SOX6 is a Novel Immunohistochemical Marker for Differential Diagnosis of Epithelioid Mesothelioma From Lung Adenocarcinoma

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间皮瘤

- 是胸膜最常见的肿瘤
- 源自胸膜间皮细胞

2015 WHO

Mesothelial tumours	
Diffuse malignant mesothelioma	
Epithelioid mesothelioma	9052/3
Sarcomatoid mesothelioma	9051/3
Desmoplastic mesothelioma	9051/3
Biphasic mesothelioma	9053/3
Localized malignant mesothelioma	
Epithelioid mesothelioma	9052/3
Sarcomatoid mesothelioma	9051/3
Biphasic mesothelioma	9053/3
Well differentiated papillary mesothelioma	9052/1*
Adenomatoid tumour	9054/0

间皮瘤	
弥漫性恶性间皮瘤	9050/3[1]
上皮样间皮瘤	9052/3
肉瘤样间皮瘤	9051/3
促结缔组织增生性间皮瘤	9051/3
	9053/3
	9050/3
瘤	
皮瘤	9052/1
	9054/0

Epithelioid mesothelioma

- **Diffuse malignant mesothelioma** (a diffuse pattern of growth over the pleural surfaces)
  -Epithelioid mesothelioma

- **Localized malignant mesothelioma** (rare ,that grossly appears as a distinctly localized nodular lesion, It shows no gross or microscopic evidence of diffuse pleural spread, but has the microscopic,immunohistochemical,and ultrastructural features of diffuse malignant mesothelioma)
  -Epithelioid mesothelioma

malignant mesothelioma

- a highly aggressive tumor with extremely poor prognosis
- its occurrence is increasing worldwide, primarily due to past and/or present occupational and/or environmental asbestos exposure（ therapeutic radiation ,fbre erionite,Germline mutations in BAP1 ）
- Malignant mesothelioma is still predominant in the developed world, including Japan, but a shift in disease occurrence is anticipated since asbestos use has recently increased in developing countries
- The latency is long, averaging 30–40 years, and few mesotheliomas are seen with latencies < 15 years
- Malignant mesothelioma is subtyped into epithelioid, sarcomatoid, and biphasic forms in the World Health Organization (WHO) classification

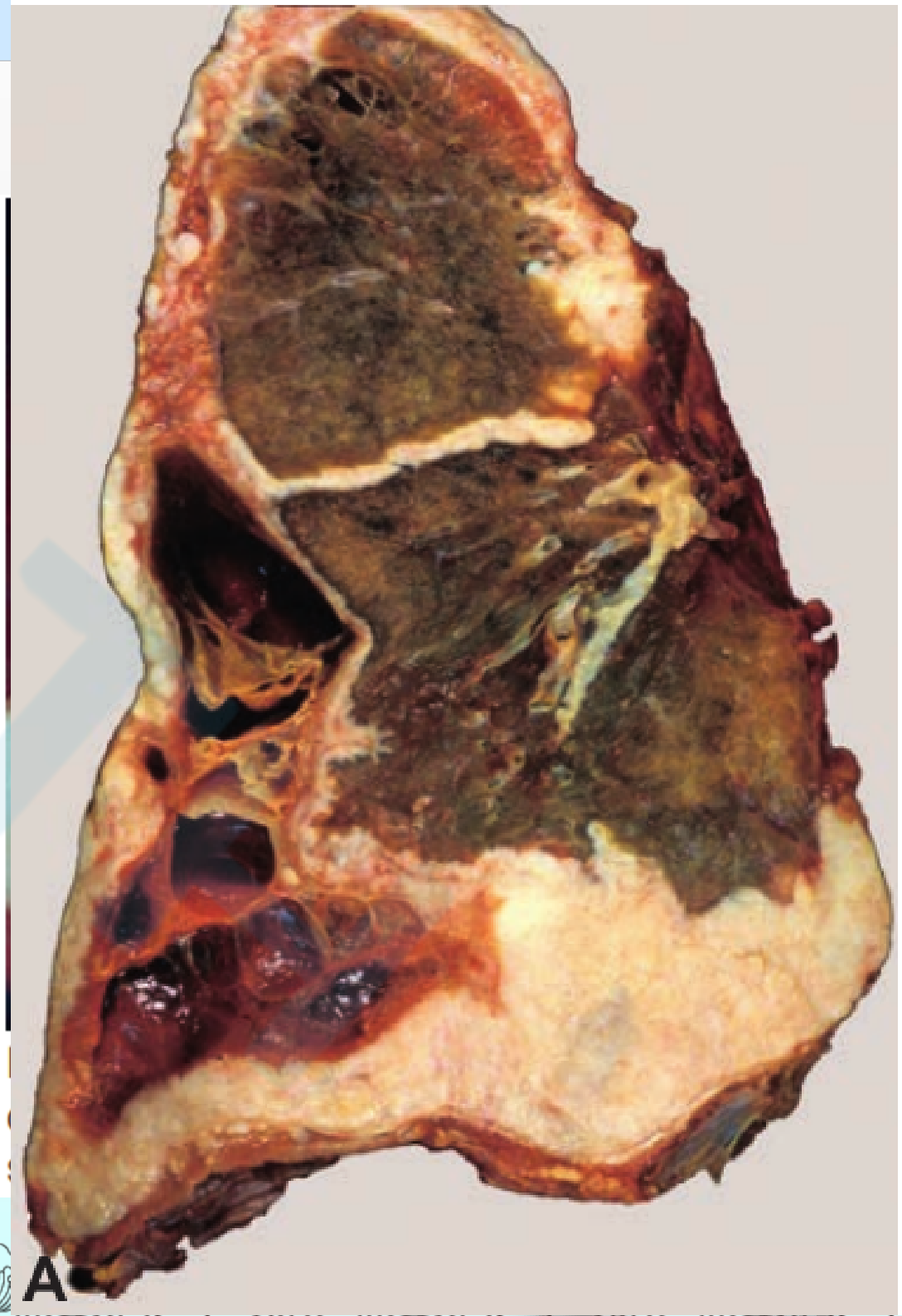
Diffuse malignant mesothelioma

-Epithelioid mesothelioma

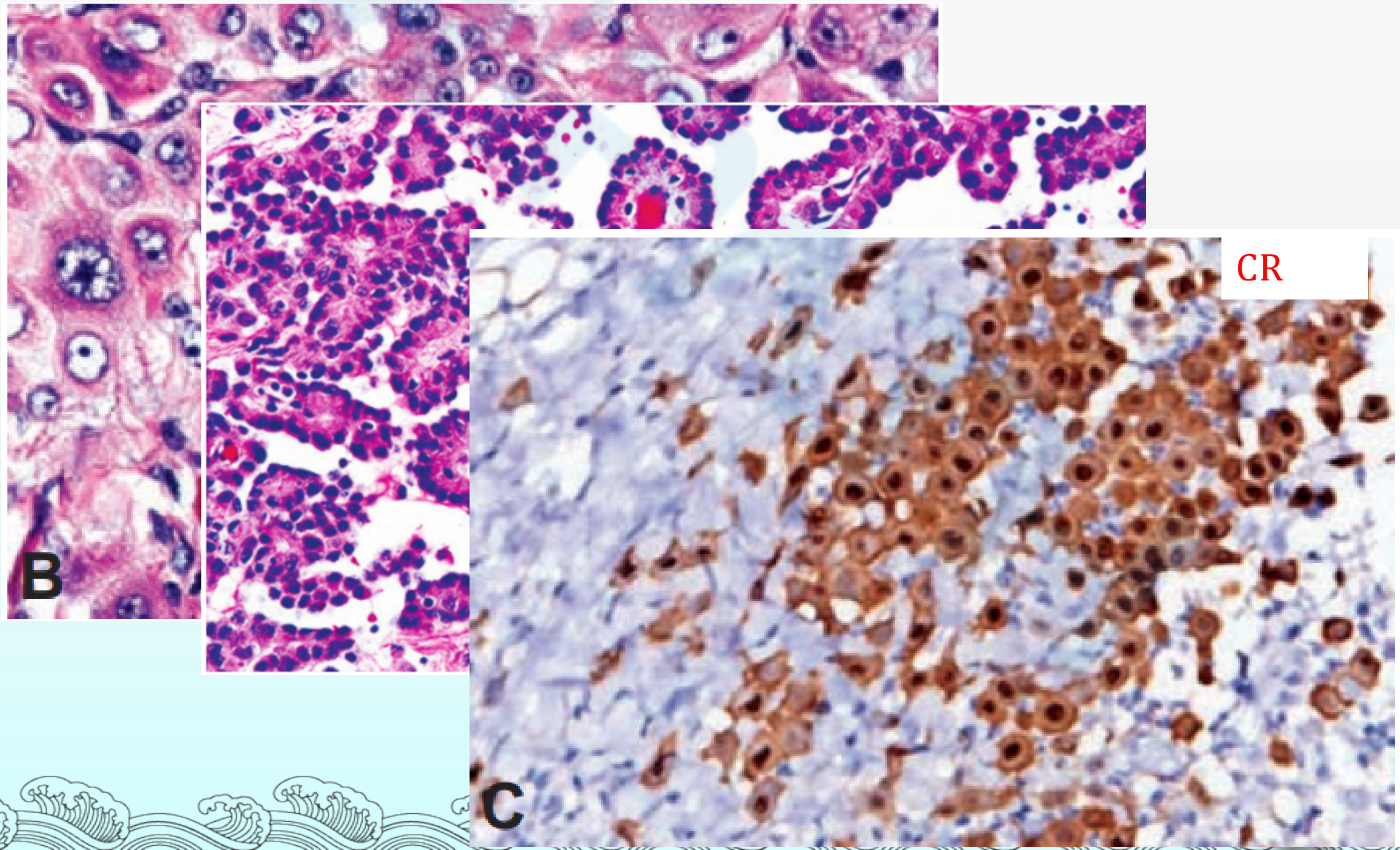
- **ICD-O code** 9052/3
- Epithelioid malignant mesothelioma; epithelial-type mesothelioma
- occur over a very wide age range. occasionally occur in children, but the vast majority of tumours are seen in patients aged ⩾ 60 years
- male:female ratio is on average 4:1
- Approximately 60–80% of malignant mesotheliomas are of the epithelioid type
- Most common symptoms are insidious onset of chest pain and/or dyspnoea , A chest wall mass, weight loss, abdominal pain, and ascites are less common presentations

Epithelioid malignant mesothelioma

- Early mesothelioma presents as small nodules distributed on the parietal and (less commonly) on the visceral pleura
- As mesothelioma progresses, the nodules coalesce to form a rind of tumour encasing the lung

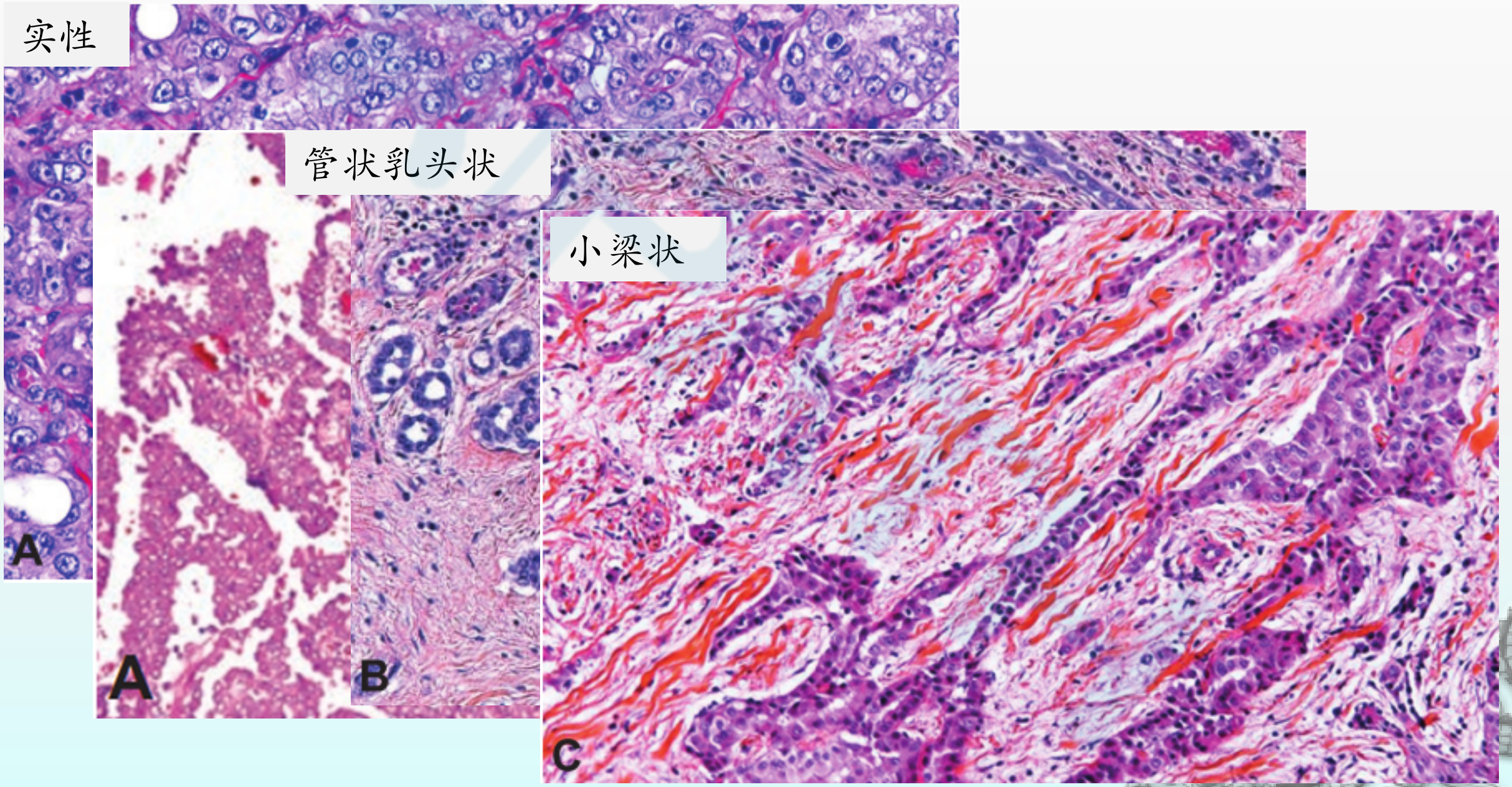


Epithelioid malignant mesothelioma



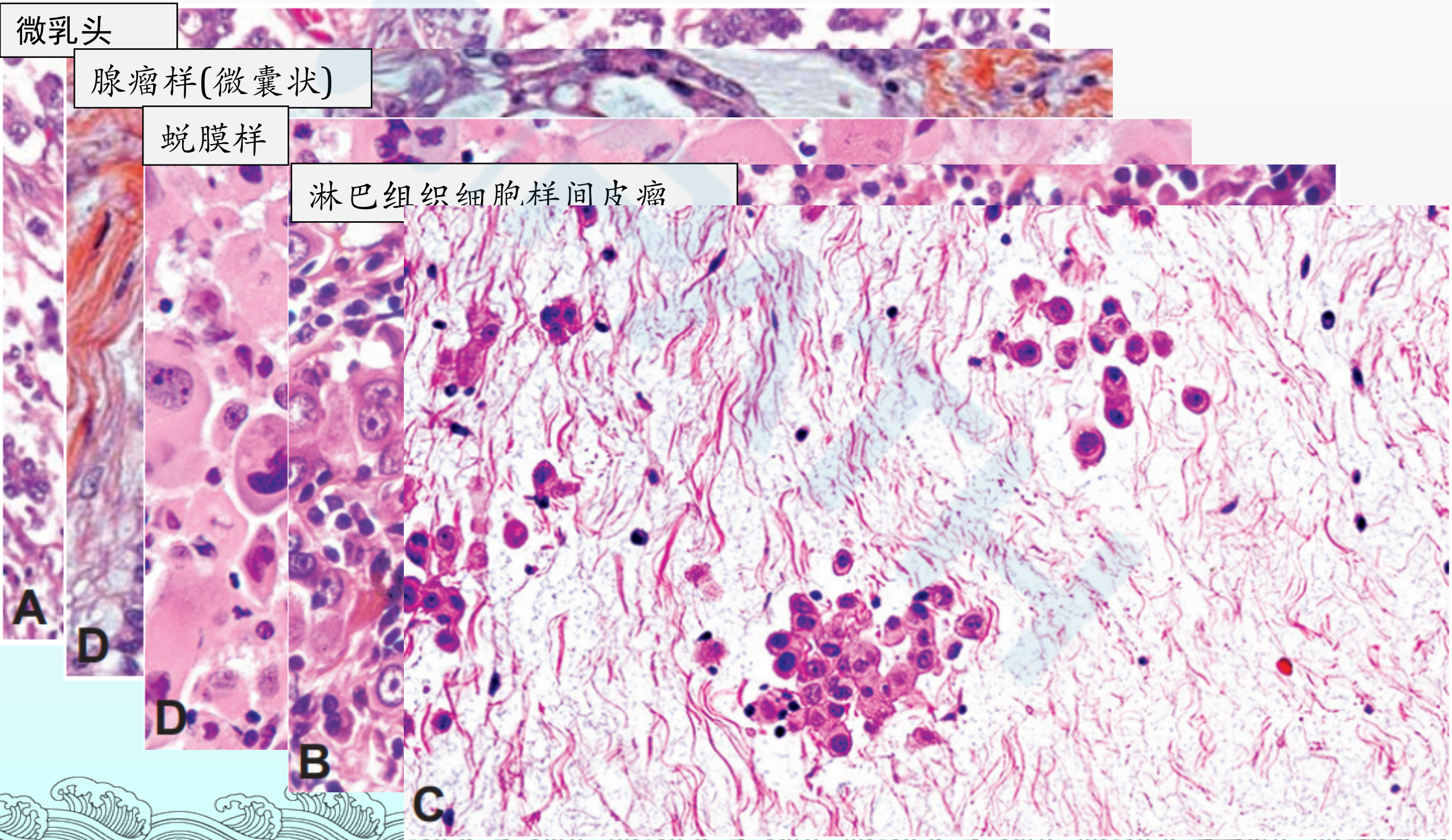
Epithelioid malignant mesothelioma

(most commonly histologic morphologic patterns)



Epithelioid malignant mesothelioma

(Less commonly histologic morphologic patterns)



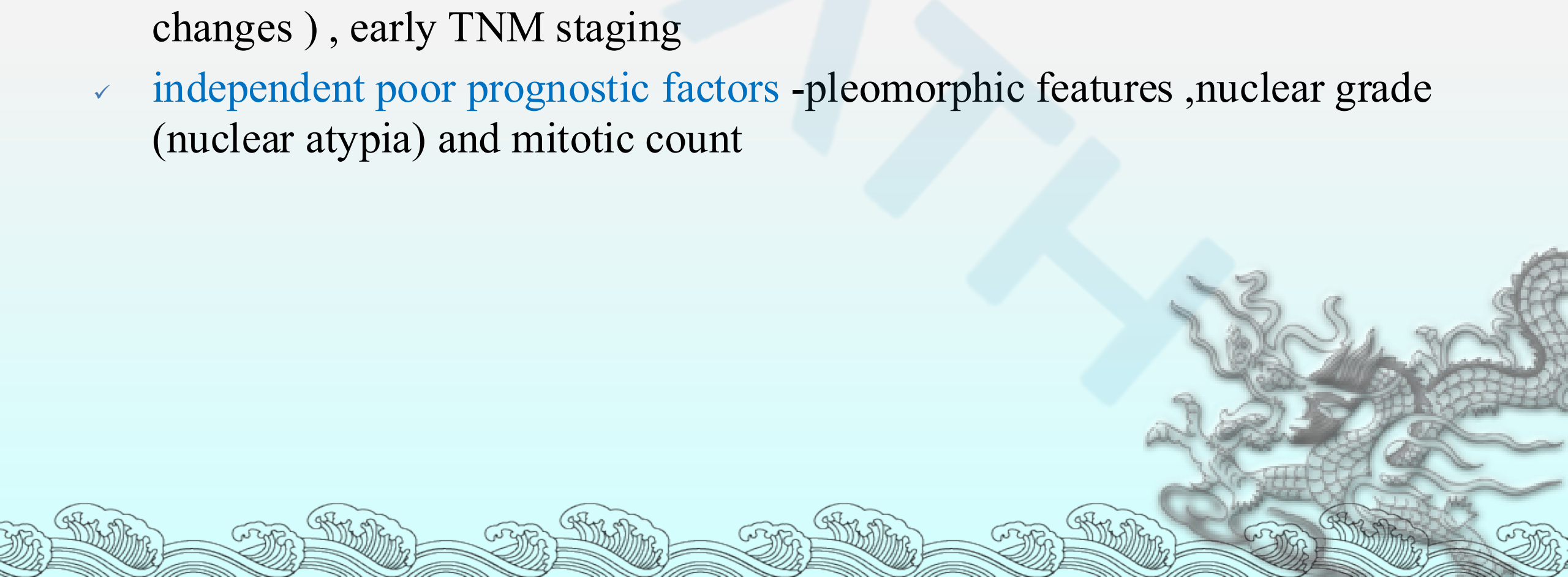
Epithelioid malignant mesothelioma

Mesothelial markers		
Markers	**Sensitivity**	**Specificity versus lung adenocarcinoma**
Calretinin	> 90%	90–95%
CK5/6	75–100%	80–90%
WT1	70–95%	~100%
D2-40	90–100%	85%

- ✓ The International Mesothelioma Interest Group (IMIG) recommends calretinin, podoplanin (D2-40), and Wilms' tumor 1 (WT1) as mesothelioma markers
- ✓ 至少联合使用两种间皮标志物

Epithelioid malignant mesothelioma

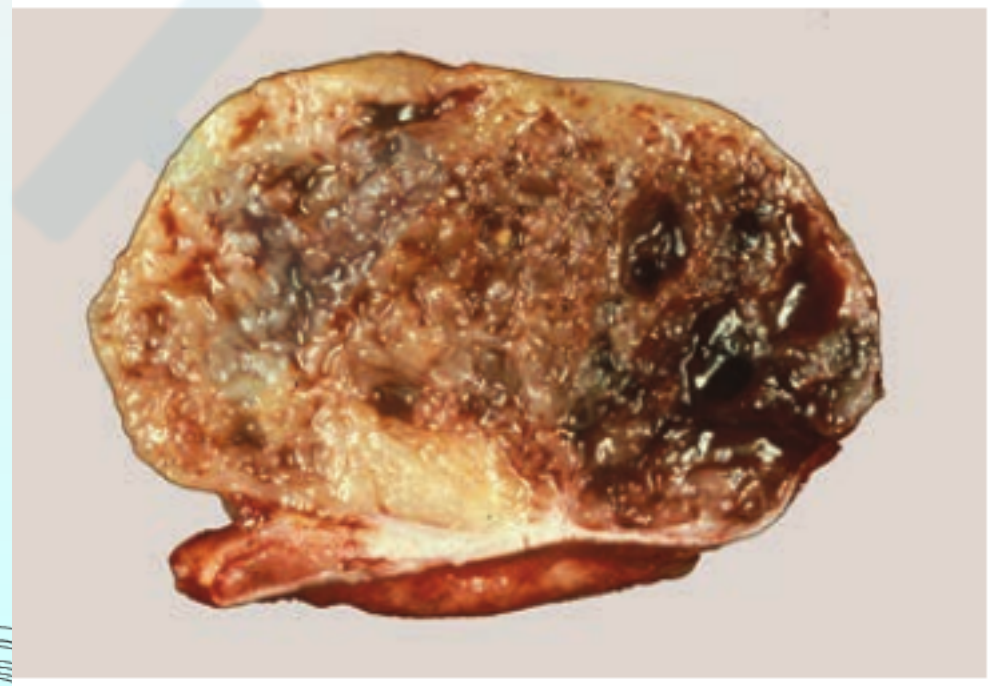
- Chromosomal losses are more common than gains
  - ✓ The most common losses： 1p, 3p, 4q, 6q, 9p, 13q, 14q, 22q
  - ✓ The most common gains： 1q, 5p, 7p, 8q, and 17q
- The long-term survival rate of patients is poor
  - ✓ good prognostic factors -younger age, epithelioid type(abundant myxoid changes) , early TNM staging
  - ✓ independent poor prognostic factors -pleomorphic features ,nuclear grade (nuclear atypia) and mitotic count



Localized malignant mesothelioma

-Epithelioid mesothelioma

- Localized mesothelioma; solitary malignant mesothelioma
- very rare; fewer than 50 cases have been reported. There is a slight male predisposition, and the mean age is between 60 and 65 years
- incidental finding. Patients may present with chest pain, dyspnoea, malaise, fever, or night sweats
- solitary,circumscribed, pleural-based mass attached to the visceral or parietal pleura
- have a better prognosis than diffuse mesotheliomas, and may be cured by surgical excision



Lung adenocarcinomas

- a malignant epithelial tumour with glandular differentiation, mucin production, or pneumocyte marker expression
- Tobacco smoking, radon in indoor environments and mines, other occupational agents, and outdoor air pollution
- Mortality and incidence rates have generally been highest in highincome countries, particularly the United States and European countries, but are now declining, particularly in younger males and females

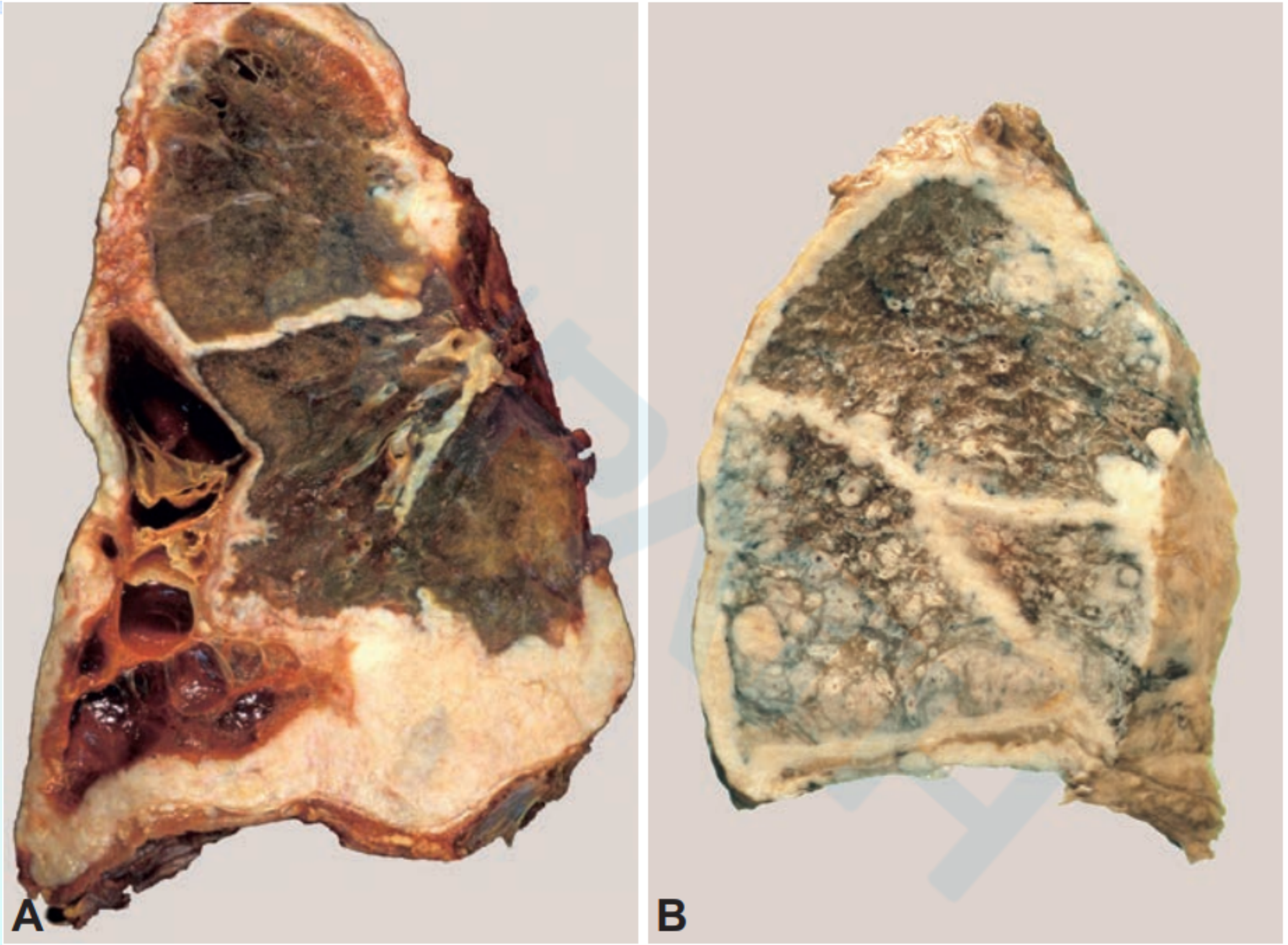
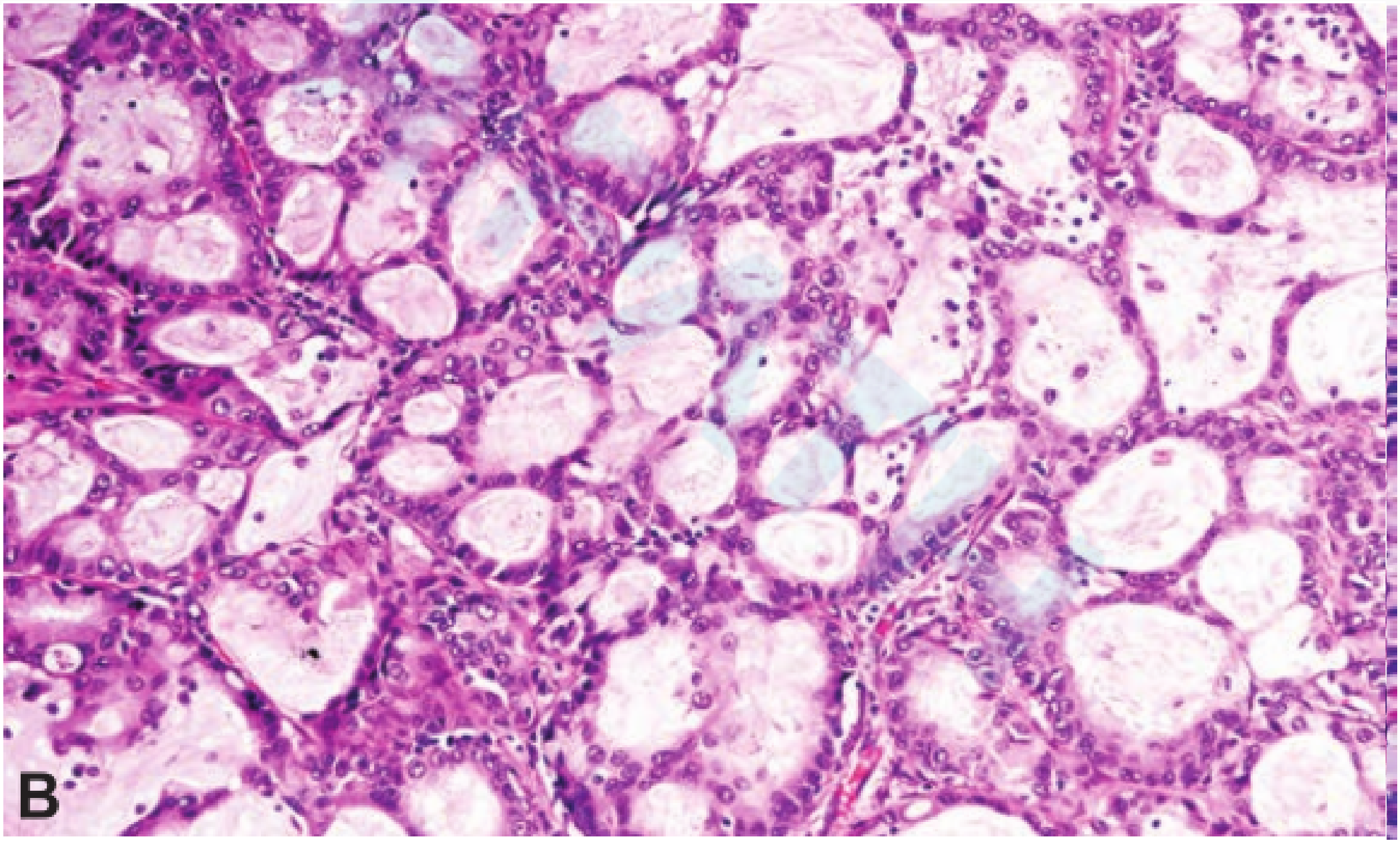
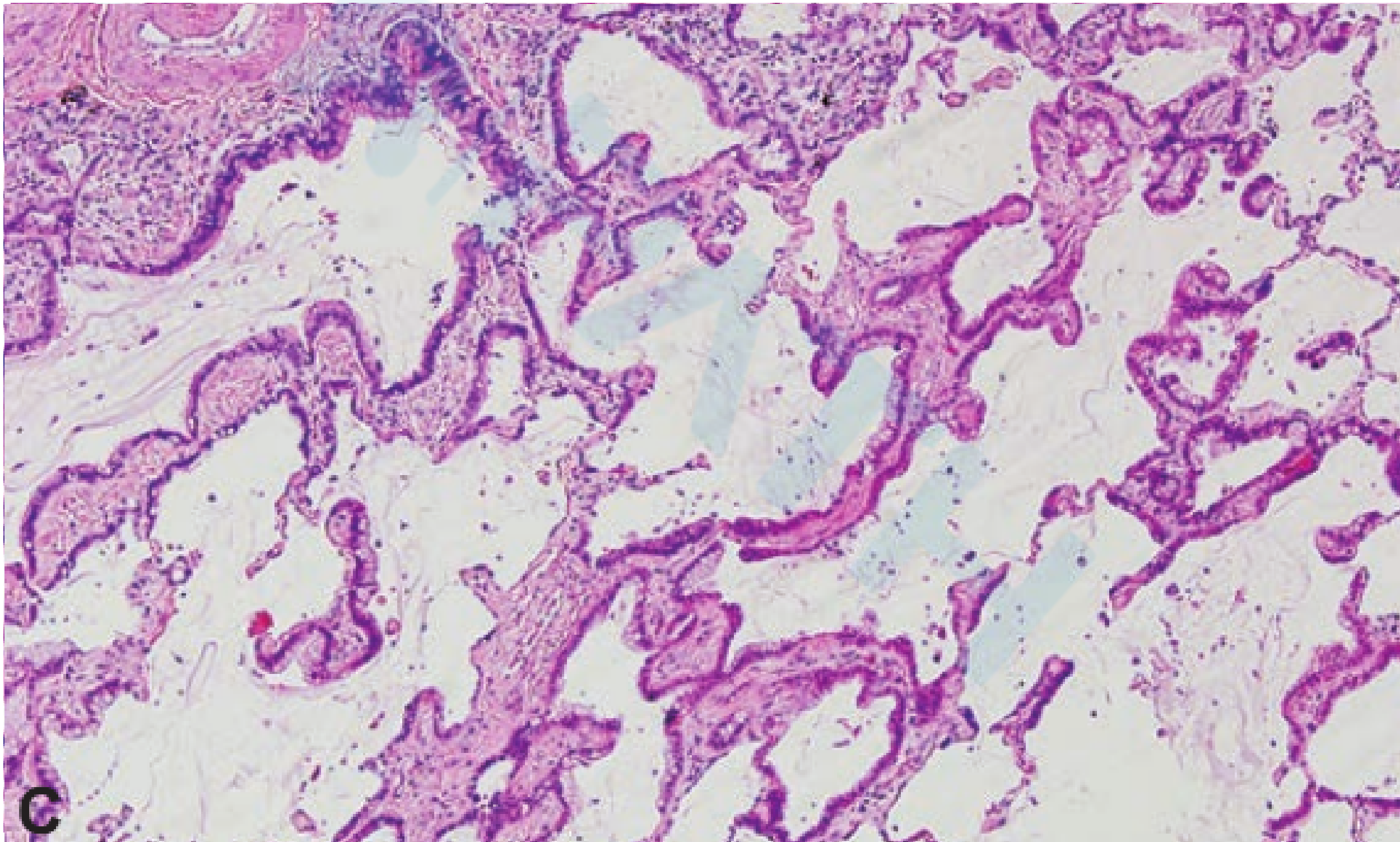


Fig. 2.05 **A** Diffuse malignant mesothelioma. The tumour encases the lung as a rind and grows along the interlobar septa, compressing the lung parenchyma. Reprinted from Galateau-Salle F {785}. **B** Pseudomesotheliomatous adenocarcinoma. The tumour encases the lung, mimicking a malignant mesothelioma. Unlike malignant mesothelioma,

Lung adenocarcinomas



Lung adenocarcinomas



- Our previous study that lung adenocarcinoma was most frequently misdiagnosed as malignant mesothelioma
- The IMIG recommends 3 mesothelioma markers,their use has improved diagnostic accuracy. However, their sensitivity and specificity for differentiating epithelioid mesothelioma from lung adenocarcinoma are not ideal
- In this study, we identified high expression levels of sex-determining region Y box 6 (SOX6) in epithelioid mesothelioma relative to lung adenocarcinoma

SOX6 (sex-determining region Y box 6)

- SOX6 is a protein that binds DNA through a highly conserved high-mobility group domain and belongs to the D subfamily of sex-determining region Y-related transcription Factors
- Recent studies have revealed that SOX6 is a tumor suppressor and is down regulated in multiple cancers,including esophageal squamous cell carcinoma, hepatocellular carcinoma, chronic myeloid leukemia, and ovarian cancers

MATERIALS AND METHODS

- **Transcriptome Analysis of Microarray Gene Expression Data**

reanalyzed previous microarray gene-expression data from 6 epithelioid mesothelioma and 6 lung adenocarcinoma samples, to identify transcripts with a >2-fold difference in expression between the 2 tumor types

- **Patients and Histologic Samples**

(the Department of Pathology, Hiroshima University archives, between 2007 and 2016)

- ✓ Fifty-four epithelioid mesothelioma （underwent video-assisted thoracoscopic biopsy,pleurectomy, decortication, or extrapleural Pneumonectomy.）
- ✓ Sixty-nine lung adenocarcinoma （randomly selected from patients who were surgically resected）
- ✓ All specimens were evaluated and confirmed independently by 3 pathologists

- **Immunohistochemistry and Evaluation of SOX6 Expression**

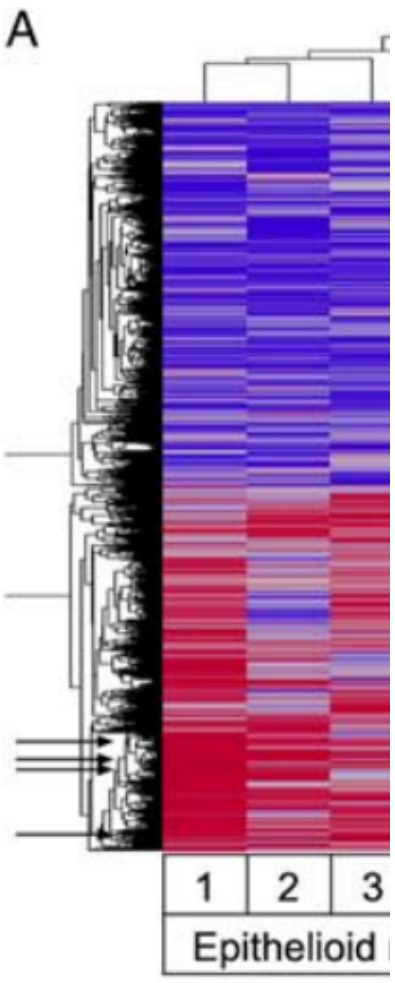
SOX6 （ (clone: A-4) positive (nuclear expression ,0 for no expression, 1+for <10%, 2+ for 10% to 50%, 3+ for >50%)

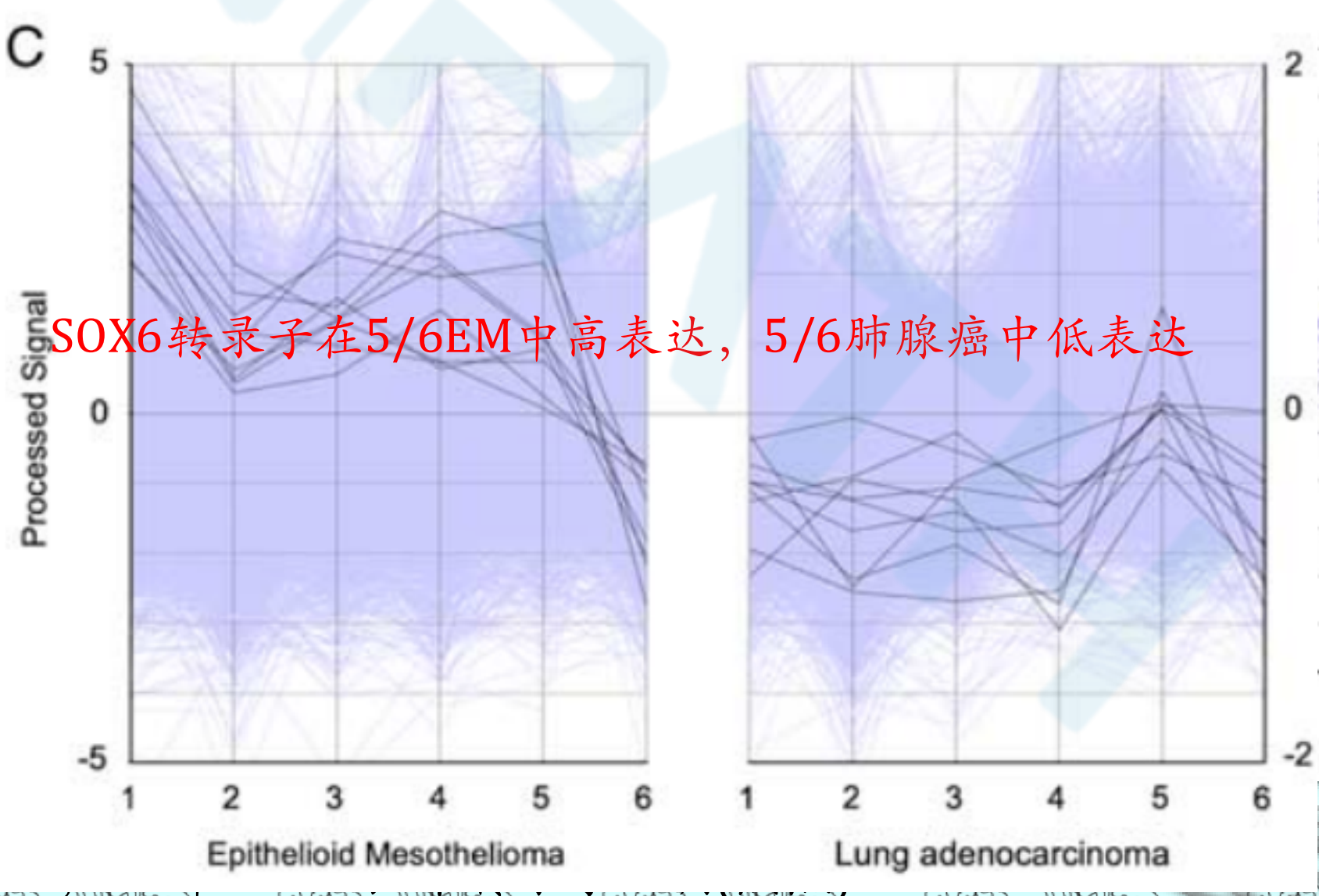
Calretinin,D2-40, WT1, CEA, claudin 4,TTF-1, napsin A

- **Statistical Analysis**

RESULTS

- **Differential Gene Expression Between Epithelioid Mesothelioma and Lung Adenocarcinoma**





SOX6转录子在5/6EM中高表达，5/6肺腺癌中低表达

A:EM中3278

B:与肺腺癌相

RESULTS

- **SOX6 ,Calretinin, D2-40, and WT1 Expression in Epithelioid Mesothelioma and Lung Adenocarcinoma**

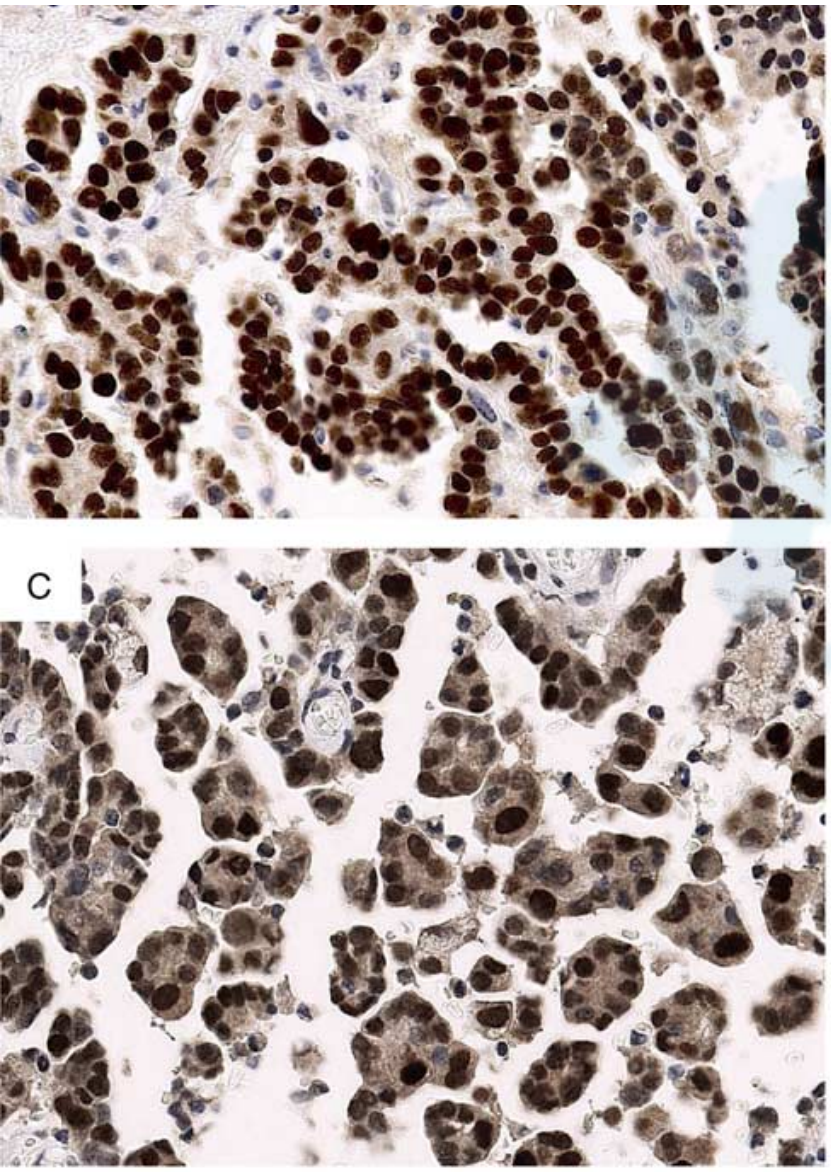
TABLE 1. Immunohistochemical Findings for Epithelioid Mesothelioma and Lung Adenocarcinoma

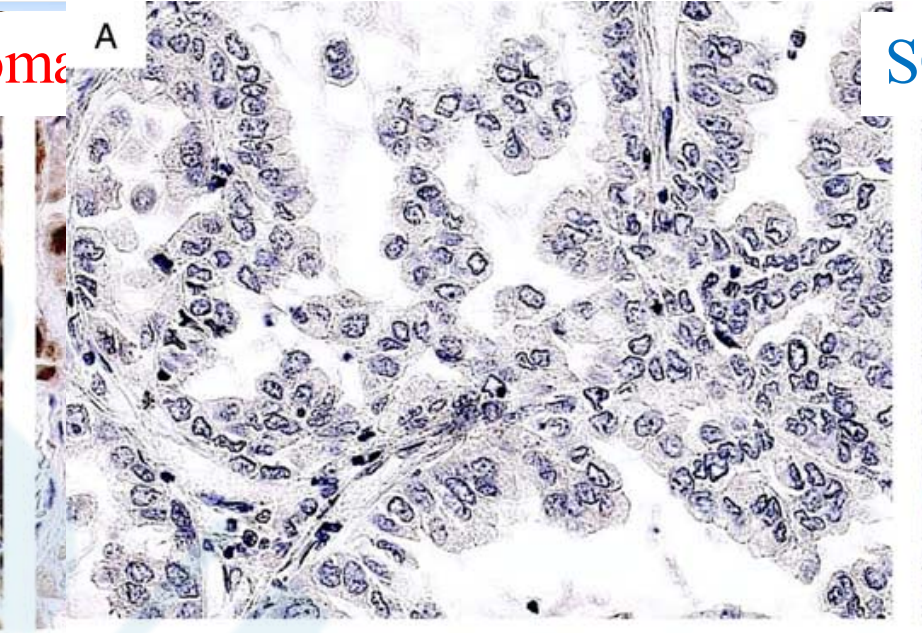
Epithelioid Mesothelioma (54 Cases)						Lung Adenocarcinoma (69 Cases)					
		Immunohistochemical Score*						Immunohistochemical Score*			
Marker	No. Positive Cases, n (%)	0	1+	2+	3+	Marker	No. Positive Cases, n (%)	0	1+	2+	3+
SOX6 核	53 (98)	1	0	5	48	SOX6	5 (7)	64	2	3	0
Calretinin 浆/核	53 (98)	1	4	0	49	Calretinin	15 (22)	54	9	6	0
D2-40 膜	53 (98)	1	2	6	45	D2-40	7 (10)	62	4	3	0
WT1 核	42 (78)	12	10	3	29	WT1	0	69	0	0	0

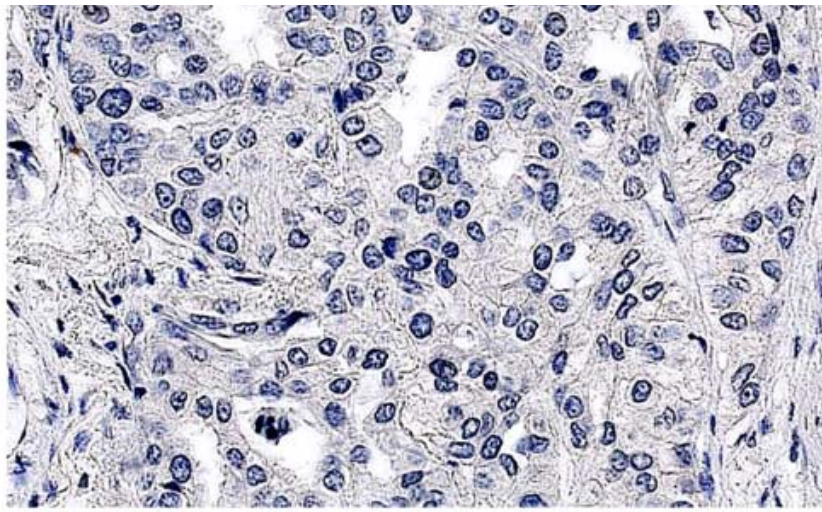
*0, negative; 1+, <10%; 2+, 10% to 50%; 3+, <50% of tumor cells with immunoreactivity.

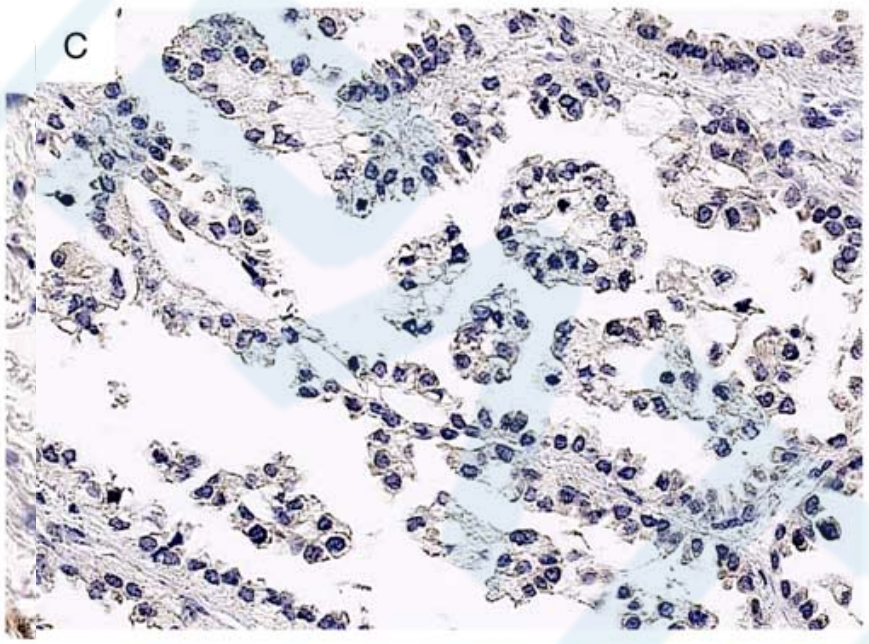
SOX6 in epithelioid mesothelioma

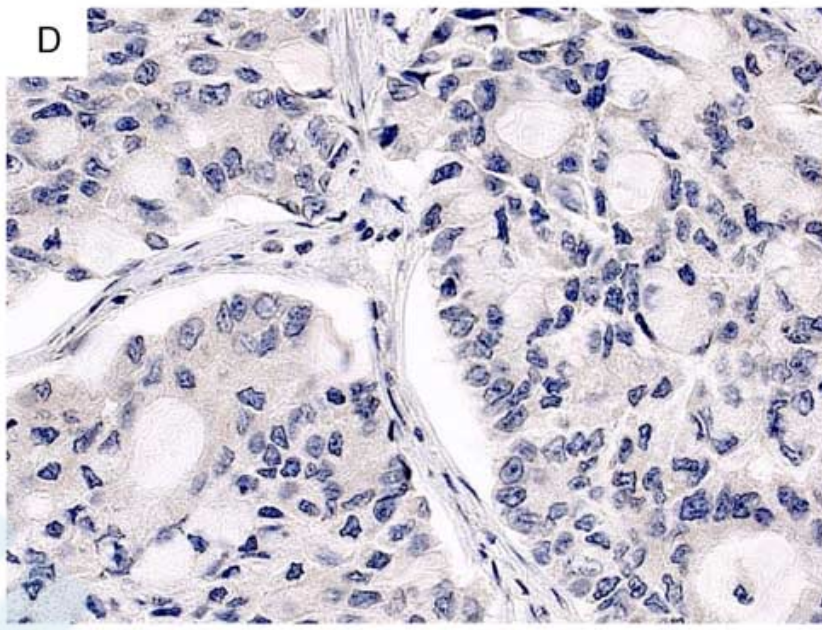
SOX6 in lung adenocarcinoma

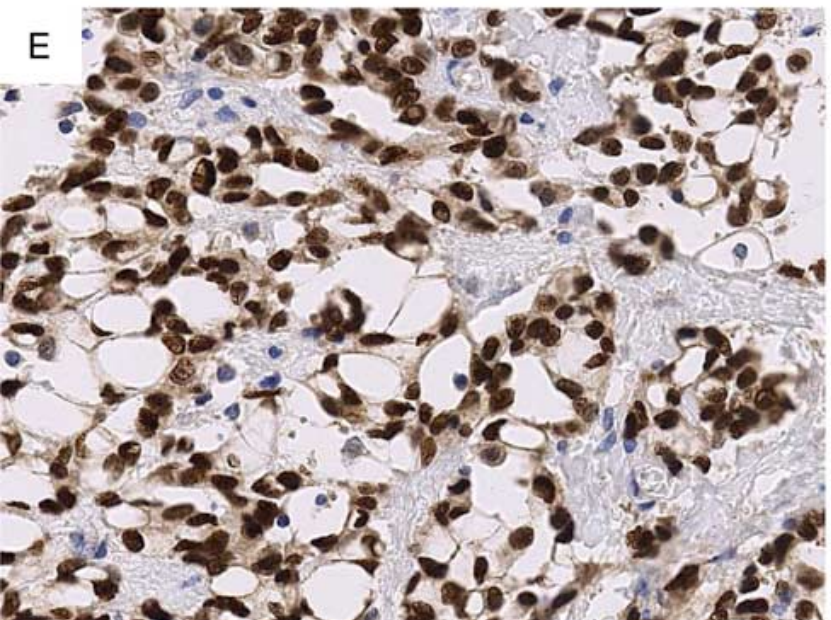


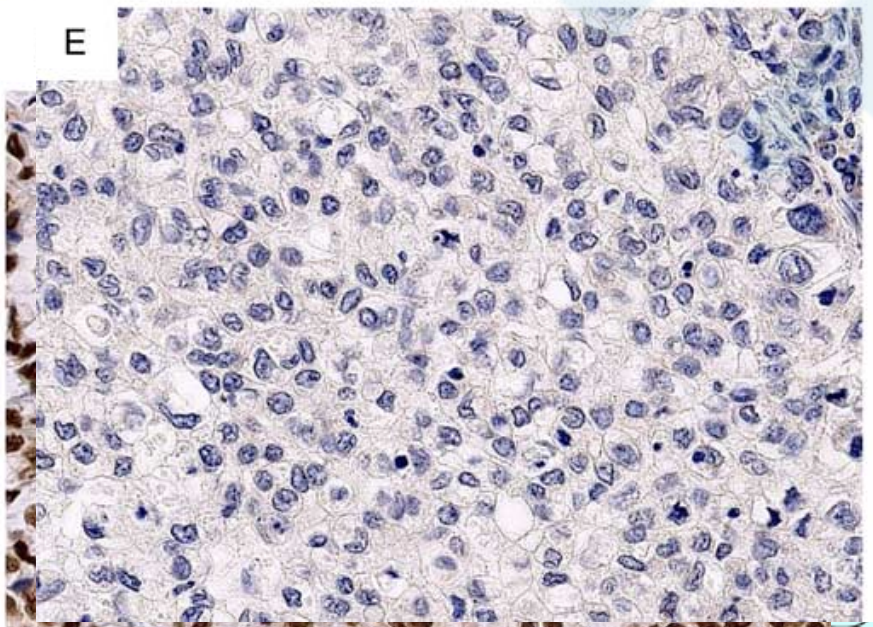


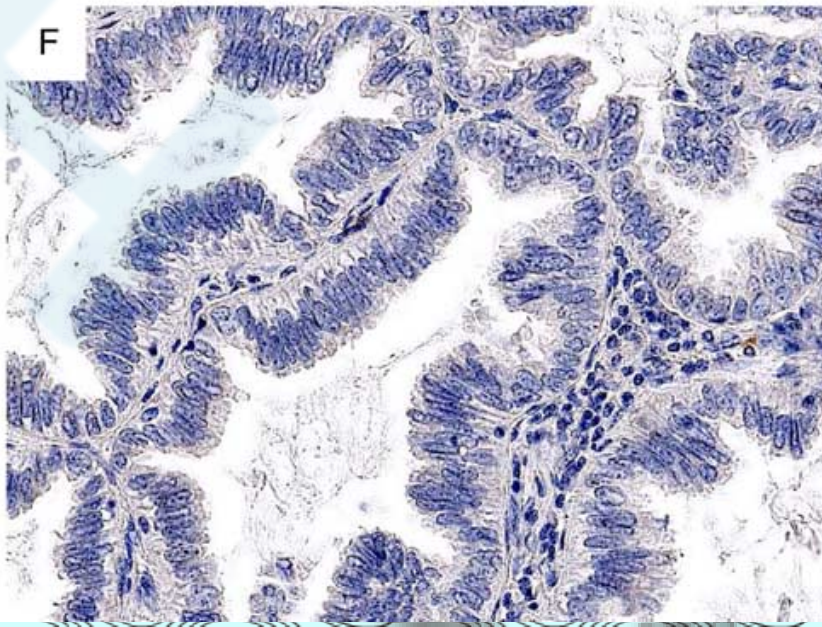












RESULTS

- **Sensitivity, Specificity, PPV,NPV of Immunohistochemical Markers for Differentiation of Epithelioid Mesothelioma From Lung Adenocarcinoma**

TABLE 2. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of Immunohistochemical Markers for Differentiation of Epithelioid Mesothelioma From Lung Adenocarcinoma

Immunohistochemical Marker	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SOX6	98	93	91	98
Calretinin	98	78	78	98
D2-40	98	90	88	98
WT1	80	100	100	85
Intelectin-1*	76	100	97	81
DAB2*	80	97	100	79
Glypican-1†	100	97	96	100

胞浆

Sensitivity and specificity values were copied from our previous publications and PPV and NPV were calculated using the results in these publications.

*Kuraoka et al.[9]

†Amatya et al.[18]

RESULTS

- **Sensitivity and Specificity of Combinations of 2 Markers**

TABLE 3. Sensitivity and Specificity of Marker Combinations for Differentiation of Epithelioid Mesothelioma From Lung Adenocarcinoma

Immunohistochemical Markers			Sensitivity (%)	Specificity (%)
SOX6	AND/OR	Calretinin	100	97
SOX6	AND/OR	D2-40	100	100
SOX6	AND/OR	WT1	100	100
Calretinin	AND/OR	D2-40	100	96
Calretinin	AND/OR	WT1	98	100
D2-40	AND/OR	WT1	100	100
SOX6	AND	Calretinin	96	74
SOX6	AND	D2-40	94	83
SOX6	AND	WT1	78	93
Calretinin	AND	D2-40	94	72
Calretinin	AND	WT1	80	93
D2-40	AND	WT1	80	90

- SOX6+ other Markers (Calretinin, D2-40, WT1) > any combinations lacking SOX6
- sensitivity (96%)--SOX6 + calretinin
- specificity(93%) --SOX6 + WT1

DISCUSSION

- The IMIG guidelines do not show ideal sensitivity or specificity. the specificities of calretinin (90% to 95%), D2-40 (85%), and WT1 (70% to 95%)
- WT1 is the only positive nuclear immunohistochemical marker for epithelioid mesothelioma
- we focused on SOX6 as a nuclear marker of epithelioidmesothelioma
- This is the first report of SOX6 immunohistochemical reactivity in mesothelioma

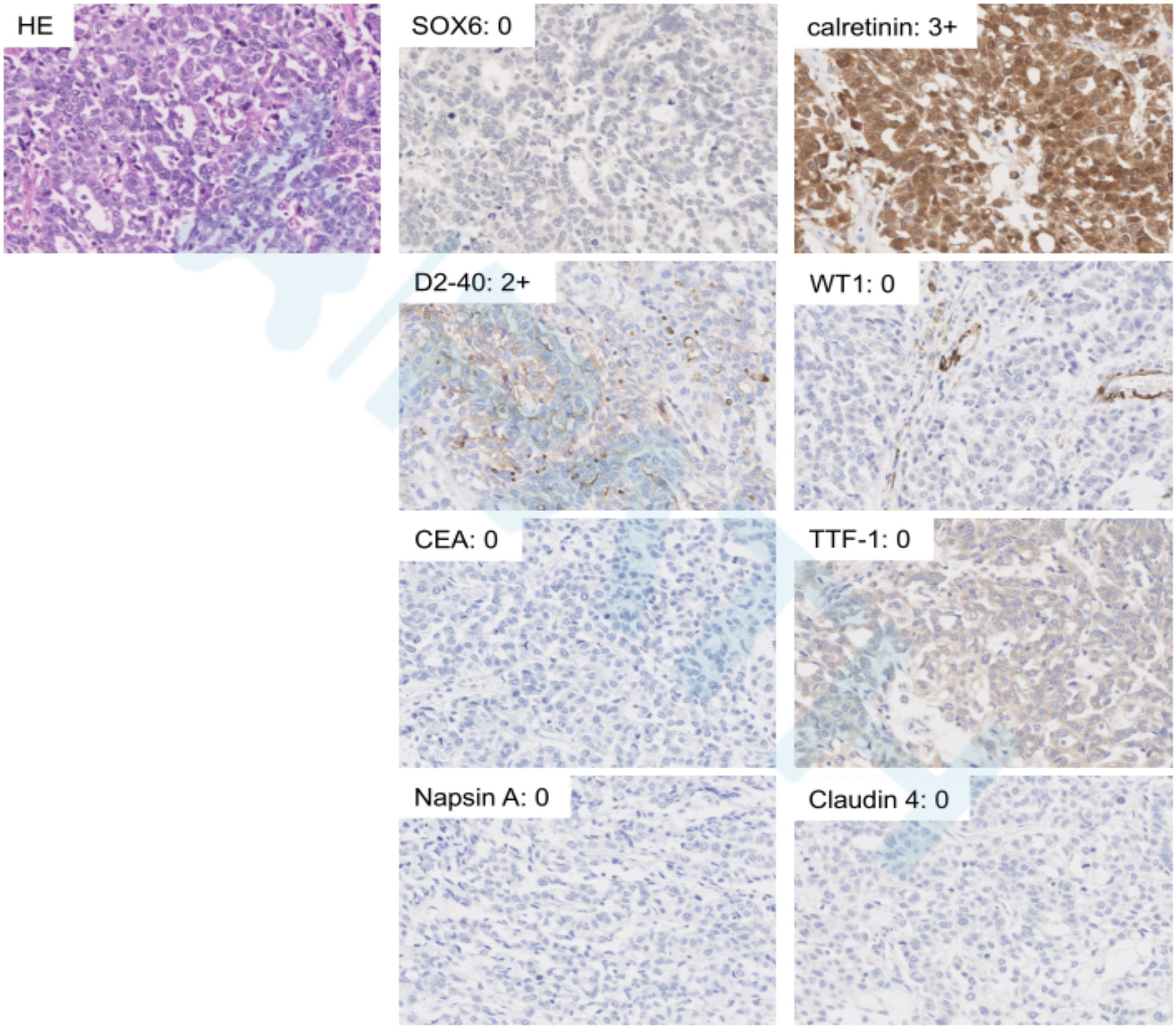
DISCUSSION

- 1 case of EM negative for SOX6 expression and 5 cases of lung adenocarcinoma positive for SOX6 expression. including the positive markers for lung adenocarcinoma: CEA,TTF-1, napsin A, and claudin 4

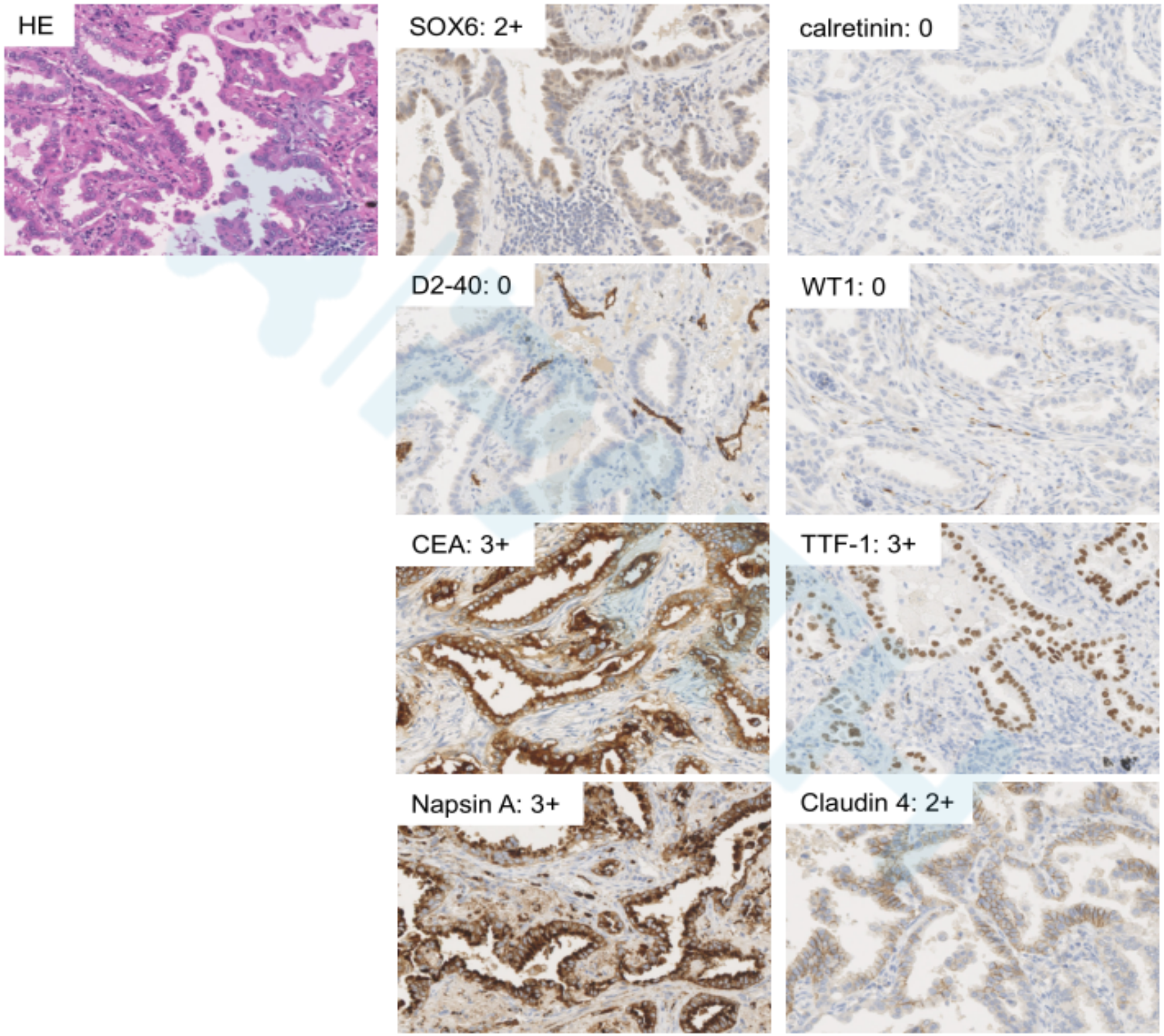
TABLE 4. Immunohistochemical Findings of 1 Case of SOX6-negative Epithelioid Mesothelioma and 5 SOX6-positive Lung Adenocarcinoma Cases, Including Positive Markers for Lung Adenocarcinoma

	SOX6	Calretinin	D2-40	WT1	CEA	TTF-1	Napsin A	Claudin 4
EM1	0	3	2	0	0	0	0	0
LAC1	1	0	0	0	3	3	3	2
LAC2	2	2	0	0	3	2	3	3
LAC3	2	0	0	0	3	3	3	2
LAC4	2	0	0	0	3	3	1	2
LAC5	1	1	0	0	3	3	3	3

Supplementary Figure 1. Immunohistochemical results for each marker of 1 epithelioid mesothelioma case, EM1, negative for SOX6. Immunoreactivity scores of 0 or 3+ are shown on each image.



Supplementary Figure 2. Immunohistochemical results for each marker representing 1 of 5 lung adenocarcinoma cases. LAC3 was positive for SOX6. Immunoreactivity scores of 0, 2+, or 3+ are shown on each image.



	SOX6	Calretinin	D2-40	WT1	CEA	TTF-1	Napsin A	Claudin 4
EM2	3	0	3	0	0	0	0	0
EM3	3	1	0	1	0	0	0	0
EM4	3	3	1	0	0	0	0	0
EM5	3	1	2	0	0	0	0	0
EM6	3	1	3	0	0	0	0	0
EM7	3	3	3	0	0	0	0	0
EM8	3	3	3	0	0	0	0	0
EM9	3	3	3	0	0	0	0	0
EM10	3	3	3	0	0	0	0	0
EM11	2	3	3	0	0	0	0	0
EM12	3	3	3	0	0	0	0	0
EM13	2	3	3	0	0	0	0	0
LAC6	0	2	0	0	3	3	3	2
LAC7	0	2	0	0	3	3	3	2
LAC8	0	2	0	0	3	3	3	3
LAC9	0	2	0	0	3	0	3	3
LAC10	0	2	0	0	3	3	1	3
LAC11	0	1	0	0	2	3	3	1
LAC12	0	1	2	0	3	3	3	3
LAC13	0	1	0	0	1	3	1	2
LAC14	0	1	0	0	1	3	0	3
LAC15	0	1	1	0	1	3	3	3
LAC16	0	1	2	0	3	3	1	2
LAC17	0	1	0	0	2	3	0	3
LAC18	0	1	0	0	2	2	0	3
LAC19	0	0	2	0	2	3	3	3
LAC20	0	0	1	0	3	3	3	3
LAC21	0	0	1	0	1	3	3	3
LAC22	0	0	1	0	3	2	3	2

EM: epithelioid mesothelioma; LAC: lung adenocarcinoma

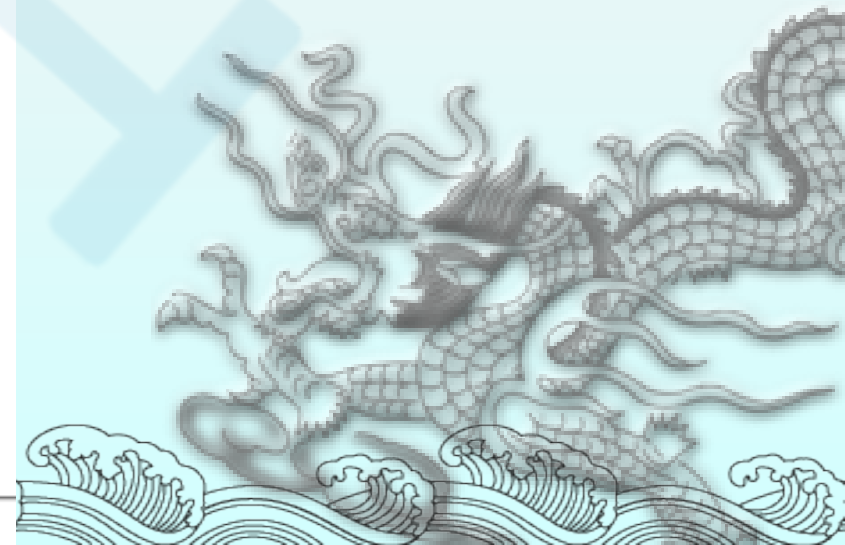
12例CR，D2-40，WT1阴性的上皮样间皮瘤及17 例CR和或 D2-40 阳性肺腺癌

·12例EM中间皮标记阴性病例

·17例肺腺癌间皮标记阳性病例

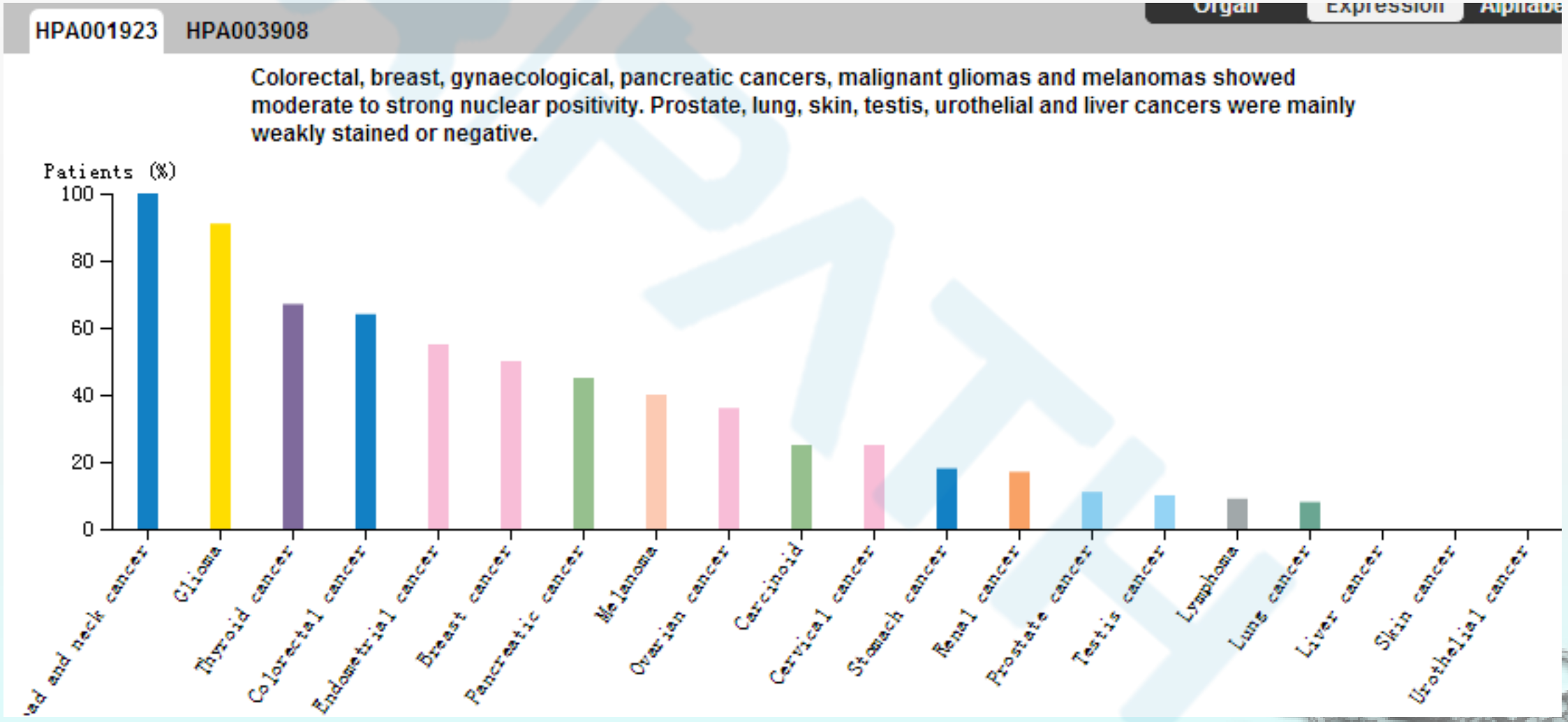
·17例肺腺癌中不表达TTF-1、NapsinA病例

•SOX6 detected true epithelioid mesotheliomas with exceptional immunohistochemical staining patterns.



DISCUSSION

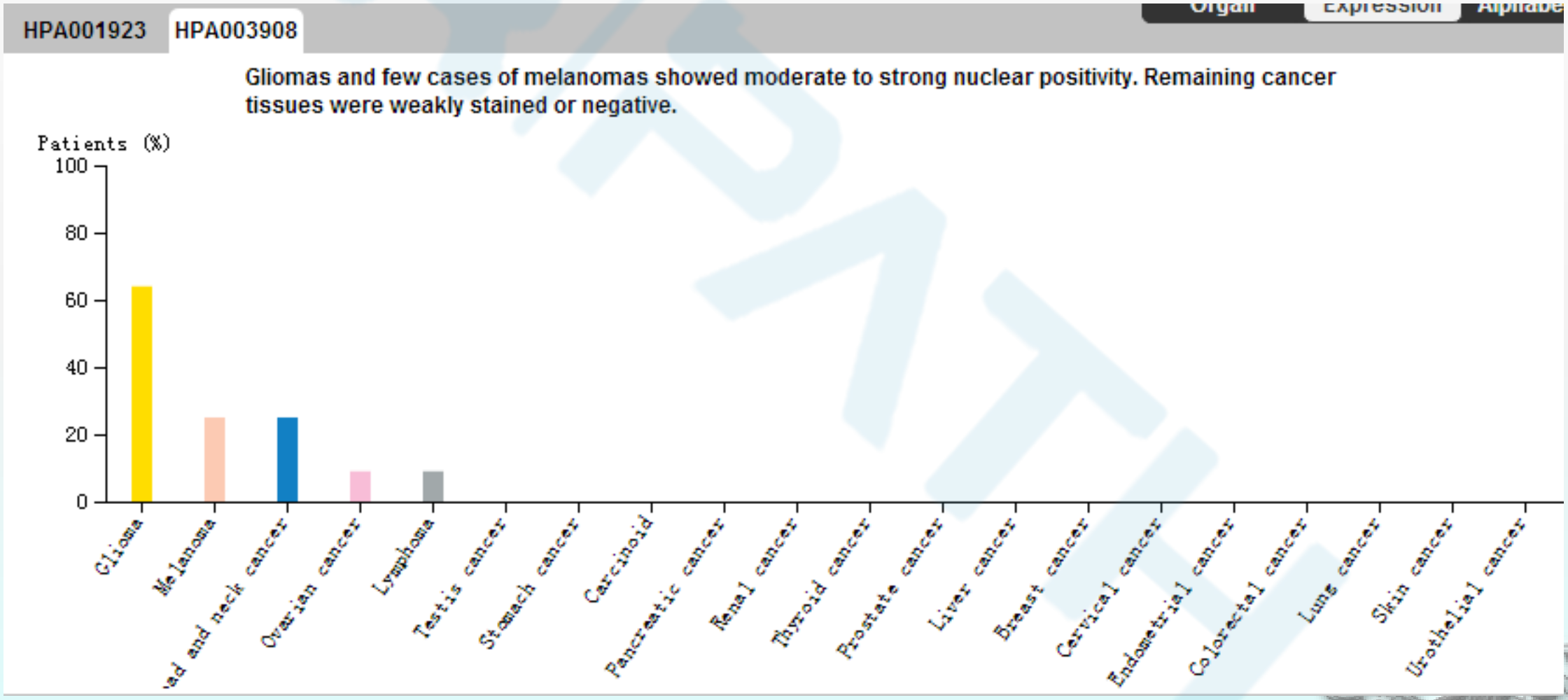
- **SOX6 expression in metastatic lung carcinoma**



SOX6中度至强核阳性肿瘤：结直肠癌、乳腺癌、卵巢癌、胰腺癌、恶性胶质瘤和黑素瘤
SOX6弱阳性或阴性肿瘤：前列腺癌、肺癌、皮肤癌、睾丸癌、尿路上皮癌和肝癌

DISCUSSION

- **SOX6 expression in metastatic lung carcinoma**



SOX6中度至强核阳性：胶质瘤、黑素瘤、头颈癌，其次卵巢癌、淋巴瘤
剩余的器官的癌组织阴性

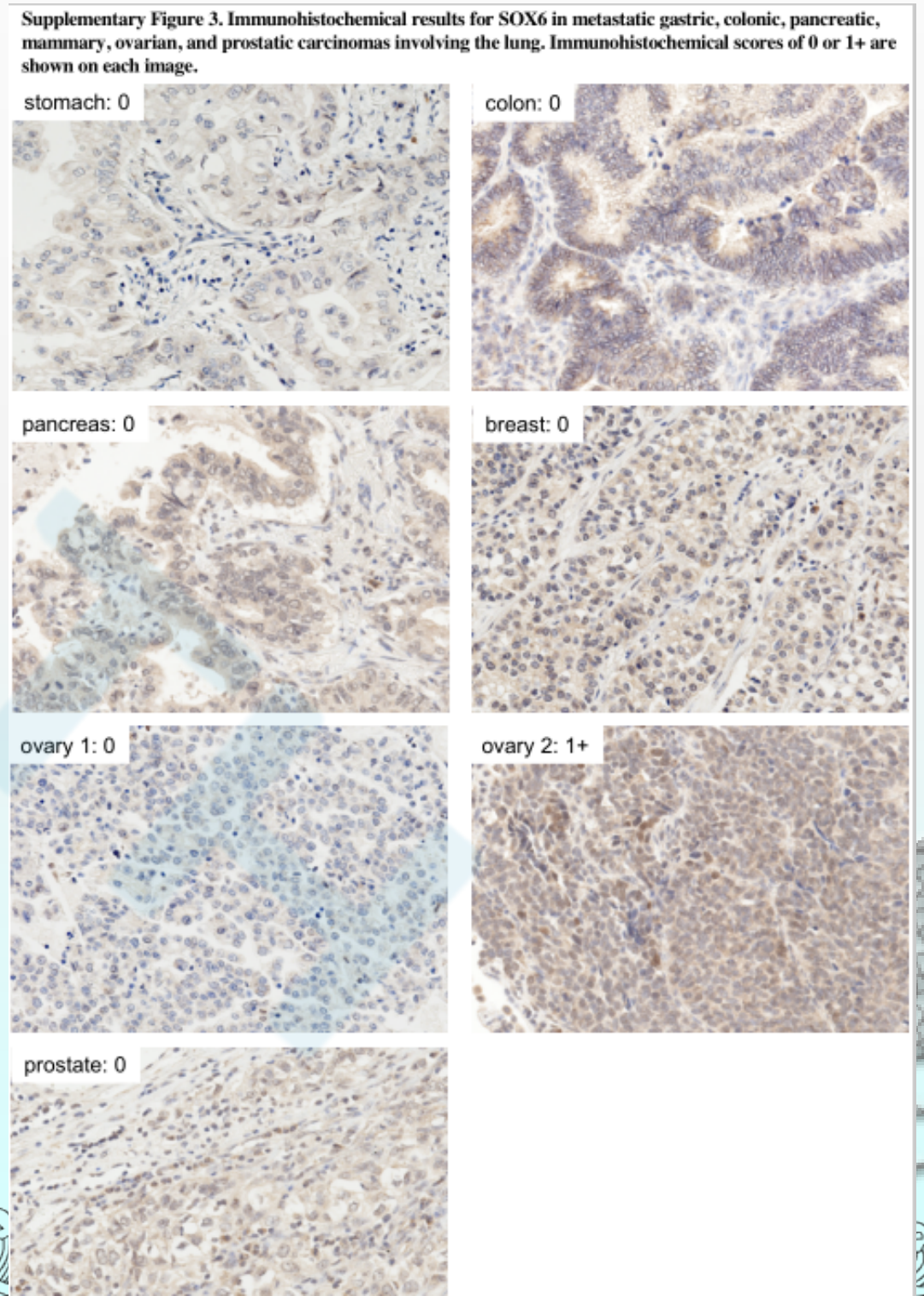
DISCUSSION

- **SOX6 expression in metastatic lung carcinoma**

✓ stomach (3 cases), colon (3 cases), pancreas (3cases),breast (3 cases), ovary (2 cases), prostate (1 case）

✓ Almost all of these cases were negative for SOX6, except for 1 ovarian carcinoma that showed focal nuclear positivity

SOX6 may be useful in the differentiation of epithelioid mesothelioma from metastatic lung carcinomas from multiple organs. Further detailed analyses with more cases are needed to reach conclusions about its utility



Supplementary Figure 3. Immunohistochemical results for SOX6 in metastatic gastric, colonic, pancreatic, mammary, ovarian, and prostatic carcinomas involving the lung. Immunohistochemical scores of 0 or 1+ are shown on each image.

DISCUSSION

- **SOX6 expression in 7 cases of sarcomatoid mesothelioma, 1 case of biphasic mesothelioma, and 15 cases of pleomorphic carcinoma of the lung**
- ✓ 2/7 sarcomatoid mesotheliomas showed SOX6 expression
- ✓ Biphasic mesothelioma showed SOX6 expression in both epithelioid and sarcomatoid components
- ✓ Pleomorphic carcinoma, the carcinomatous component was all negative for SOX6, but in 2/15 cases, sarcomatoid component showed SOX6 expression

SOX6 is involved in the regulation of mesenchymal transformation in mesothelioma cells, but further study is required

CONCLUSION

I. **SOX6 as a novel mesothelioma marker by gene expression microarray analysis of epithelioid mesothelioma and lung adenocarcinoma.**

II. **The differentiation of epithelioid mesothelioma from lung adenocarcinoma**

   ① **SOX6 nuclear staining is a positive**

   ② **SOX6 immunohistochemistry showed high sensitivity and specificity**

III. **Further validation of this marker by others institutes is warranted to verify its practical use**

THANK YOU

Mesothelial markers		
Markers	**Sensitivity**	**Specificity versus lung adenocarcinoma**
Calretinin	> 90%	90–95%
CK5/6	75–100%	80–90%
WT1	70–95%	~100%
D2-40	90–100%	85%
Adenocarcinoma (positive epithelial markers)		
Markers	**Sensitivity**	**Specificity versus malignant mesothelioma**
MOC31	95–100%	85–98%
BerEP4	95–100%	74–87%
BG8 (Lewis Y)	90–100%	93–97%
B72.3	25–85%	> 95%
Monoclonal carcinoembryonic antigen	80–100%	> 95%
Organ specific – lung		
Markers	**Sensitivity**	**Specificity versus malignant mesothelioma**
TTF1 (8G7G3/1)	~80%	High
Napsin A	~80%	High

Epithelioid malignant mesothelioma

Histological features		Atypical mesothelial hyperplasia	Malignant mesothelioma
Major criteria	主要标准		
Stromal i			
Cellularity	细胞	Confined to the pleural surface	Dense, with stromal reaction
Papillae	乳头	Simple, lined by single-cell layer	Complex, with cellular stratification
Growth pattern	生长模式	Surface growth	Expansile nodules, complex and disorganized pattern
Zonation	分区	Process becomes less cellular towards chest wall	No zonation of process, often more cellular away from effusion
Vascularity	血管分布	Capillaries are perpendicular to the surface	Irregular and haphazard
Minor criteria	次要标准		
Cytological atypia	细胞不典型	Confined to areas of organizing effusion	Present in any area, but many cells are deceptively bland and relatively monotonous
Necrosis	坏死	Rare (necrosis may be within pleural exudate)	Necrosis of tumour area is usually a sign of malignancy
Mitoses	核分裂象	Mitoses may be plentiful	Many mesotheliomas show very few mitoses (but atypical mitoses favour malignancy)

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