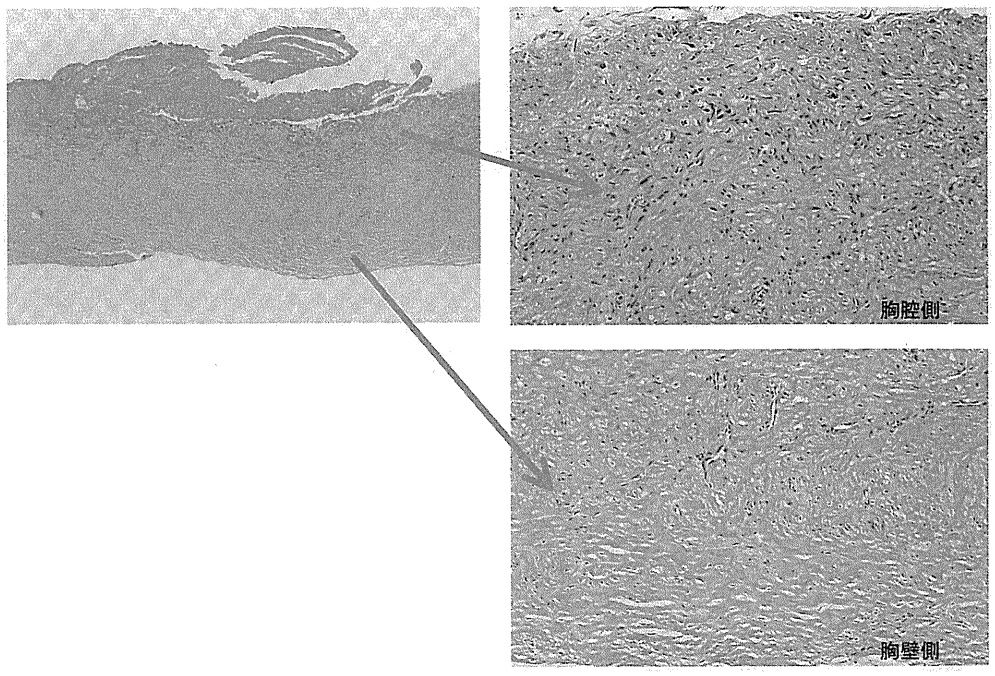

図5　線維性胸膜炎の組織像（HE 染色，弱拡大と強拡大）

表7　線維性胸膜炎と線維形成型中皮腫との鑑別

線維性胸膜炎	線維形成型中皮腫
いわゆる zonation（+） 表層側→細胞密度が高い 細胞異型性を認める 深部側→細胞密度が低い 細胞異型性に乏しい	いわゆる zonation（−）
胸膜表面に垂直な capillary（+）	Capillary（−）
Bland necrosis（−）	Bland necrosis（+）
Sarcomatous foci（−）	Sarcomatous foci（+）
Nodular expansion（−）	Nodular expansion（+）

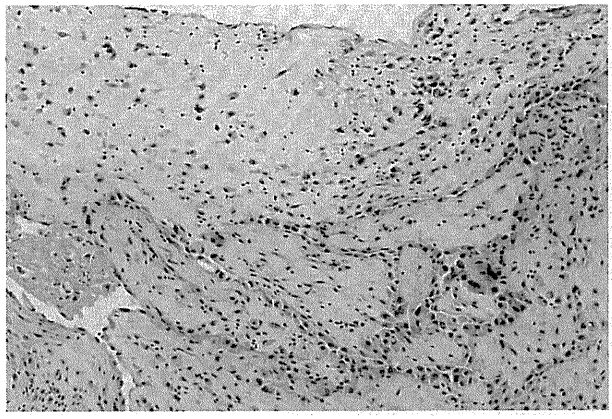

図6　異型的な中皮細胞過形成の組織像（HE 染色，中拡大）

率が高く，これらを中皮腫の陰性マーカーとして用いる[10]．

肉腫型では cytokeratin（CAM5.2，AE1/AE3）に対する抗体が陽性マーカーとなる．上皮型であげた中皮細胞マーカーである calretinin などの肉腫型での陽性率は低く，陽性マーカーとしては用いにくい．他の肉腫それぞれのマーカー，例えば，平滑筋肉腫における desmin や h-caldesmon，横紋筋肉腫における myoD1 や myoglobin，SFT における CD34 などは，中皮腫での陽性率は低く陰性マーカーとなる[11]．

4. 中皮細胞の増殖病変の良悪性の鑑別

表6にあげる中皮細胞の増殖病変の良悪性の鑑別診断は，患者に適格な治療を決める意味できわめて重要である．早期の中皮腫例では，胸膜肺全摘術という侵襲の大きい手術が適応となる為，その重要性も増している．

まず，頻度の高い例として，線維性胸膜炎（図5）と線維形成型中皮腫の鑑別があげられる．この両者の鑑別ではとくに表層のみから得られた小さな生検材料の場合，きわめて難しい．両者の鑑別点としては表7に項目をあげるが，この中ではいわゆる“zonation”が信頼度が高い[12]．しかし，これも胸膜の表層から胸壁までの胸膜全層にわたる生検材料が提供されないと判断できない．この両者の鑑別に，紡錘形細胞の免疫組織化学的染色が有用な場合がある．中皮細胞マーカーである calretinin や cytokeratin（CAM5.2 あるいは AE1/AE3）は両者とも陽性であるが，desmin は線維性胸膜炎では陽性であるが，線維形成型中皮腫では陰性である．α-SMA は両方とも陽性であることが多い．

次いで，早期の上皮型中皮腫と反応性の異型的な中皮細胞過形成（図6）との鑑別も重要である．この場合，浸潤像の有無，すなわち中皮細胞増殖がみられる範囲が最も重要な鑑別のポイントとなるが（図7），小さな生検で表層の組織しか得られていない場合，両者の鑑別には苦