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Immunohistochemistry of cytokeratins 7, 8, 17, 18, and 19, and GLUT-1 aids differentiation of desmoplastic malignant mesothelioma from fibrous pleuritis

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Summary. It is difficult to distinguish desmoplastic malignant mesothelioma (DMM) from fibrous pleuritis (FP). We investigated the utility of immunohistochemistry as a way of differentiating between DMM and FP. We examined 11 DMMs and 46 FPs with the aid of antibodies against 18 cytokeratin (CK) subtypes, calponin, caldesmon, desmin, and GLUT-1. The best sensitivity and specificity cut-off values in the receiver operating characteristic curves (ROC) for CKs 7, 8, 17, 18, and 19, and GLUT-1 were each above 60%. When cases with either DMM or FP were partitioned by the staining score associated with the best sensitivity and specificity cut-off values in ROC, the incidence of a positive expression for CKs 7, 8, 17, 18, and 19, and GLUT-1 was significantly higher in DMM than in FP. In conclusion, immunohistochemistry for CKs 7, 8, 17, 18, and 19, and GLUT-1 may be useful, alongside histological characteristics, for separating DMM from FP.

Key words: Immunohistochemistry, Desmoplastic malignant mesothelioma, Fibrous pleurisy, Cytokeratins, GLUT-1

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Introduction

Malignant mesothelioma is a relatively rare tumor that originates from the serosal membrane of the pleura, peritoneum, pericardium, or tunica vaginalis. The latent period between asbestos exposure and onset of mesothelioma is reported to be between 15 and 60 years (Bianchi et al., 1997; McElvenny et al., 2005). In Japan, the Ministry of Health, Labor, and Welfare has disclosed that the number of deaths due to mesothelioma increased gradually from 500 in 1995 to 1156 in 2009 (Ministry of Health, Labour and Welfare 2010). On the above basis, mesothelioma cases are expected to continue to increase in Japan, and to peak in about the year 2025 because large amounts of asbestos were used in Japan between 1960 and 1975.

In the 2004 WHO classification, malignant mesotheliomas were essentially classified as epithelioid, biphasic, sarcomatoid, or desmoplastic. Although the desmoplastic type had been classified as a subtype of sarcomatoid mesothelioma until 2004, it is now considered a new entity because it is characterized by a shorter survival than either epithelioid or sarcomatoid mesothelioma (Churg et al., 2004). To qualify for a diagnosis of desmoplastic mesothelioma (DMM), the paucicellular collagen-rich tissue must occupy at least 50% of a tissue specimen. In addition to the above findings, a diagnosis of DMM requires a storiform pattern or the "patternless pattern" of Stout (Stout,

1965), plus one or more of the following four findings: invasion of chest wall or lung, bland necrosis, frankly sarcomatoid areas, and distant metastases. However, it is difficult to distinguish the histological features of DMM from those of fibrous pleuritis (FP) because the inflammation and hyperplasia of connective tissue cause a change in the form of the epithelioid cell so that it shows cytological atypia.

In recent years, a number of immunohistochemical markers -- including antibodies to cytokeratin (CK) 5/6, calretinin, Wilm's tumour-1 (WT-1), and thrombomodulin -- have become available for the diagnosis of mesothelioma. These markers have proven very useful for differentiating epithelioid mesothelioma from lung adenocarcinoma (Cury et al., 2000; Oates and Edwards 2000; Carella et al., 2001; Ordóñez 2003; Suster and Moran 2006; Addis and Roche 2009; Husain et al., 2009). In contrast, the frequency and degree of expression of these markers in DMM has not been characterized, and the effectiveness of immunohistochemistry for differentiating DMM from FP is unclear. To address these issues, we used antibodies against 18 cytokeratins, calponin, caldesmon, desmin, and GLUT-1 to examine a series of 11 DMMs and 46 FPs.

Materials and methods

We obtained surgically resected or autopsied specimens from patients with DMM (11 specimens) or FP (46 specimens). The DMM specimens were collected in 7 different hospitals across Japan. The DMM patients were 9 men and 2 women (mean age 68.3 years; range 57 to 83). Among them, seven patients had had previous occupational or environmental exposure to asbestos. Ten patients had pleural effusion with a high hyaluronic acid concentration, and 2 cases had positive cytology. Seven patients (excluding the 4 autopsy cases) died after an operation, such as panpleuropneumonectomy, pleurectomy or pleural biopsy. The four autopsy cases died at 1 to 8 months after the onset of symptoms.

Macroscopically, pleural thickening and adhesion were seen in DMM. Microscopically, DMM was characterized by the presence, in at least 50% of the tumor, of dense collagenized tissue separated by atypical tumor cells arranged in a storiform or "patternless" pattern. These areas included micronodular proliferation and foci of bland necrosis. For the definitive diagnosis of mesothelioma, we performed immunohistochemistry using 8 antibodies. Tumor cells were positive for

Table 1. Antibodies used in this study.

Antibody/ antigen	Type*/clone	Source	Dilution	Pretreatment
CK† 1	G	Acris Antibodies GmbH, Herford, Germany	1/25	autoclave in 0.05M Tris buffer, pH10.0, for 20 min
CK 2	M/BM5091	Acris Antibodies GmbH, Herford, Germany	1/100	incubate in 0.05M Tris-0.01% protease (Sigma type XXIV), for 30 min at room temperature
CK 3	M/AE5	Enzo Life Sciences, Plymouth Meeting, PA	1/1,000	boil in 0.05M citrate-0.002M EDTA buffer, pH6.0, for 60 min
CK 4	M/EP1599	Novus Biologicals, Littleton, CO	1/100	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
CK 5	M/XM26	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/300	autoclave in 0.05M Tris buffer, pH10.0, for 20 min
CK 6	M/LHK6B	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/20	boil in 0.01M Tris buffer, pH 10.0, for 60 min
CK 7	M/OV-TL 12/30	Nichirei Biosciences Inc, Tokyo, Japan	1/5	incubate in 0.05M Tris-0.01% protease (Sigma type XXIV), for 30 min at room temperature
CK 8	M/DE-K	DAKO, Glostrup, Denmark	1/2	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
CK 9	M/Ks9.70+Ks9.216	Progen Biotechnik GmbH, Heidelberg, Germany	1/10	autoclave in 0.05M Tris buffer, pH10.0, for 20 min
CK 10	M/LHP1	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/400	incubate in 0.05M Tris-0.01% protease (Sigma type XXIV), for 30 min at room temperature
CK 12	R	TransGenic Inc, Hyogo, Japan	1/500	autoclave in 0.05M Tris buffer, pH10.0, for 20 min
CK 13	M/KS-1A3	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/500	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
CK 14	M/LL002	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/200	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
CK 15	M/LHK15	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/300	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
CK 16	M/LL025	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/200	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
CK 17	M/E3	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/200	autoclave in 0.05M citrate-0.002M EDTA buffer, pH6.0, for 20 min
CK 18	M/DC-10	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/200	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
CK 19	M/b170	Novocastra Laboratories Ltd, Tyne and Wear, UK	1/1000	boil in 0.01M Tris-0.001M EDTA buffer, pH 9.0, for 60 min
Calponin	M/ CALP	DAKO, Glostrup, Denmark	1/400	incubate in 0.05M Tris-0.01% protease (Sigma type XXIV), for 30 min at room temperature
Caldesmon	M/ h-CD	DAKO, Glostrup, Denmark	1/200	boil in 0.05M citrate-0.002M EDTA buffer, pH6.0, for 60 min
Desmin	M/ D33	Nichirei Biosciences Inc, Tokyo, Japan	ready to use	No pretreatment
GLUT-1	R	Immuno-Biological Laboratories Co. Ltd, Fujioka, Japan	1/50	No pretreatment

*: G, guinea pig: M, mouse: R, rabbit; †: cytokeratin.

calretinin and AE1/AE3 in all cases, D2-40 in 9 cases, and WT-1 in 7 cases, but negative for CEA, BerEP4, MOC31, and TTF-1 in all cases. Since the first four antibodies yield positive reactions in mesothelioma, while the last four yield positive reactions in pulmonary adenocarcinoma, we diagnosed DMM in all 11 cases.

Immunohistochemistry

We used the polymer-peroxidase method (EnVision+/HRP; Dako Cytomation, Denmark) on deparaffinized sections of DMM and FP. All antibodies were incubated overnight at 4 degree Celsius. The monoclonal and polyclonal antibodies used are listed in Table 1, together with the antigen-retrieval conditions.

For the analysis of immunoreactivity, the extent of moderate-to-strong staining was scored as: 0, indicating negative reaction of tumor cells; 1, ≤10% of tumor area stained; 2, 11 to 25% stained; 3, 26 to 50% stained; 4, 51 to 75% stained; or 5, ≥76% stained. For each antibody, a

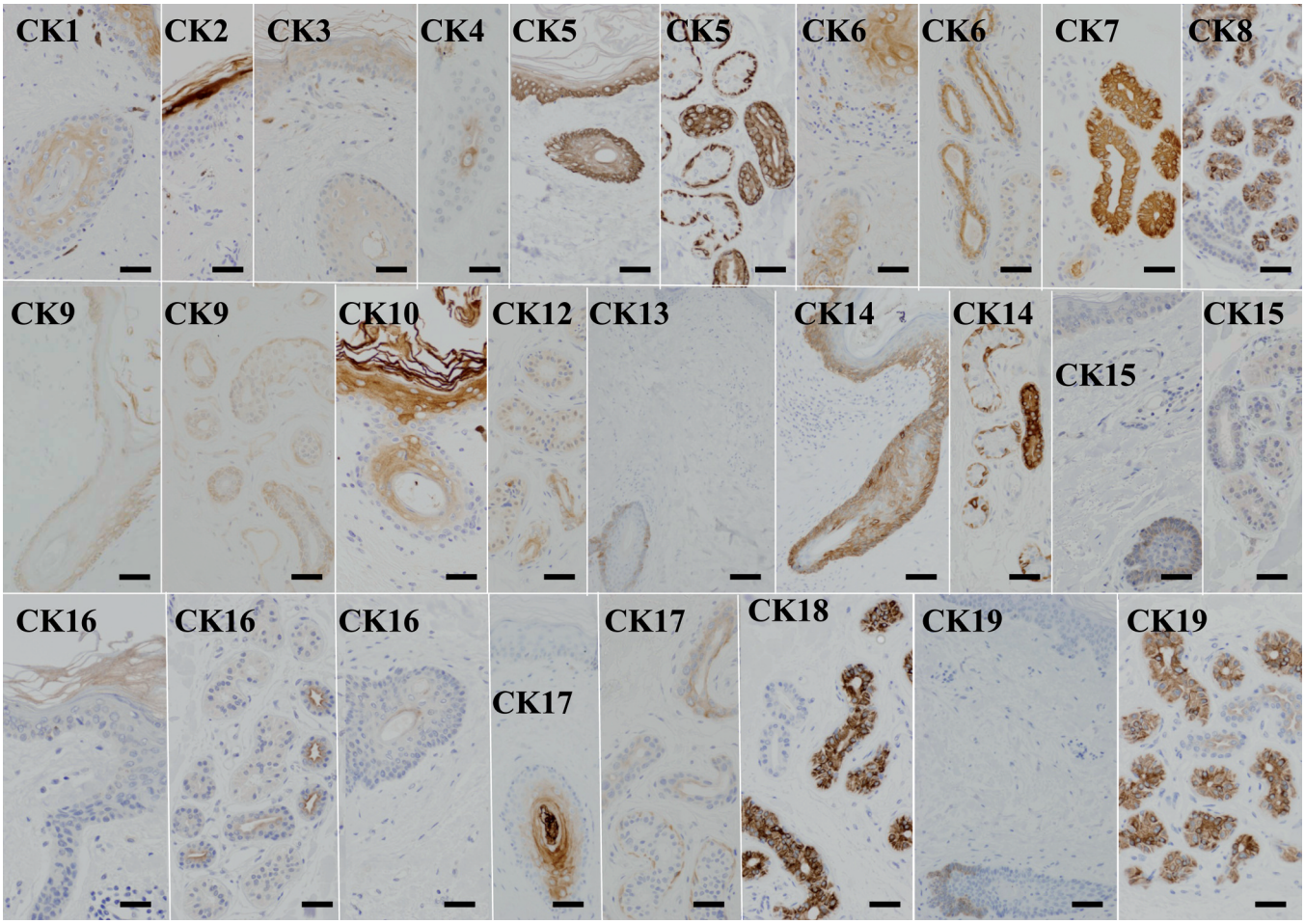

Fig. 1. Immunohistochemistry of cytokeratins within normal skin. CK1 was revealed in squamous, granular, and cornified layers of the epidermis, and inner root hair sheath, CK2 in granular and cornified layers of the epidermis, CK3 in squamous, granular, and cornified layers of the epidermis, and inner root hair sheath, CK4 in inner root hair sheath, CK5 in basal, squamous, and granular layers of the epidermis, outer root hair sheath, inner and outer layers of the eccrine ducts, and myoepithelial cells of the secretory glands, CK6 in squamous, granular, and cornified layers of the epidermis, inner root hair sheath, and inner layer of the eccrine ducts, CK7 in inner layer of the eccrine ducts, and secretory and myoepithelial cells of the secretory glands, CK8 in secretory and myoepithelial cells of the secretory glands, CK9 in basal and cornified layers of the epidermis, outer root hair sheath, outer layers of the eccrine ducts, and myoepithelial cells of the secretory glands, CK10 in granular and cornified layers of the epidermis, and inner root hair sheath, CK12 in inner layer of the eccrine ducts, and secretory and myoepithelial cells of the secretory glands, CK13 in outer root hair sheath, CK14 in basal and squamous layers of the epidermis, outer root hair sheath, inner and outer layers of the eccrine ducts, and myoepithelial cells of the secretory glands, CK15 in outer root hair sheath, and secretory and myoepithelial cells of the secretory glands, CK16 in cornified layer of the epidermis, inner root hair sheath, and inner layers of the eccrine ducts, CK17 in inner root hair sheath, inner layer of the eccrine ducts, and myoepithelial cells of the secretory glands, CK18 in secretory and myoepithelial cells of the secretory glands, and CK19 in outer root hair sheath, inner layer of the eccrine ducts, and secretory and myoepithelial cells of the secretory glands. Bars: 50 μm.

Table 2. Cytokeratin (CK) expression in the skin.

	CK 1	CK 2	CK 3	CK 4	CK 5	CK 6	CK 7	CK 8	CK 9	CK 10	CK 12	CK 13	CK 14	CK 15	CK 16	CK 17	CK 18	CK 19
Epidermis																		
Basal layer	-	-	-	-	+	-	-	-	+	-	-	±	+	-	-	-	-	-
Squamous layer	+	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
Granular layer	+	+	+	-	±	+	-	-	-	+	-	-	-	-	-	-	-	-
Cornified layer	+	+	+	-	-	+	-	-	+	+	+	-	-	-	±	-	-	-
Hair sheath																		
Inner root sheath	+	-	±	±	-	+	-	-	-	±	+	-	-	-	+	+	-	-
Outer root sheath	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	+
Eccrine duct																		
Inner layer	-	-	-	-	+	+	±	-	-	-	+	-	+	-	+	±	-	+
Outer layer	-	-	-	-	±	-	-	-	+	-	-	-	±	-	-	-	-	-
Secretory gland																		
Secretory cells	-	-	-	-	-	-	+	+	-	-	±	-	-	±	-	-	+	+
Myoepithelial cells	-	-	-	-	+	-	±	+	±	-	±	-	+	±	-	+	+	+

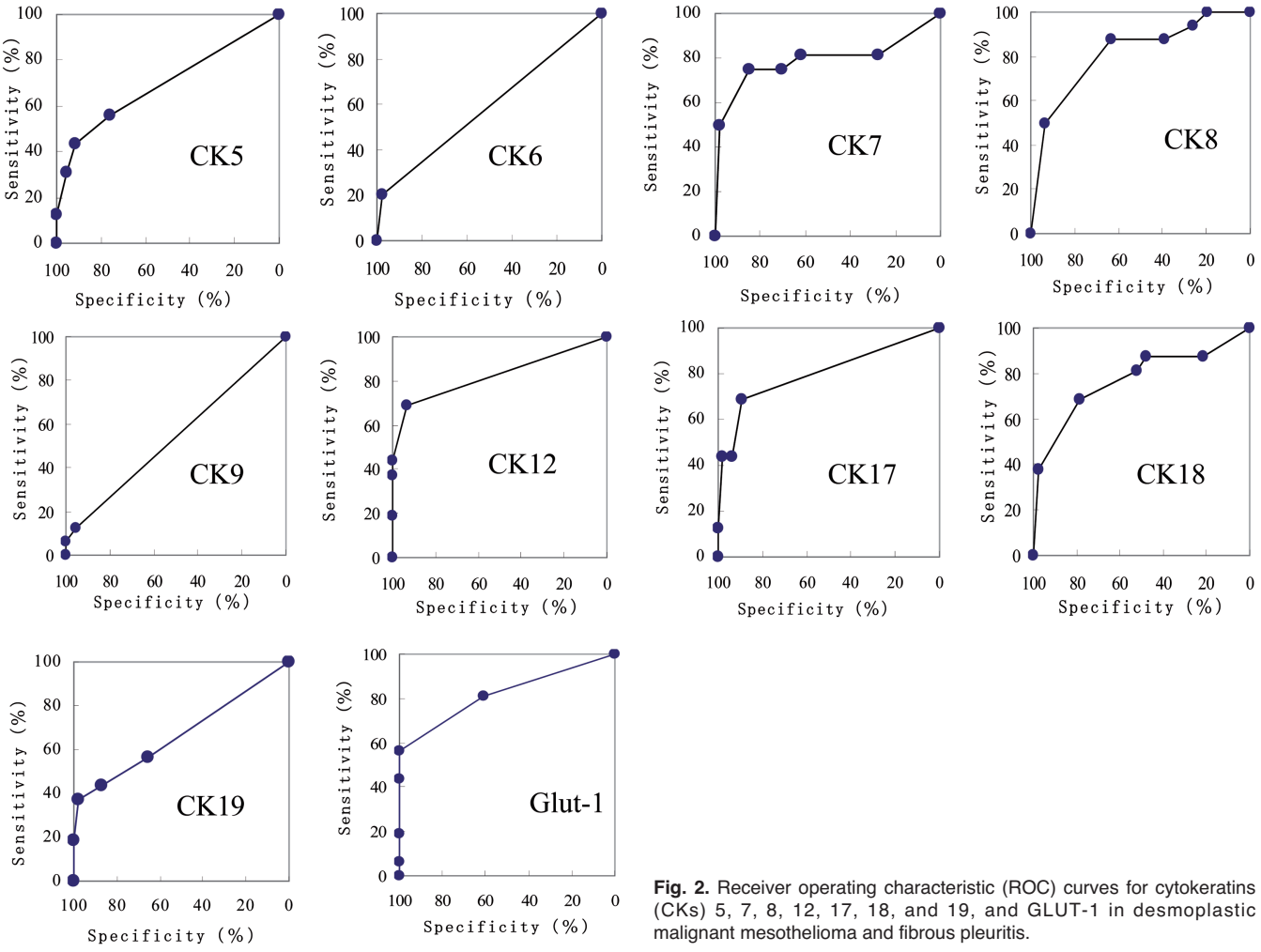

Fig. 2. Receiver operating characteristic (ROC) curves for cytokeratins (CKs) 5, 7, 8, 12, 17, 18, and 19, and GLUT-1 in desmoplastic malignant mesothelioma and fibrous pleuritis.

receiver operating characteristic (ROC) curve was employed to identify the best cut-off values for sensitivity and specificity (13). Then, tumors with a staining score equal to or above that associated with the best sensitivity and specificity were graded as positive.

Data analysis

Statistical analysis of the difference in incidence between two groups was performed using the Chi-square analysis. A p value of less than 0.05 was considered significant.

Results

By immunohistochemistry, each CK was revealed in the cytoplasm of positive cells in the epidermis, dermis, eccrine ducts, and/or secretory glands within normal skin (Table 2, Fig. 1). Calponin, caldesmon, and desmin were detected in the cytoplasm of both vascular smooth muscle cells and bronchial surface epithelial cells within the normal lung. GLUT-1 was found in the membrane of red blood cells within blood vessels and in the membrane and cytoplasm of bronchial surface epithelial cells, each within the normal lung. Expressions of CKs 5, 7, 8, 9, 12, 17, 18, and 19, calponin, caldesmon, and desmin were confined to the cytoplasm of DMM tumor cells and of reactive spindle cells in FP, while GLUT-1 expression was detected in the membrane and cytoplasm of DMM tumor cells and reactive spindle cells in FP (Fig. 2). However, the staining intensity of their proteins sometimes varied within a given case. No ROC curve could be obtained for CKs 1, 2, 3, 4, 6, 9, 10, 13, 14, 15, or 16, or for calponin, caldesmon, or desmin.

Among CKs 5, 7, 8, 12, 17, 18, and 19, and GLUT-1, the best sensitivity and specificity cut-off values in the ROC curves were above 60% for each of CKs 7, 8, 17, 18, and 19, and GLUT-1 (Table 3, Fig. 3). The incidence of a positive expression for CK5, CK12, or CK17 was significantly higher in DMM than in FP (tumors were graded as positive if 1% or more of their cells showed staining) (Table 4; p=0.046, p=0.001, or p<0.0001, respectively). The incidence of a positive expression for CK7 or CK18 was significantly higher in DMM than in FP (tumors were graded as positive if 51% or more of their cells showed staining) (p<0.0001 or p=0.0001, respectively). The incidence of a positive expression for CK18 was significantly higher in DMM than in FP (tumors were graded as positive if 76% or more of their cells showed staining) (p=0.0001). Further, the incidence of a positive expression for GLUT-1 was significantly higher in DMM than in FP (tumors were graded as positive if 11% or more of their cells showed staining) (p<0.0001). Given the best sensitivity and specificity cut-off values in the ROC curves, and the statistical analysis of the difference in incidence between the two groups, CKs 7, 8, 17, 18, and 19, and GLUT-1 were identified as potentially useful markers for diagnosis between DMM and FP.

Table 3. Sensitivity and specificity data for cytokeratins (CKs) and GLUT-1.

	CK5	CK7	CK8	CK12	CK17	CK18	CK19	GLUT-1
Sensitivity*	55	82	64	55	91	82	64	73
Specificity*	76	85	94	94	89	78	87	100

*: CK5, CK12, CK17: positive if staining score >1, CK19, GLUT-1: positive if staining score ≥2, CK7, CK18: positive if staining score >4, CK8: positive if staining score >5

Discussion

Although several proteins -- such as CK5/6, calretinin, WT-1, thrombomodulin, and mesothelin -- are useful markers for distinguishing epithelioid mesothelioma from pulmonary adenocarcinoma (Cury et al., 2000; Oates and Edwards 2000; Carella et al., 2001; Ordóñez 2003), no immunohistochemical marker for

Table 4. Expressions of cytokeratins (CKs) and GLUT-1 in desmoplastic malignant mesothelioma (DMM) and fibrous pleuritis (FP).

	DMM	FP	p value*
CK5			
>1%	6	11	0.046
0%	5	35	
CK7			
>51%	9	7	<0.0001
<50%	2	39	
CK8			
>76%	7	3	<0.0001
<75%	4	43	
CK12			
>1%	6	3	0.001
0%	5	43	
CK17			
>1%	10	5	<0.0001
0%	1	41	
CK18			
>51%	9	10	0.0001
<50%	2	36	
CK19			
>11%	7	6	0.0047
<10%	4	40	
GLUT-1			
>11%	8	0	<0.0001
<10%	3	46	

*: Statistical analysis of the difference in incidence between two groups was performed using the Chi-square analysis.

differential diagnosis between DMM and FP has been reported. In the present study, in which we examined 18 CKs, calponin, caldesmon, desmin, and GLUT-1 in 11 DMMs and 46 FPs, we observed that the best sensitivity and specificity cut-off values in the ROC curves were above 60% for each of CKs 7, 8, 17, 18, and 19, and GLUT-1, and that for each of these, the incidence of a positive expression was significantly higher in DMM than in FP. On that basis, immunohistochemistry for CKs 7, 8, 17, 18, and 19, and GLUT-1 may provide useful markers for separating DMM from FP, alongside such histological characteristics as cellular atypia, storiform

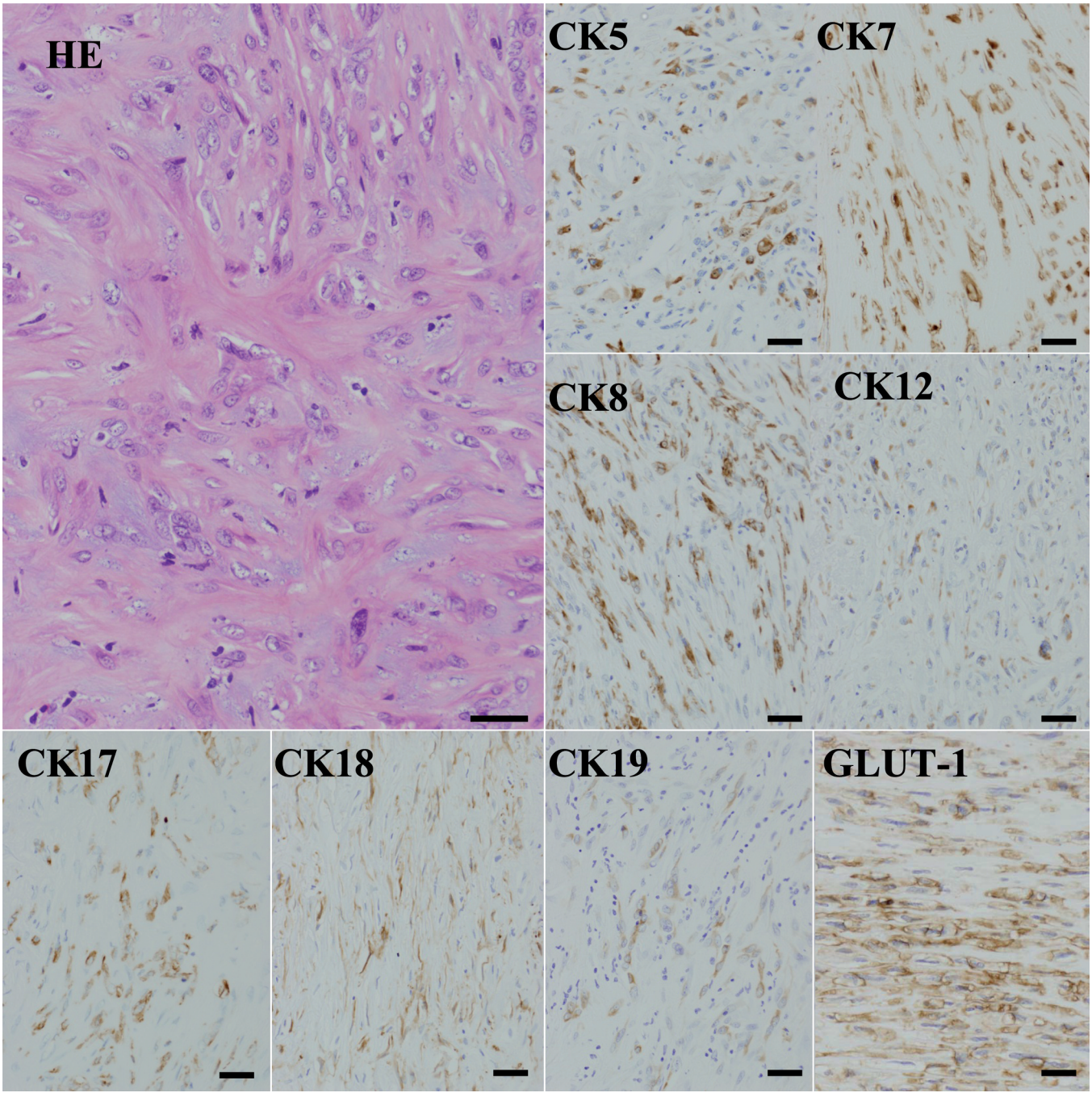

Fig. 3. Hematoxylin-eosin (HE) staining and immunohistochemistry of cytokeratins (CKs) 5, 7, 8, 12, 17, 18, and 19, and GLUT-1 in desmoplastic malignant mesothelioma (DMM). Bars: 50 μm.

pattern and/or bland necrosis.

It was previously reported that immunohistochemical staining for CK5/6, calretinin, WT-1, and mesothelin was useful in the diagnosis of sarcomatoid mesothelioma, as well as of epithelioid mesothelioma (Cury et al., 2000; Oates and Edwards 2000; Carella et al., 2001; Lucas et al., 2003; Ordóñez 2003; Suster and Moran 2006; Addis and Roche 2009; Husain et al., 2009). In epithelioid mesothelioma, CK5/6, calretinin, WT-1, and mesothelin have been found to be positive in 55-100%, 42-100%, 43-95%, and 100% of cases, respectively (Ordóñez 2003; Suster and Moran 2006). As far as we can tell, however, these positive expressions are lower in incidence in sarcomatous mesothelioma than in epithelioid mesothelioma, since CK5/6, calretinin, and WT-1 were detected in 0-29%, 39-100%, and 0-50%, respectively, in sarcomatoid mesothelioma (Suster and Moran 2006). However, there is little reported immunohistochemistry for DMMs (Lucas et al., 2003), although in routine practice pathologists frequently have need to differentiate DMM from FP.

It is well known that CKs are a family of intermediate filaments involved in epithelial differentiation, and several CKs are useful tools for differential diagnosis in surgical pathology (Quinlan et al., 1985; Moll et al., 2008; Klebe et al., 2010). So far, at least 20 distinct CKs have been identified. In mesothelioma, Bolen et al (1986), who examined 9 epithelial, 5 sarcomatoid, and 2 desmoplastic malignant mesotheliomas, using antibodies of both low molecular weight CKs (2 antibodies of 44 and 54 kDa, and 46, 52, and 54 kDa) and high molecular weight CKs (one antibody of 57 and 66 kDa), demonstrated that epithelial mesotheliomas were all positive for low and high molecular weight CKs, while sarcomatoid and desmoplastic mesotheliomas were all positive for low molecular weight CKs, but negative for high molecular weight CKs, except for one sarcomatous mesothelioma. In the present study, we investigated 18 CKs for their utility in differentiating DMM from FP. In the ROC curves for low molecular weight CKs (less than 55kDa), such as CKs 7, 8, 17, 18, and 19, sensitivity and specificity tended to be above 60%, while for high molecular weight CKs (equal or more than 55kDa), such as CKs 1, 2, 3, 4, 5, 6, 9, 10, 11, and 12, one or both of them tended to be below 60%. When tumors were graded as positive if 1% or more of their cells showed staining for CK17, if 11% or more of their cells showed staining for CK19, if 51% or more of their cells showed staining for CK7 or CK18, or if 76% or more of their cells showed staining for CK8 (criteria based on the sensitivity and specificity data in their ROC curves), we demonstrated that positive expressions of CKs 7, 8, 17, 18, and 19 were significantly more frequent in DMMs than in FPs. To judge from that finding, immunostaining for CKs 7, 8, 17, 18, and 19 may not only help to identify the presence of invasion into adipose tissue or invasion into the underlying lung in DMM, but also aid the diagnosis of DMM.

In the present study, we demonstrated that GLUT-1 immunohistochemistry was useful for separating DMM from FP. It is generally accepted that GLUT-1 is one of 14 members of the mammalian facilitative glucose transporter (GLUT) family of passive carriers that function as an energy-independent system for the transport of glucose down a concentration gradient (Olson and Pessin, 2005). Although GLUT-1 is not detectable in a large proportion of cells within normal tissues or benign lesions, it is expressed in various cancers (Macheda et al., 2005). In recent studies, GLUT-1 expression has been found to be useful for distinguishing malignant mesothelioma from reactive mesothelial hyperplasia (Kato et al., 2007; Acurio et al., 2008). In fact: (a) Kato et al. (2007) found that its immunoreactivity was negative in 40 reactive mesothelial cases, but positive in all of 40 malignant mesotheliomas, and (b) Acurio et al. (2008) found that 40 benign mesothelial tissues (20 normal, 20 reactive cases) were negative, while 34 of 45 malignant mesotheliomas were positive (unpublished observations). On the basis of the present data and those from the above two studies, GLUT-1 may be a useful marker for separating DMM from FP, as well as for separating malignant mesothelioma from FP or reactive mesothelial hyperplasia.

In conclusion, immunohistochemistry for CKs 7, 8, 17, 18, and 19, and GLUT-1 may be useful for differentiating DMM from FP, alongside their characteristic histological features. Even so, accurately diagnosing DMM in routine practice will require careful consideration of all the available findings by the pathologists.

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References

Acurio A., Arif Q., Gattuso P., McIntere M., Krausz T. and Husain A.N. (2008). Value of immunohistochemical markers in differentiating benign from malignant mesothelial lesions: United States and Canadian Academy of Pathology annual meeting [abstract 1527]. Mod. Pathol. 21, 334A.

Addis B. and Roche H. (2009). Problems in mesothelioma diagnosis. Histopathology 54, 55-68.

Bianchi C., Giarelli L., Grandi G., Brollo A., Ramani L. and Zuch C. (1997). Latency periods in asbestos-related mesothelioma of the pleura. Eur. J. Cancer Prev. 6, 162-166.

Bolen J.W., Hammar S.P. and McNutt M.A. (1986). Reactive and neoplastic serosal tissue: a light-microscopic, ultrastructural, and immunocytochemical study. Am. J. Surg. Pathol. 10, 34-47.

Carella R., Deleonardi G., D'Errico A., Salerno A., Egarter-Vigl E., Seebacher C., Donazzan G. and Grigioni W.F. (2001). Immunohistochemical panels for differentiating epithelial malignant mesothelioma from lung adenocarcinoma: a study with logistic regression analysis. Am. J. Surg. Pathol. 25, 43-50.

Churg A, Roggli V, Galateau-Salle F Cagle P.T., Gibbs A.R., Hasleton P.S., Henderson D.W., Vignaud J.M., Inai K., Praet M., Ordóñez N.G., Hammar S.P., Testa J.R., Gazdar A.F., Saracci R., Pugatch R., Samet J.M., WeillH., Rusch V., Colby T.V., Vogt P., Brambilla E. and Travis W.D. (2004). Mesothelioma. In: Pathology and genetics of tumours of the lung, pleura, thymus and heart. Travis W.D., Brambilla E., Muller-Hermelink H.K. and Harris C.C. (eds). Lyon. IARC pp 128-136.

Cury P.M., Butcher D.N., Fisher C., Corrin B. and Nicholson A.G. (2000). Value of the mesothelium-associated antibodies thrombomodulin, cytokeratin 5/6, calretinin, and CD44H in distinguishing epithelioid pleural mesothelioma from adenocarcinoma metastatic to the pleura. Mod. Pathol. 13, 107-112.

Husain A.N., Colby T.V., Ordóñez N.G., Krausz T., Borczuk A., Cagle P.T., Chirieac L.R., Churg A., Galateau-Salle F., Gibbs A.R., Gown A.M., Hammar S.P., Litzky L.A., Roggli V.L., Travis W.D. and Wick M.R. (2009). Guidelines for pathologic diagnosis of malignant mesothelioma: a consensus statement from the International Mesothelioma Interest Group. Arch. Pathol. Lab. Med. 133, 1317-1331.

John R., Lifshitz M.S., Jhang J. and Fink D. (2007). Post-analysis: medical decision-marking. In: Henry's clinical diagnosis and management by laboratory methods. 21st Edition. McPherson R.A. and Pincus M.R. (eds). Saunders Elsevier. China pp 68-75.

Kato Y., Tsuta K., Seki K., Maeshima A.M., Watanabe S., Suzuki K., Asamura H., Tsuchiya R. and Matsuno Y. (2007). Immunohistochemical detection of GLUT-1 can discriminate between reactive mesothelium and malignant mesothelioma. Mod. Pathol. 20, 215-220.

Klebe S., Brownlee N.A., Mahar A., Burchette J.L., Sporn T.A., Vollmer R.T. and Roggli V.L. (2010). Sarcomatoid mesothelioma: a clinical-pathologic correlation of 326 cases. Mod. Pathol. 23, 470-479.

Lucas D.R., Pass H.I., Madan S.K., Adsay N.V., Wali A., Tabaczka P. and Lonardo F. (2003). Sarcomatoid mesothelioma and its histological mimics: a comparative immunohistochemical study. Histopathology 42, 270-279.

Macheda M.L., Rogers S. and Best J.D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J. Cell. Physiol. 202, 654-662.

McElvenny D.M., Darnton A.J., Price M.J. and Hodgson J.T. (2005). Mesothelioma mortality in Great Britain from 1968 to 2001. Occup Med 55, 79-87.

Ministry of Health, Labour and Welfare. (2010). Vital statistics of japan 2010. Tokyo, Japan. Ministry of Health, Labour and Welfare.

Moll R., Divo M. and Langbein L. (2008). The human keratins: biology and pathology. Histochem. Cell Biol. 129, 705-733.

Oates J. and Edwards C. (2000). HBME-1, MOC-31, WT1 and calretinin: an assessment of recently described markers for mesothelioma and adenocarcinoma. Histopathology 36, 341-347.

Olson A.L. and Pessin J.E. (2005). Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. Annu. Rev. Nutr. 16, 235-256.

Ordóñez N.G. (2003). The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am. J. Surg. Pathol. 27, 1031-1051.

Quinlan R.A., Schiller D.L., Quinlan R.A., Schiller D.L., Hatzfeld M., Achtstätter T., Moll R., Jorcano J.L., Magin T.M. and Franke W.W. (1985). Patterns of expression and organization of cytokeratin intermediate filaments. Ann. NY Acad. Sci. 455:282-306.?

Stout A. (1965). Biological effects of asbestos. Ann. NY Acad. Sci. 132, 680-682.

Suster S. and Moran C.A. (2006). Applications and limitations of immunohistochemistry in the diagnosis of malignant mesothelioma. Adv. Anat. Pathol. 13,316-329.

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