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Original Article

Immunohistochemical marker panels for distinguishing between epithelioid mesothelioma and lung adenocarcinoma

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The distinction between epithelioid mesothelioma and lung adenocarcinoma remains an important diagnostic challenge for surgical pathologists. The aim of the present study was to select a limited and appropriate panel of antibodies that can differentiate between epithelioid mesothelioma and lung adenocarcinoma. Specimens of 90 epithelioid mesotheliomas and 51 lung adenocarcinomas obtained from Japanese cases were examined using calretinin, WT1, AE1/AE3, CAM5.2, cytokeratin (CK) 5/6, vimentin, epithelial membrane antigen (EMA), thrombomodulin, CEA, CA19-9, and CA125. Ninety-six percent of epithelioid mesotheliomas were positive for calretinin; 99% for WT1; 100% for AE1/AE; 97% for CAM5.2; 70% for CK 5/6; 91% for vimentin; 96% for EMA; 71% for thrombomodulin; 77% for mesothelin; 7% for CEA; 17% for CA19-9; and 85% for CA125. In contrast, 33% of lung adenocarcinomas were positive for calretinin; 16% for WT1; 100% for AE1/AE3, CAM5.2, and EMA; 41% for CK 5/6; 47% for vimentin; 20% for thrombomodulin; 69% for mesothelin; 98% for CEA; 73% for CA19-9; and 80% for CA125. For distinguishing between epithelioid mesothelioma and lung adenocarcinoma, the combination of CEA, calretinin and each WT1 or thrombomodulin was suggested to be the best panel of immunohistochemical markers.

Key words: adenocarcinoma, calretinin, CEA, immunohistochemistry, mesothelioma, thrombomodulin, WT1

The differential diagnosis between epithelioid mesothelioma and lung adenocarcinoma is a well-known diagnostic challenge in surgical pathology, and it is of critical importance for proper clinical management and in view of the increasing number of claims involving job-related asbestos exposure. Both the tumors may involve the pleural surfaces and, in some instances, their overlapping histological features preclude a firm diagnosis based on conventional light microscopic observations. Several ancillary diagnostic techniques, including histochemistry, electron microscopy, and immunohistochemistry (IHC), have been proposed to assist in the diagnosis of epithelioid mesothelioma. The diagnostic utility of conventional histochemical stains is limited. Lung adenocarcinomas are not consistently positive for intracytoplasmic mucicarmine and PAS after diastase digestion. Furthermore, this reaction has been observed in a few epithelioid mesotheliomas.[1] The alcian blue-positive, hyaluronidase-sensitive reaction has also been reported in lung adenocarcinomas.[1] Electron microscopy has proven to be useful and is often considered as the gold standard in the diagnosis of epithelioid mesothelioma.[2,3] However, electron microscopic study generally requires great expense and a lot of time compared with the other diagnostic techniques, and the morphological ultrastructural features of mesothelial differentiation may not be apparent in the less-differentiated tumors.

Since the last decade, various immunohistochemical markers that can facilitate the diagnosis of epithelioid mesothelioma have become available.[4,5] A particular issue is the lack of reliable positive markers for mesothelial cells in formalin-fixed, paraffin-embedded sections, although several reports have claimed variable results.[4,5] To date, many of the routinely used probes such as CEA, BerEP4, B72.3, and CD15 stain the carcinoma cells in adenocarcinomas but not those in mesotheliomas. Over the past few years, a number of markers that react with epithelioid mesotheliomas but not with adenocarcinomas have become commercially available; but the number of studies that have evaluated the practical use of these antibodies is limited, and the results are controversial.[5]

In the present study, 12 of the most promising commercially available antibodies were examined on routine histological specimens of epithelioid mesotheliomas and lung adenocarcinomas obtained from Japanese cases. These antibodies were tested to clarify the contribution of IHC in the differential diagnosis of these two tumor types and to confirm a specific type of battery of antibodies that can be used in any pathological department or laboratory.

MATERIALS AND METHODS

Patients and histological samples

We used paraffin-embedded specimens from 90 patients with a definite histological diagnosis of mesothelioma who had undergone thoracoscopic pleural biopsy, percutaneous needle biopsy, surgical decortication, or autopsy conducted between 1995 and 2005. These specimens were retrieved from the archives of the Department of Pathology at Hiroshima University and from 36 other institutes. These 90 cases were divided into 71 cases of epithelioid mesotheliomas and 19 cases of biphasic mesotheliomas. In the present study, immunohistochemical evaluation for the sarcomatoid component of the biphasic mesotheliomas was excluded. Paraffin-embedded histological samples of the surgical specimens from 51 patients with a histological diagnosis of primary lung adenocarcinoma were obtained at segmentectomy, lobectomy, or pneumonectomy conducted between 2003 and 2005. These samples were retrieved from the archives of the Department of Pathology at Hiroshima University.

Each of the tumor specimens was reviewed by three pathologists (K. I., Y. T., and K. K.), and all mesothelioma cases were diagnosed using the currently accepted histological criteria combined with the immunohistochemical features.

Immunohistochemical procedures

Immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections using the avidin–biotin–peroxidase method (ABC) with Histofine SAB-PO kit (Nichirei, Tokyo, Japan). The primary antibodies used in the present study are as follows: calretinin, WT1, pan-cytokeratin (AE1/AE3), CAM5.2, cytokeratin 5/6 (CK 5/6), vimentin, epithelial membrane antigen (EMA), thrombomodulin, mesothelin, CEA, CA19-9 and CA125. Dilution and incubation times of each marker are shown in Table 1.

Immunoreactivity was scored as negative (no immunostaining) or positive. The grade of the immunostained cells was recorded as follows: 1+, 1–25%; 2+, 26–50%; 3+, 51–100%. The scoring was performed based on the extent of positive cells, regardless of intensity. Several combinations of specific and sensitive immunohistochemical findings were analyzed in order to identify the most specific and sensitive combination that can be used for the differential diagnosis of these two tumor types.

RESULTS

Immunohistochemical profiles of epithelioid mesotheliomas and lung adenocarcinomas

The immunohistochemical results are summarized in Tables 2 and 3, and a brief description of the reactivity of each antibody is presented here.

Calretinin

In epithelioid mesotheliomas, the staining reaction was generally strong and diffuse, and a positive finding was observed in both the cytoplasm and the nucleus (Fig. 1a). In contrast, the staining reaction was limited to <10% of the carcinoma cells in most lung adenocarcinomas.

WT1

In the majority of epithelioid mesotheliomas, the staining reaction was strong and diffuse, and it was confined to the nuclei (Fig. 1b). In all eight lung adenocarcinomas that had positive reactivity, the reaction was focal (1+) and weak.

AE1/AE3 and CAM5.2

All epithelioid mesotheliomas and most lung adenocarcinomas were positive for AE1/AE3 and CAM5.2. A cytoplasmic staining pattern was observed.

CK 5/6

The majority of epithelioid mesotheliomas had a staining reaction that was limited to <50% of the tumor cells. In most of the lung adenocarcinomas, the reaction was focal (1+).

Vimentin

In both epithelioid mesotheliomas and lung adenocarcinomas, vimentin was expressed throughout the cytoplasm.

EMA and thrombomodulin

In both epithelioid mesotheliomas and lung adenocarcinomas, a cytoplasmic staining pattern was observed with accentuation of the reaction along the cell membranes (Fig. 1c).

Mesothelin

In epithelioid mesotheliomas, the staining reaction was usually strong and diffuse, and it was characterized by thick membranous reactivity, particularly along the apical cell membrane (Fig. 2a). In lung adenocarcinomas, the reaction was focal and weak, and its pattern was less consistent; that is, in some cases the reaction was observed along the apical cell membrane, and in others it was cytoplasmic or mixed membranous and cytoplasmic (Fig. 2b).

CEA, CA19-9, and CA125

In lung adenocarcinomas, a cytoplasmic staining pattern was observed with accentuation of the reaction along the cell membrane (Fig. 3a). The grade was 1+ in a few epithelioid mesotheliomas that had a positive reaction.

Specificity and sensitivity of each immunohistochemical antibody for epithelioid mesothelioma

The comparison of the immunoreactivity between epithelioid mesotheliomas and lung adenocarcinomas is shown in Table 4. The results indicated that calretinin, CK 5/6, vimentin, and thrombomodulin are the positive markers for epithelioid mesothelioma, and CEA and CA19-9 are the negative markers.

The sensitivity and specificity of one, two, or three antibodies for the diagnosis of epithelioid mesothelioma are indicated in Tables 5–7.

When each positive marker was observed, WT1 had the highest sensitivity and specificity in epithelioid mesotheliomas, and it was suggested to be the most useful positive marker of epithelioid mesothelioma. The sensitivity of calretinin was as high as WT1, but it was inferior to WT1 with respect to specificity. Thrombomodulin followed WT1 with respect to high specificity but it was inferior to WT1 and calretinin. Among the positive markers of lung adenocarcinoma, CEA had the highest sensitivity and specificity.

Among the combinations of two antibodies, the combination of WT1 and CEA (either WT1 positivity or CEA negativity) had the highest sensitivity, and the combination of calretinin and CEA (both calretinin positivity and CEA negativity) had the highest specificity (Table 6).

Among the combinations of three antibodies, the combination of WT1, calretinin, and thrombomodulin (WT1-positivity and (calretinin positivity or thrombomodulin positivity)) had the highest sensitivity, but it was inferior to the combination of WT1 and CEA. Both the combination of CEA, calretinin and WT1 (CEA negativity and (calretinin positivity or WT1 positivity)) and the combination of CEA, calretinin and thrombomodulin (CEA negativity and (calretinin positivity or thrombomodulin positivity)) had the highest specificity (Table 7).

In the present study, the proportion of epithelioid mesotheliomas that had partial reactivity to calretinin, WT1, and cytokeratin 5/6 (graded as 1+ or 2+) was higher than that recently reported by Ordóñez.[6] Precisely, these markers generally had diffuse (graded as 3+) and dense positive findings in well-differentiated epithelioid mesotheliomas, which have a distinct papillary or tubulopapillary growth pattern (Fig. 4a). In contrast, in the poorly differentiated cases, which have a solid and diffuse growth pattern and lack papillary or tubulopapillary structure (Fig. 4b), these antibodies had weak and localized (graded as 1+ or 2+) reactions. In addition, calretinin and WT1 produced a nuclear-positive pattern in well-differentiated cases, whereas they produced a cytoplasmic pattern in the poorly differentiated cases. Based on the fact noted here, the discrepancy between the present results and that of the Ordóñez report may be due to the variation in the cases selected, that is, the proportion of the poorly differentiated cases included in the present study may be higher than that in the Ordóñez study. In addition, there might be some technical problems, for example, the time lapse after the sections were cut into thin sections, the vagaries of IHC or interlaboratory variability.

The comparison of the immunoreactivity between epithelioid mesotheliomas and epithelioid components of biphasic mesotheliomas is given in Table 8. CK 5/6 and mesothelin had wider reactivity in epithelioid mesothelioma. In contrast, vimentin had wider reactivity in the epithelioid component of biphasic mesothelioma. These findings suggest that biphasic mesothelioma indicated the loss or decrease of mesothelial phenotypes along the progression.

In the present study we could not obtain complete data on asbestos exposure of each patient. With regard to the data we could obtain, there were no significant differences in expression patterns with the presence and absence of asbestos exposure.

DISCUSSION

Among the so-called ‘positive’ markers of mesothelioma, calretinin is one of the most frequently used markers for the diagnosis of epithelioid mesothelioma. The results of the present study confirm those of other investigations.[6,7] However, the percentage of calretinin-positive lung adenocarcinoma cases in the present study was much higher than that previously reported.

Recent studies have suggested that *WT1* suppressor gene plays an important role in both the development of the mesothelium and in the pathogenesis of mesothelioma.[8–13] The present results are similar to those obtained in other investigations in which 6F-H2 anti-WT1 mAb used was the same as that used in the present study.[6] Although the percentage of WT1-positive lung adenocarcinomas in the present study was higher than that previously reported, positive findings were observed only in a small area or a few cells (graded as 1+) in all the WT1-positive lung

adenocarcinomas. In addition, WT1 had the highest sensitivity and specificity among all positive markers. Therefore, it is evident that WT1 is the most useful positive marker for the pathological diagnosis of epithelioid mesothelioma. However, when compared with calretinin, WT1 reactivity tends to be within a limited area (graded as 1+ or 2+). In addition, the density of WT1 reactivity tends to be weaker than calretinin reactivity. Therefore, it is possible that calretinin, rather than WT1, is more useful in distinguishing between these two malignancies, particularly in a small biopsy specimen.

In 1992 Collins *et al*. were the first to suggest that thrombomodulin (CD141) could be a useful positive immunohistochemical marker for the diagnosis of epithelioid mesothelioma.[14] In their study, thrombomodulin reactivity was reported in all 31 epithelioid mesotheliomas, whereas only four of the 48 lung adenocarcinomas were positive and only one of the four cases exhibited strong positivity. Since then, many other reports have been published and although the majority of the reports have confirmed the usefulness of thrombomodulin in distinguishing epithelioid mesotheliomas from lung adenocarcinomas,[14–18] others have not.[6,19–21] The results of the present study including the grading of reactivity are in almost complete agreement with the observation reported by Ordóñez.[6]

In 1985, using gel electrophoresis, Blobel *et al*. were the first to demonstrate significant differences in the cytokeratin expression pattern between epithelioid mesotheliomas and lung adenocarcinomas.[22] These investigators demonstrated that although both these malignancies expressed simple epithelial-type CK peptides (CK 7, 8, 18, and 19), other CK, including CK 5 and CK 6, were present in epithelioid mesotheliomas but not in lung adenocarcinomas. In 1989, Moll *et al*. confirmed this observation by immunofluorescence methods using the AE14 anti-CK 5 antibody.[23] Additional comparative studies on the expression of CK peptide 5 and 6 in epithelioid mesotheliomas and lung adenocarcinomas have become possible only recently with the introduction of the commercially available D5/16B4 anti-CK 5/6 mAb. In the present study, 70% of epithelioid mesotheliomas had reactivity to CK 5/6. This finding is in agreement with the observation reported by Chu and Weiss;[24] but this value is lower than those reported in other investigations.[6,7,16,25,26] In contrast, 41.2% of lung adenocarcinomas also expressed this marker. In the present study, the percentage of CK 5/6-positive lung adenocarcinomas was much higher than those previously reported. However, the reaction was focal (1+) and weak in most cases.

Although a large number of published reports have advocated the utility of CEA in the diagnosis of epithelioid mesotheliomas, some controversy still exists regarding the expression of this protein in epithelioid mesotheliomas.[6,16,17,19,20,27–50] In some earlier studies, the percentage of CEA positivity in epithelioid mesothelioma was reported to be as high as one-third to nearly one half of the cases.[30,36,42] At present, it is believed that these high values were due to the use of anti-CEA antibodies that cross-reacted with non-CEA antigens. However, in recent investigations, CEA expression has been consistently reported in epithelioid mesotheliomas, but in much lower percentages, ranging from 1% to 10% of the cases.[16,20,27,29,34,44] In the present study only 6.8% of epithelioid mesotheliomas had reactivity for CEA. This finding confirms those of other recent investigations.[16,20,27,29,34,44] The findings related to the grading of reactivity are also very similar to those reported by Ordóñez.[6] Among the negative markers of epithelioid mesothelioma, CEA had the highest sensitivity and specificity. Due to its high sensitivity and specificity, CEA continues to be one of the best negative markers of mesothelioma.

The first investigation on the potential of CA19-9 as a marker for distinguishing between epithelioid mesothelioma and lung adenocarcinoma was conducted by Ordóñez in 1989.[41] In his study, CA19-9 positivity was reported in nine (39%) of the 23 lung adenocarcinomas, but not in any of the 19 epithelioid mesotheliomas. Since then, several other studies have been published.[27,51–53] In the most recent study, which is a comparative investigation of a variety of markers, CA19-9 reactivity was reported in 16 (53%) of the 30 adenocarcinomas at various sites, but not in any of the 28 epithelioid mesotheliomas. Ordóñez concluded that CA19-9 was useful and should be part of the four-marker panel recommended for distinguishing between epithelioid mesotheliomas and adenocarcinomas. The present results indicated that this marker is one of the negative markers of epithelioid mesothelioma; but its sensitivity and specificity in epithelioid mesothelioma is not sufficiently high to distinguish between epithelioid mesothelioma and lung adenocarcinoma.

In 1992, using the K1 anti-mesothelin antibody on frozen tissue specimens, Chang *et al*. reported the expression of this marker in all 15 epithelioid mesotheliomas, but not in any of the 23 lung adenocarcinomas.[54] These investigators concluded that mesothelin could be a useful immunohistochemical marker for discriminating between these malignancies. However, recent studies have shown that mesothelin is strongly expressed in other carcinomas, particularly serous carcinomas of the ovary, pancreatic adenocarcinomas, and in some squamous cell carcinomas.[55–59] In the present study there was no significant difference with respect to the immunoreactivity between epithelioid mesotheliomas and lung adenocarcinomas. These results indicate that this marker is not useful in discriminating these tumors.

Since the first investigation on the potential of vimentin immunostaining in the diagnosis of epithelioid mesothelioma by Churg in 1985,[60] a large number of reports have been published with conflicting conclusions, but some controversy remains regarding its potential in the diagnosis of epithelioid mesothelioma.[6,19,37,39,41,43,49,50,61] The results of the present study indicate that this marker is useful as one of the positive markers of epithelioid mesothelioma. However, its

specificity in epithelioid mesothelioma is not sufficiently high to distinguish between epithelioid mesothelioma and lung adenocarcinoma.

To date, there are few reports that have examined the sensitivity and specificity of various combinations of antibodies for their usefulness in diagnosis of epithelioid mesotheliomas. In previous investigations Riera *et al*. recommended the combination of CEA, BG8, and BerEP4,[62] Abutaily *et al*. recommended E-cadherin and TTF-1,[7] and Yaziji *et al*. recommended calretinin, BG8, and MOC-31 as the first-line antibodies.[63] In the present study, among the combinations of two or three antibodies, the combination of WT1 and CEA had the highest sensitivity, and the combination of CEA, calretinin and either WT1 or thrombomodulin had the highest specificity. The combination of WT1 and CEA had 100% sensitivity, but specificity of this combination was insufficient to distinguish lung adenocarcinoma from mesothelioma. Consequently, the present results suggest that the first-line antibodies for the differential diagnosis of epithelioid mesothelioma and lung adenocarcinoma should be CEA, calretinin and either WT1 or thrombomodulin.

In the present study we did not evaluate podoplanin or D2-40 mAb, which appear to be highly specific and sensitive mesothelial markers.[64–66] The two reports describing podoplanin expression in epithelioid mesothelioma and adenocarcinoma suggest that it is highly specific for epithelioid mesothelioma.[65,66] However, D2-40 also recognized ovarian serous carcinomas in one of the two previous studies,[64] and therefore, does not appear to be highly specific for epithelioid mesothelioma. So far, it should be considered that their utility in routine diagnostic work has not yet been completely determined. Further research on these antibodies would clarify their utility in discriminating between these tumors.

CONCLUSION

It is suggested that the combination of CEA, calretinin and either WT1 or thrombomodulin is the most useful antibody panel for distinguishing between epithelioid mesothelioma and lung adenocarcinoma. Further studies, including whole genome expression profiles of epithelioid mesothelioma and lung adenocarcinoma, will clarify several useful positive and negative markers for the differential diagnosis of these tumors in the near future.[67]

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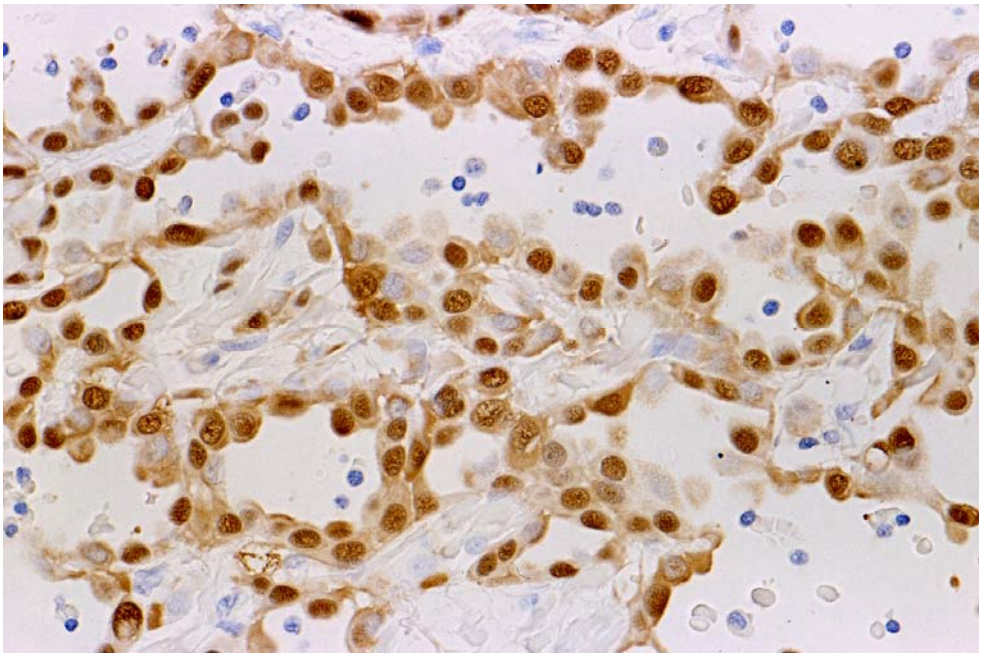
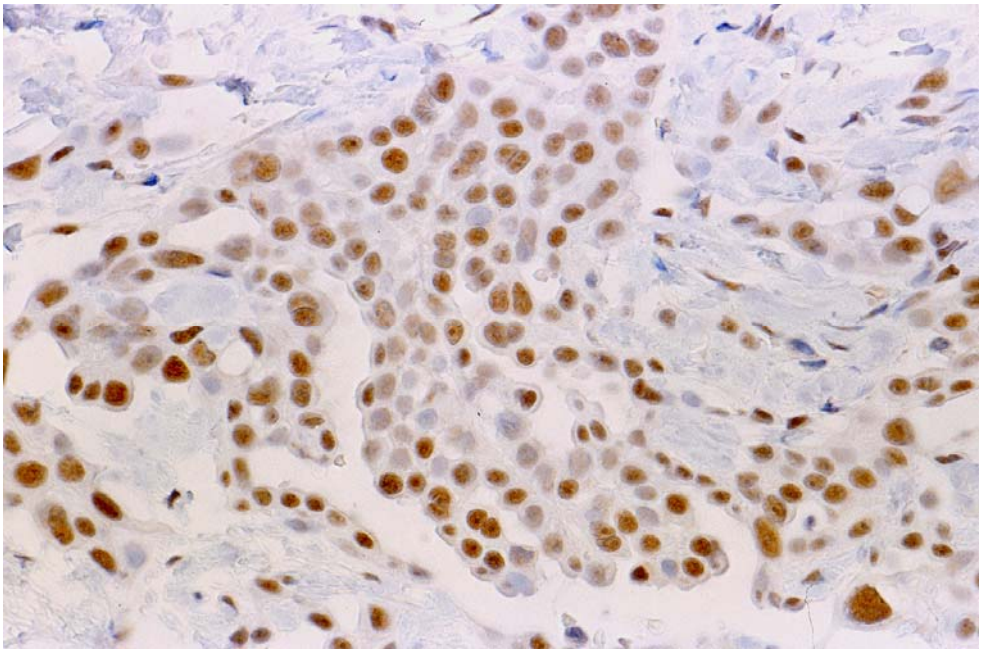


(**a**)



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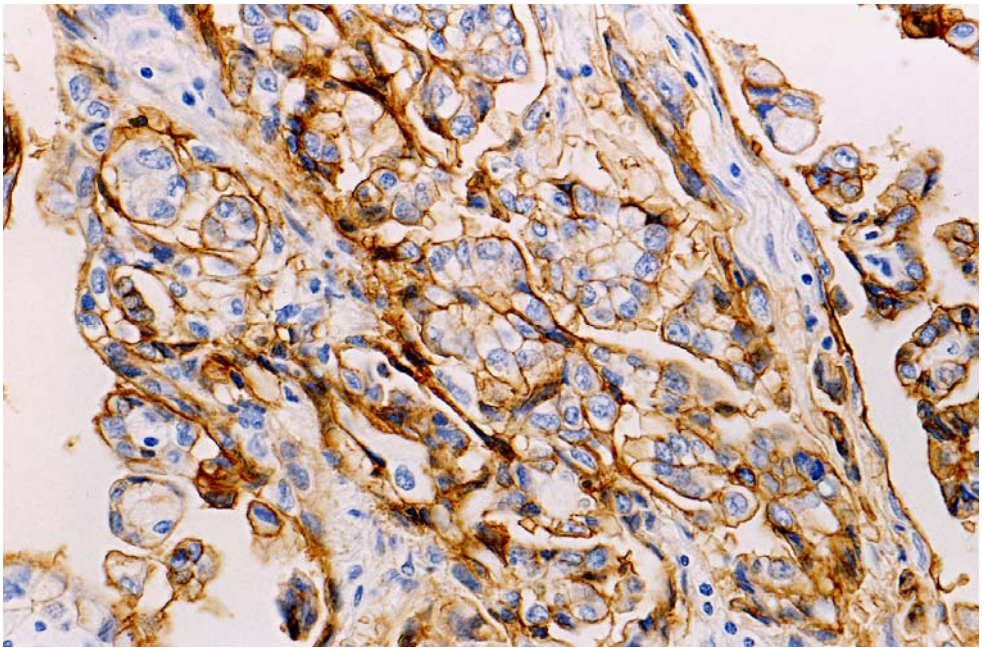
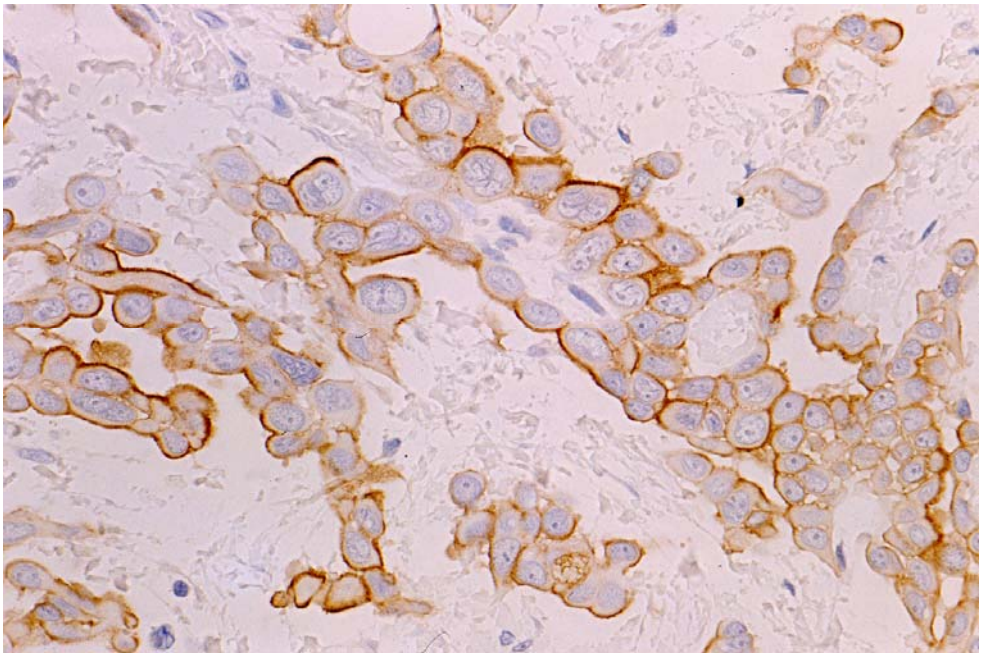


(**c**)

Figure 1 Mesothelioma showing (**a**) strong nuclear and cytoplasmic positivity for calretinin; (**b**) strong nuclear positivity for WT1; and (**c**) membranous positivity for thrombomodulin. The staining is particularly strong along the apical surface of the cells.



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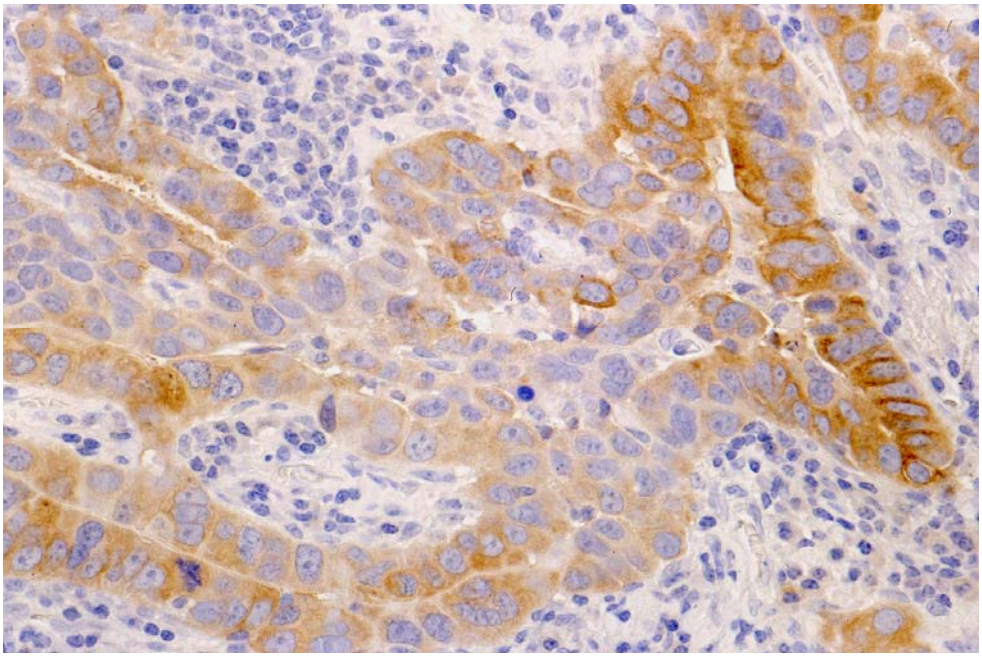
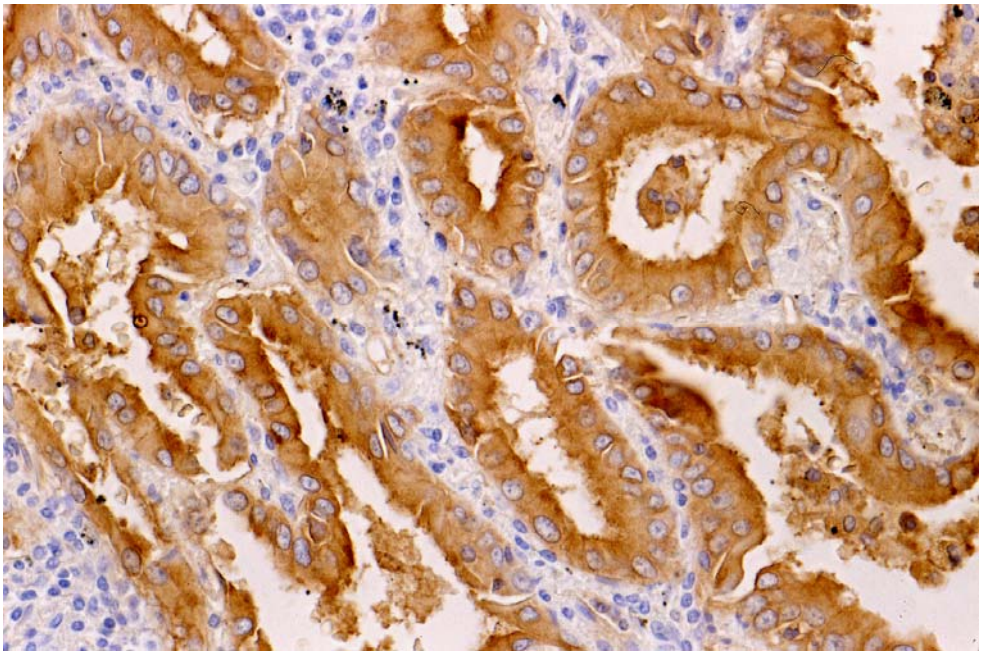


(**b**)

Figure 2 (**a**) Mesothelioma with strong positivity for mesothelin. The staining is particularly strong along the apical surface of the cells. (**b**) Adenocarcinoma with mesothelin positivity.



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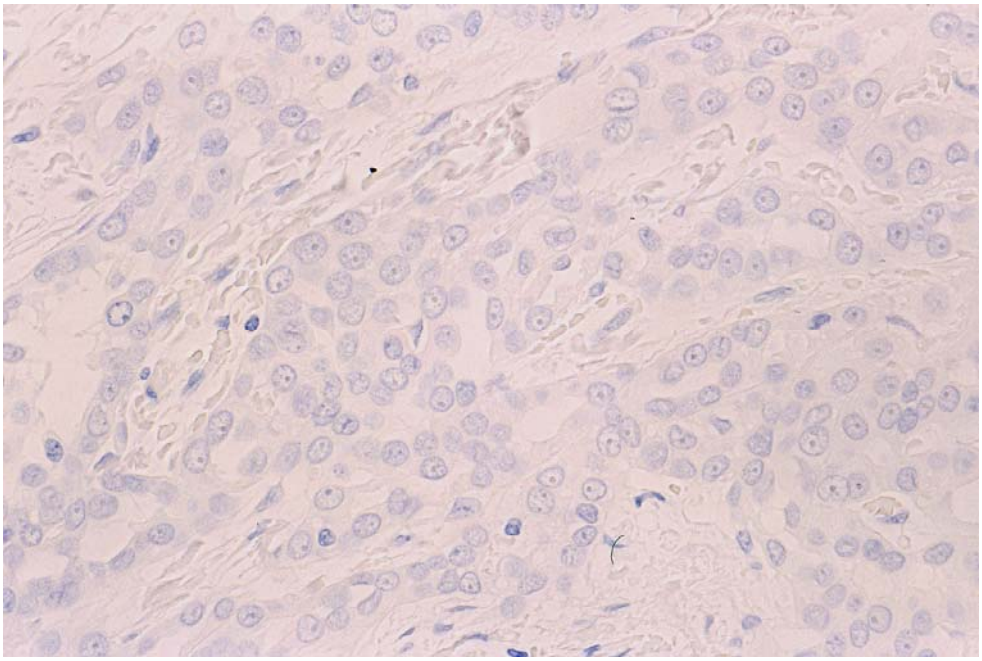
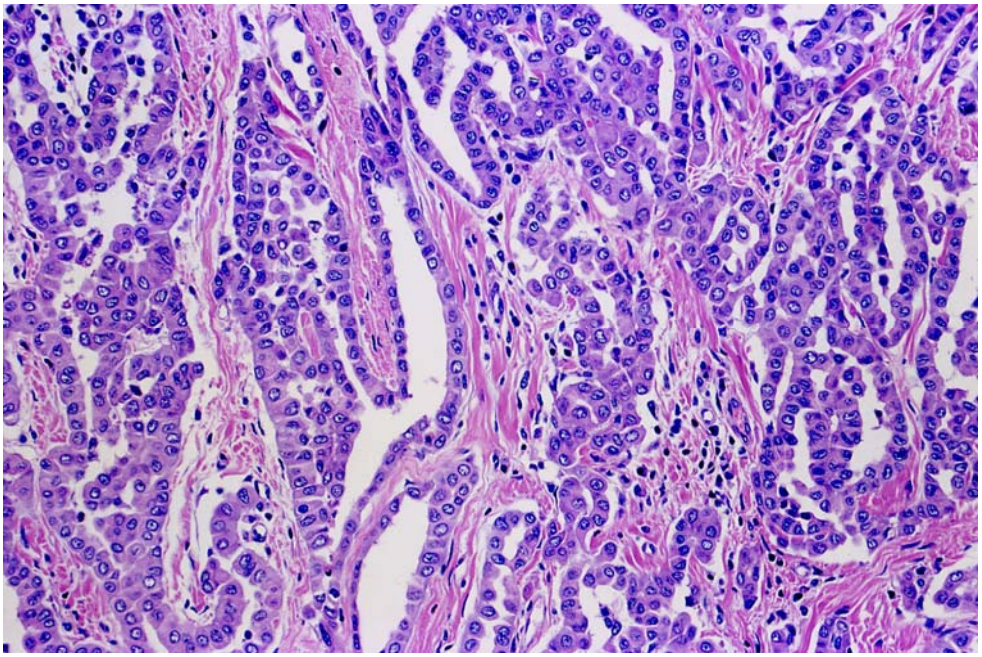
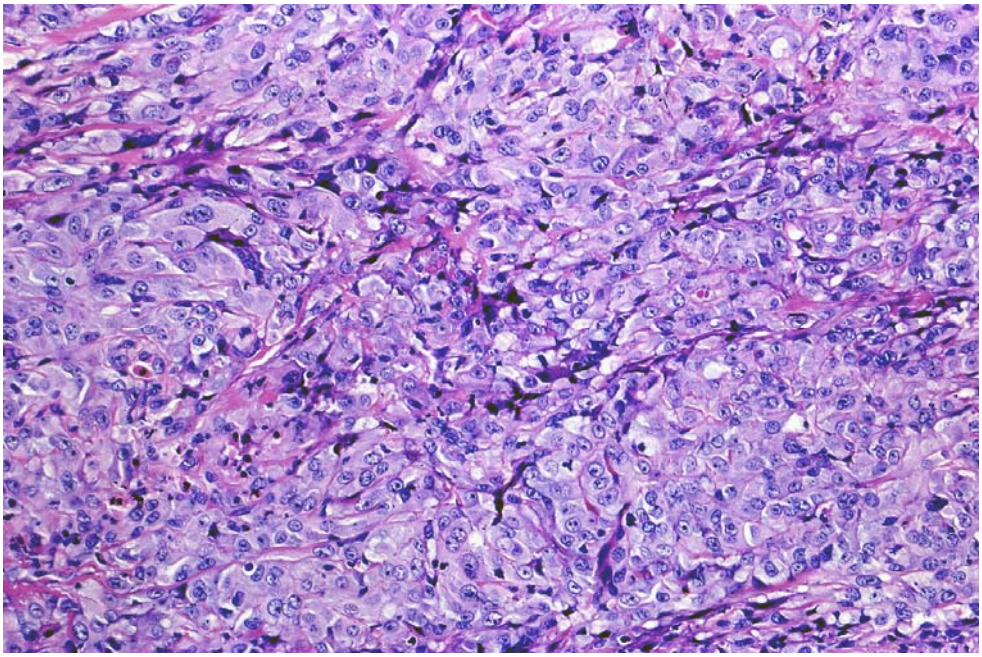


(**b**)

Figure 3 (**a**) Adenocarcinoma showing strong membranous and cytoplasmic positivity for CEA. (**b**) Mesothelioma cells are negative for CEA.



(**a**)



(**b**)

Figure 4 (**a**) Well-differentiated epithelioid mesothelioma showing distinct tubulopapillary growth pattern. (**b**) Poorly differentiated epithelioid mesothelioma showing solid and diffuse growth pattern and lack of papillary or tubulopapillary structure.

Table 1 Antibodies used in this study

Antibody to	Clone	Source	Dilution	Retrieval†
Calretinin	Poly	Zymed, San Francisca, CA, USA	1:100	MW, 5 min
WT1	6F-H12	DakoCytomation, Glostup, Denmark	1:400	AC, 20 min
Cytokeratin-multi	AE1/AE3	Novocastra, Newcastle-upon-tyne, UK	1:100	MW, 5 min
Cytokeratin (CAM5.2)	2A4	Becton-Dickinson, Mountainview, CA, USA	Pre-diluted	MW, 5 min
CK 5/6	D5/16B4	DakoCytomation, Glostup, Denmark	1:50	MW, 5 min
Vimentin	V9	DakoCytomation, Glostup, Denmark	1:100	MW, 5 min
EMA	E29	DakoCytomation, Glostup, Denmark	1:100	MW, 5 min
Thrombomodulin	1009	DakoCytomation, Glostup, Denmark	1:1600	No retrieval
Mesothelin	5B2	Novocastra, Newcastle-upon-tyne, UK	1:20	MW, 5 min
CEA	Poly	Immuno-Biomedical Laboratories, Takasaki, Japan	1:40	MW, 5 min
CA19-9	OV185:1	TFB, Tokyo, Japan	1:1	MW, 5 min
CA125	116NS19-9	TFB, Tokyo, Japan	Pre-diluted	MW, 5 min

†In citrate buffer (pH 6.0).

AC, autoclave; CK, cytokeratin; EMA, epithelial membrane antigen; MW, microwave; WT1, Wilms' tumor gene product.

Table 2 Immunohistochemical findings in mesothelioma

	Positive cases		Grading of reactivity			
Markers	*n*	%	0	1+	2+	3+
Calretinin	85/89	95.5	4	13	8	64
WT1	85/86	98.8	1	23	14	48
AE1/AE3	89/89	100	0	6	5	78
CAM5.2	85/88	96.6	3	3	6	76
CK 5/6	56/80	70	24	28	14	14
Vimentin	81/89	91.0	8	26	18	37
EMA	85/89	95.5	4	17	17	51
Thrombomodulin	60/85	70.6	25	38	13	9
Mesothelin	65/84	77.4	19	5	15	45
CEA	6/88	6.8	82	4	1	1
CA19-9	7/41	17.1	34	5	2	0
CA125	35/41	85.4	6	6	10	19

CK, cytokeratin; EMA, epithelial membrane antigen.

Table 3 Immunohistochemical findings in adenocarcinoma

	Positive cases		Grading of reactivity			
Markers	*n*	%	0	1+	2+	3+
Calretinin	17/51	33.3	34	12	3	2
WT1	8/51	15.7	43	8	0	0
AE1/AE3	51/51	100	0	1	0	50
CAM5.2	51/51	100	0	0	0	51
CK 5/6	21/51	41.2	30	17	4	0
Vimentin	24/51	47.1	27	10	11	3
EMA	51/51	100	0	1	0	50
Thrombomodulin	10/51	19.6	41	6	3	1
Mesothelin	35/51	68.6	17	19	8	7
CEA	50/51	98.0	1	3	6	41
CA19-9	37/51	72.5	14	22	5	10
CA125	41/51	80.4	10	12	10	19

CK, cytokeratin; EMA, epithelial membrane antigen.

Table 4 Comparison of immunohistochemical findings between mesotheliomas and adenocarcinomas

Markers	Positive cases				*P*
	Mesotheliomas		Adenocarcinomas		(Fisher's exact test)
	n	(%)	*n*	(%)	
Calretinin	85/89	95.5	17/51	33.3	<0.001
WT1	85/86	98.8	8/51	15.7	<0.001
AE1/AE3	89/89	100	51/51	100	–
CAM5.2	85/88	96.6	51/51	100	0.251
CK 5/6	56/80	70	21/51	41.2	0.001
Vimentin	81/89	91.0	24/51	47.1	<0.001
EMA	85/89	95.5	51/51	100	0.159
Thrombomodulin	60/85	70.6	10/51	19.6	<0.001
Mesothelin	65/84	77.4	35/51	68.6	0.907
CEA	6/88	6.8	50/51	98.0	<0.001
CA19-9	7/41	17.1	37/51	72.5	<0.001
CA125	35/41	85.4	41/51	80.4	0.366

CK, cytokeratin; EMA, epithelial membrane antigen.

Table 5 Sensitivity and specificity of immunohistochemistry in mesotheliomas by one marker

One marker	Sensitivity (%)	Specificity (%)
Calretinin(+)	95.5	66.7
WT1(+)	98.8	84.3
CK 5/6(+)	70	58.8
Vimentin(+)	91.0	52.9
Thrombomodulin(+)	70.6	80.4
CEA(–)	93.2	98.0
CA19-9(–)	82.9	72.5

CK, cytokeratin.

Table 6 Sensitivity and specificity of immunohistochemistry in mesotheliomas by two markers

Two markers	Sensitivity (%)	Specificity (%)
Calretinin(+) or WT1(+)	100	62.7
Calretinin(+) or TM(+)	98.8	54.9
Calretinin(+) or CEA(–)	97.7	64.7
WT1(+) or TM(+)	97.6	70.6
WT1(+) or CEA(–)	100	82.4
TM(+) or CEA(–)	97.6	78.4
Calretinin(+) and WT1(+)	95.3	88.2
Calretinin(+) and TM(+)	70.5	92.2
Calretinin(+) and CEA(–)	91.8	100
WT1(+) and TM(+)	73.1	94.1
WT1(+) and CEA(–)	90.4	100
TM(+) and CEA(–)	69.1	100

TM, thrombomodulin.

Table 7 Sensitivity and specificity of immunohistochemistry in mesotheliomas by three markers

Three markers	Sensitivity (%)	Specificity (%)
WT1(+) and (Cal(+) or CEA(–))	96.4	88.2
WT1(+) and (Cal(+) or TM(+))	97.6	86.3
WT1(+) and (TM(+) or CEA(–))	96.3	94.1
Cal(+) and (WT1(+) or CEA(–))	96.4	88.2
Cal(+) and (WT1(+) or TM(+))	95.2	84.3
Cal(+) and (TM(+) or CEA(–))	94.0	92.2
CEA(–) and (Cal(+) or WT1(+))	92.9	100
CEA(–) and (Cal(+) or TM(+))	92.9	100
CEA(–) and (WT1(+) or TM(+))	91.5	100

Cal, calretinin; TM, thrombomodulin.

Table 8 Comparison of immunoreactivity between epithelioid mesothelioma and epithelioid component of biphasic mesothelioma

	Epithelioid mesothelioma						Epithelioid component of biphasic mesothelioma						
	Positive cases		Grading of reactivity				Positive cases		Grading of reactivity				
Markers	*n*	%	0	1+	2+	3+	*n*	%	0	1+	2+	3+	*P*†
Calretinin	69/70	98.6	1	9	7	53	16/19	84.2	3	4	1	11	0.058
WT1	66/67	98.5	1	15	11	40	19/19	100	0	8	3	8	0.137
AE1/AE3	70/70	100	0	5	4	61	19/19	100	0	1	1	17	0.779
CAM5.2	66/69	95.7	3	3	3	60	19/19	95.7	0	0	3	16	0.878
CK 5/6	46/62	74.2	16	21	11	14	10/18	55.6	8	7	3	0	0.030
Vimentin	62/70	88.6	8	22	14	26	19/19	100	0	4	4	11	0.049
EMA	68/70	97.1	2	15	12	41	17/19	89.5	2	2	5	10	0.703
Thrombomodulin	47/66	71.2	19	30	10	7	13/19	68.4	6	8	3	2	0.902
Mesothelin	56/66	84.8	10	3	13	40	9/18	50	9	2	2	5	0.002
CEA	3/69	4.3	66	3	0	0	3/19	15.8	16	1	1	1	0.070
CA19-9	6/32	18.8	26	4	2	0	1/9	11.1	8	1	0	0	0.564
CA125	29/32	90.6	3	5	8	16	6/9	66.7	3	1	2	3	0.202

†Mann–Whitney *U*-test.
CK, cytokeratin; EMA, epithelial membrane antigen