全　文　要　約

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

(SOX6 は上皮型中皮腫と肺腺癌の鑑別診断における新規免疫組織化学マーカーである)

神原　貴大

(医歯薬保健学研究科　博士課程医歯薬学専攻)

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

Abstract:

The differential diagnosis of epithelioid mesothelioma from lung adenocarcinoma using immunohistochemistry is improving. However, immunohistochemical markers with high sensitivity and specificity have yet to be identified. In this study, we investigated the utility of SOX6 as a novel immunohistochemical marker, identified by analyzing previous gene expression data. Immunohistochemically, SOX6 expression was present in 53 of 54 (98%) cases of epithelioid mesothelioma, compared to its expression in only 5 of 69 (7%) cases of lung adenocarcinoma. The sensitivity and specificity of SOX6 expression for differentiating epithelioid mesothelioma and lung adenocarcinoma were 98 and 93%, respectively. SOX6 expression showed similar sensitivity and far better specificity than those of calretinin or podoplanin (D2-40). In addition, SOX6 expression was more sensitive than Wilms' tumor 1 (WT1) expression. The combination of SOX6 with other markers showed comparable or better sensitivity and specificity relative to other combinations. In particular, the sensitivity of positivity for both SOX6 and calretinin (96%) and the specificity of positivity for both SOX6 and WT1 (93%) were higher than those of the other combinations. In conclusion, SOX6 is a novel candidate immunohistochemical marker for differentiating epithelioid mesothelioma from lung adenocarcinoma.

INTRODUCTION

Malignant mesothelioma is 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.[1] 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.[2] Malignant mesothelioma is subtyped into epithelioid, sarcomatoid, and biphasic forms in the WHO classification, with multiple morphological patterns. Epithelioid mesothelioma shows multiple histological morphological patterns, including tubulopapillary, micropapillary, acinar, adenomatoid tumor-like, solid, trabecular, pleomorphic, clear cell, deciduoid, adenoid cystic, signet ring, small cell, rhabdoid, and transitional areas.[3] Lung adenocarcinomas also show multiple subtypes: lepidic, papillary, micropapillary, acinar, solid, invasive mucinous, colloid, fetal, and enteric.[4, 5] This histological diversity can cause difficulty in distinguishing lung adenocarcinoma from epithelioid mesothelioma.[4] Our previous study on diagnoses of patients who died from malignant mesothelioma in Japan revealed that lung adenocarcinoma was most frequently misdiagnosed as malignant mesothelioma.[6] As treatment protocols and the prognosis of epithelioid mesothelioma and lung adenocarcinoma differ, it is very important that they are accurately diagnosed. The International Mesothelioma Interest Group (IMIG) recommends

calretinin, podoplanin (D2-40), and Wilms' Tumor 1 (WT1) as mesothelioma markers; their use has improved diagnostic accuracy. However, their sensitivity and specificity for differentiating epithelioid mesothelioma from lung adenocarcinoma are not ideal.[7]

We recently analyzed gene expression in formalin-fixed paraffin-embedded (FFPE) sections from epithelioid mesothelioma and lung adenocarcinoma, which led us to propose novel markers for this differential diagnosis. The markers positive for epithelioid mesothelioma included intelectin-1 and disabled homolog 2 (DAB2), while markers negative for epithelioid mesothelioma included mucin 21 (MUC21) and mucin 4 (MUC4).[8, 9] In the same study, we identified high expression levels of sex-determining region Y box 6 (SOX6) in epithelioid mesothelioma relative to lung adenocarcinoma.

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. [10-12] Recent studies have revealed that SOX6 is a tumor suppressor and is downregulated in multiple cancers, including esophageal squamous cell carcinoma, hepatocellular carcinoma, chronic myeloid leukemia, and ovarian cancers.[13-16] In this study, we evaluated the utility of SOX6 as a novel immunohistochemical marker for the differentiation of epithelioid mesothelioma from lung adenocarcinoma.

MATERIALS AND METHODS

Transcriptome Analysis of Microarray Gene Expression Data

We reanalyzed previous microarray gene-expression data from 6 epithelioid mesothelioma and 6 lung adenocarcinoma samples, using Subio (Subio, Amami-shi, Japan) to identify transcripts with a greater than two-fold difference in expression between the two tumor types[9].

Patients and Histological Samples

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks from epithelioid mesothelioma and lung adenocarcinoma were retrieved from the Department of Pathology, Hiroshima University archives. Fifty-four epithelioid mesothelioma specimens were derived from patients who underwent video-assisted thoracoscopic biopsy, pleurectomy, decortication, or extrapleural pneumonectomy between 2007 and 2016. Sixty-nine lung adenocarcinoma specimens were randomly selected from patients whose tumors were surgically resected between 2007 and 2016. All specimens were evaluated and confirmed independently by 3 pathologists (K.K., V.J.A, and Y.T.); all cases with inconsistent diagnoses were determined by consensus. Diagnoses were made through histological features and immunohistochemical marker panels according to the consensus guidelines established by the 2017 International Mesothelioma Interest Group (IMIG) meeting[7] and the 2015 World Health Organization histological classification of lung tumors.[3, 5] This study was performed in accordance with the Ethics Guidelines for Human Genome/Gene Research enacted by the Japanese government for the collection of tissue specimens and was approved by the institutional ethics review committee (Hiroshima University E-974).

Immunohistochemistry and Evaluation of SOX6 Expression

Immunohistochemical staining was performed on 3-μm thick tissue sections from representative FFPE blocks of epithelioid mesothelioma and lung adenocarcinoma using a BenchMark GX automated immunohistochemical station (Roche-Ventana Diagnostics, KK, Tokyo, Japan) with the ultraView Universal DAB Detection Kit (Roche-Ventana). In brief, the antigen was retrieved with Cell Conditioning buffer (CC1; Roche-Ventana) at 95 °C for 30 min. The primary antibody was an anti-SOX6 antibody (clone: A-4, Santa Cruz Biotechnology, Dallas, TX, USA), diluted 100 times with an antibody diluent (Roche-Ventana). Sections were incubated with the primary antibody for 32 min at 37°C, followed by amplification with an Amplification Kit (Roche-Ventana). Calretinin, D2-40, WT1, CEA, Claudin 4, TTF-1, and Napsin A immunohistochemistry was performed as previously described.[8, 17] Immunoreactivity of nuclear SOX6 expression was considered positive and the positive rate of immunostaining in tumor cells was scored as: 0 for no expression of SOX6 in tumor cells, 1+ for <10% positive tumor cells, 2+ for 10-50% positive tumor cells, and 3+ for >50% positive tumor cells.

Statistical Analysis

In this study, sensitivity is defined as the ability of immunohistochemical markers to identify epithelioid mesothelioma, calculated as the percentage of true positive cases for the given markers. Specificity is the ability of the immunohistochemical markers to exclude lung adenocarcinoma, calculated as the percentage of true negative cases for the given markers. The sensitivity, specificity, positive predictive values, and negative predictive values were calculated using a contingency table model.

RESULTS

Differential Gene Expression between Epithelioid Mesothelioma and Lung Adenocarcinoma

Microarray gene expression analysis at the exon level showed that 3278 transcripts were upregulated and 3446 were downregulated in epithelioid mesothelioma when compared to lung adenocarcinoma, based on at least two-fold differential expression between the 2 conditions (**Figure 1A**). Nine SOX6 transcripts were upregulated in epithelioid mesothelioma with respect to lung adenocarcinoma, as shown in the scatter plot (**Figure 1B**). SOX6 transcripts showed higher expression in 5 of 6 epithelioid mesotheliomas and lower expression in 5 of 6 lung adenocarcinomas, as shown in the line diagram **(Fig. 1C).**

SOX6 Expression in Epithelioid Mesothelioma and Lung Adenocarcinoma

The numbers of positive cases and immunohistochemical scores of SOX6, calretinin, D2-40, and WT1 in epithelioid mesothelioma and lung adenocarcinoma samples are shown in **Table 1**. SOX6 expression was predominantly localized in nuclei; however, weak cytoplasmic expression was also present. SOX6 expression was also focally identified in basal cells of the bronchial and bronchiolar epithelium, which were used as internal positive controls when available. Nuclear SOX6 expression was present in 53 of 54 cases (98%) of epithelioid mesothelioma. Representative cases are shown in **Figure 2**. Forty-eight cases had an immunohistochemical score of 3+ and 5 cases had a score of 2+. No epithelioid

mesothelioma samples had an immunohistochemical score of 1+. On the other hand, only 5 of 69 (7%) lung adenocarcinoma samples had weak nuclear expression of SOX6; representative cases are shown in **Figure 3**. Of the 5 positive cases, 3 had scores of 2+ and two had scores of 1+. SOX6 expression was able to differentiate epithelioid mesothelioma from lung adenocarcinoma with 98% sensitivity and 93% specificity (**Table 2**).

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

Nuclear expression of calretinin was present in 53 of 54 cases (98%) of epithelioid mesothelioma, with an immunohistochemical score of 3+ in 49 cases and 1+ in 4 cases. Calretinin was also expressed in 15 of 69 cases (22%) of lung adenocarcinoma, with an immunohistochemical score of 2+ in 6 cases and 1+ in 9 cases. Membranous expression of D2-40 was present in 53 of 54 cases (98%) of epithelioid mesothelioma, with scores of 3+ in 45 cases, 2+ in 6 cases, and 1+ in 2 cases. D2-40 was also expressed in 7 of 69 (10%) cases of lung adenocarcinoma, with scores of 2+ in 4 cases and 1+ in three cases. Nuclear WT1 expression was present in 42 of 55 cases (78%) of epithelioid mesothelioma, with scores of 3+ in 29 cases, 2+ in 3 cases, and 1+ in ten cases; all the lung adenocarcinoma cases were negative. Both calretinin and D2-40 expression showed a sensitivity of 98%; however, their specificities were 78% and 90%, respectively. WT1 expression showed a sensitivity of 78% and specificity of 100% (**Table 2**).

Sensitivity and Specificity of Combinations of Two Markers

The sensitivities and specificities of combinations of any two markers of SOX6, calretinin, D2-40, and WT1 for the differentiation of epithelial mesothelioma and lung adenocarcinoma are shown in **Table 3**. Combining SOX6 with other markers yielded comparable or better sensitivities and specificities than did combinations lacking SOX6. In particular, the sensitivity of positive expression of both SOX6 and calretinin (96%) and the specificity of positive expression of both SOX6 and WT1 (93%) were higher than those of other combinations.

DISCUSSION

Both epithelioid mesothelioma of the pleura and lung adenocarcinoma show multiple subtypes that cause difficulty in their differential diagnosis. The treatment protocols and the prognosis of epithelioid mesothelioma and lung adenocarcinoma differ significantly, requiring accurate diagnosis for effective treatment. Therefore, the International Mesothelioma Interest Group guidelines recommend a panel of immunohistochemical markers for accurate diagnosis.[7] However, the markers recommended in this guideline do not show ideal sensitivity or specificity. In particular, the specificities of calretinin (90-95%) , D2-40 (85%) , and WT1 (70-95%) are not high enough.[7] The sensitivities and specificities of calretinin, D2-40, and WT1 measured in this study were similar to those in the IMIG guidelines, except for calretinin, which had only 78% specificity. The specificity of calretinin in our previous reports ranged from 71-81%.

We considered this discrepancy to be caused by the use of an automated immunohistochemical station and the anti-calretinin antibody, clone SP65, from Ventana-Roche. To improve diagnostic accuracy, we previously reported 3 positive immunohistochemical markers for epithelioid mesothelioma: Intelectin-1, DAB2, and Glypican-1[9, 18]. In these reports, although Intelectin-1 and DAB2 showed high specificities, their sensitivities were not high enough, while Glypican-1 showed both high sensitivity and specificity **(Table 2)**. All of these markers were expressed in the cytoplasm of epithelioid mesothelioma samples. Given that WT1 is the only positive nuclear immunohistochemical marker for epithelioid mesothelioma, we set out to identify additional nuclear immunohistochemical markers. Therefore, we focused on SOX6 as a nuclear marker of epithelioid mesothelioma. This is the first report of SOX6 immunohistochemical reactivity in mesothelioma.

The expression of SOX6 showed high sensitivity (98%), high specificity (93%), a positive predictive value of 91%, and a negative predictive value of 98% (**Table 2**). The positive predictive values of calretinin and D2-40 were also lower than those of SOX6. Therefore, SOX6 has similar or better utility for the differentiation of epithelioid mesothelioma from lung adenocarcinoma than calretinin and D2-40.

Nuclear SOX6 expression was present in 53 of 54 epithelioid mesothelioma samples and only 5 cases of lung adenocarcinomas, indicating high sensitivity and specificity for differential diagnosis. We found 1 case of epithelioid mesothelioma negative for SOX6 expression and 5 cases of lung adenocarcinoma positive for SOX6 expression. Immunohistochemical findings for these 6 cases are shown in **Table 4** as EM1 and LAC1 to LAC5, including the data for positive markers for lung adenocarcinoma: CEA, TTF-1, Napsin A, and Claudin 4. [8, 17] One epithelioid mesothelioma, negative for SOX6, showed a solid growth pattern and immunohistochemical reactivity for calretinin (2+) and D2-40 (3+), but was negative for WT1. In this case, EM1 was also not positive for any of the 4 lung adenocarcinoma markers (CEA, TTF-1, Napsin A, and Claudin 4), as shown in **Supplementary Figure 1**. Among the 5 cases of lung adenocarcinoma showing SOX6 expression, all were negative for D2-40 and WT1, and 2 were positive for calretinin expression. All 5 cases were positive for lung adenocarcinoma markers; 1 representative case, LAC3, is shown in **Supplementary Figure 2**. Of 69 cases of lung adenocarcinoma analyzed, 13 of 15 calretinin-positive cases and all 7 D2-40 positive cases were negative for nuclear SOX6 expression. Furthermore, 3 cases of lung adenocarcinoma with both calretinin and D2-40 expression were negative for SOX6. Immunohistochemical findings for 12 epithelioid mesothelioma cases negative for calretinin, D2-40, and WT1, as well as 17 lung adenocarcinoma cases positive for calretinin and/or D2-40 are shown in **Supplementary Table 1** as EM2 to EM13 and LAC6 to LAC22. Thus, SOX6 detected true epithelioid mesotheliomas with exceptional immunohistochemical staining patterns.

We investigated SOX6 for the purpose of differentiation of epithelioid mesothelioma from lung adenocarcinoma. In addition, we investigated SOX6 expression in metastatic lung carcinoma. SOX6 expression in multiple human malignancies has been reported in The Human Protein Atlas (https://www.proteinatlas.org/ENSG00000110693-SOX6/pathology). In this atlas, most adenocarcinomas from multiple organs show no nuclear reactivity for SOX6. We also analyzed SOX6 expression in

metastatic carcinomas in the lung that originated from the stomach (3 cases), colon (3 cases), pancreas (3 cases), breast (3 cases), ovary (2 cases), and prostate (1 case). Almost all of these cases were negative for SOX6, except for 1 ovarian carcinoma that showed focal nuclear positivity (**Supplementary Figure 3)**. These results suggest that 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.

Additionally, we investigated SOX6 expression in 7 cases of sarcomatoid mesothelioma, 1 case of biphasic mesothelioma, and 15 cases of pleomorphic carcinoma of the lung. Of 7 sarcomatoid mesotheliomas, only 2 showed SOX6 expression. Biphasic mesothelioma showed SOX6 expression in both epithelioid and sarcomatoid components. In pleomorphic carcinoma, the carcinomatous component was all negative for SOX6, but in 2 cases, sarcomatoid component showed SOX6 expression. These data suggest that SOX6 is involved in the regulation of mesenchymal transformation in mesothelioma cells, but further study is required.

In conclusion, we identified SOX6 as a novel mesothelioma marker by gene expression microarray analysis of epithelioid mesothelioma and lung adenocarcinoma. SOX6 immunohistochemistry showed high sensitivity and specificity for the differentiation of epithelioid mesothelioma from lung adenocarcinoma; SOX6 nuclear staining is a positive immunohistochemical marker for the differential diagnosis of epithelioid mesothelioma from lung adenocarcinoma. Further validation of this marker by 0thers institutes is warranted to verify its practical use.

REFERENCES

1. Robinson BW, Lake RA. Advances in malignant mesothelioma. *N Engl J Med.* 2005;353:1591-1603.

2. Delgermaa V, Takahashi K, Park EK, et al. Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. *Bull World Health Organ.* 2011;89:716-724, 724A-724C.

3. Galateau-Salle F, Churg A, Roggli V, et al. Epithelioid mesothelioma. In: Travis WD, Brambilla E, Burke AP, et al., eds. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon: IARC; 2015:156-164.

4. Attanoos RL, Gibbs AR. 'Pseudomesotheliomatous' carcinomas of the pleura: a 10-year analysis of cases from the Environmental Lung Disease Research Group, Cardiff. *Histopathology.* 2003;43:444-452.

5. Travis WD, Noguchi M, Yatabe Y, et al. Adenocarcinoma. In: Travis WD, Brambilla E, Burke AP, et al., eds. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon: IARC; 2015:26-37.

6. Takeshima Y, Inai K, Amatya VJ, et al. Accuracy of pathological diagnosis of mesothelioma cases in Japan: clinicopathological analysis of 382 cases. *Lung Cancer.* 2009;66:191-197.

7. Husain AN, Colby TV, Ordóñez NG, et al. Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. *Arch Pathol Lab Med.* 2018;142:89-108.

8. Kai Y, Amatya VJ, Kushitani K, et al. Mucin 21 is a novel, negative immunohistochemical marker for epithelioid mesothelioma for its differentiation from lung adenocarcinoma. *Histopathology.* 2019;74:545-554.

9. Kuraoka M, Amatya VJ, Kushitani K, et al. Identification of DAB2 and Intelectin-1 as Novel Positive Immunohistochemical Markers of Epithelioid Mesothelioma by Transcriptome Microarray Analysis for Its Differentiation From Pulmonary Adenocarcinoma. *Am J Surg Pathol.* 2017;41:1045-1052.

10. Hamada-Kanazawa M, Ishikawa K, Ogawa D, et al. Suppression of Sox6 in P19 cells leads to failure of neuronal differentiation by retinoic acid and induces retinoic acid-dependent apoptosis. *FEBS Lett.* 2004;577:60-66.

11. Iguchi H, Urashima Y, Inagaki Y, et al. SOX6 suppresses cyclin D1 promoter activity by interacting with beta-catenin and histone deacetylase 1, and its down-regulation induces pancreatic beta-cell proliferation. *J Biol Chem.* 2007;282:19052-19061.

12. Zhou Y, Zheng X, Chen LJ, et al. microRNA-181b suppresses the metastasis of lung cancer cells by targeting sex determining region Y-related high mobility group-box 6 (Sox6). *Pathol Res Pract.* 2019;215:335-342.

13. Guo X, Yang M, Gu H, et al. Decreased expression of SOX6 confers a poor prognosis in hepatocellular carcinoma. *Cancer Epidemiol.* 2013;37:732-736.

14. Li Y, Xiao M, Guo F. The role of Sox6 and Netrin-1 in ovarian cancer cell growth, invasiveness, and angiogenesis. *Tumour Biol.* 2017;39:1010428317705508.

15. Qin YR, Tang H, Xie F, et al. Characterization of tumor-suppressive function of SOX6 in human esophageal squamous cell carcinoma. *Clin Cancer Res.* 2011;17:46-55.

16. Wang J, Ding S, Duan Z, et al. Role of p14ARF-HDM2-p53 axis in SOX6-mediated tumor suppression. *Oncogene.* 2016;35:1692-1702.

17. Mawas AS, Amatya VJ, Kushitani K, et al. MUC4 immunohistochemistry is useful in distinguishing epithelioid mesothelioma from adenocarcinoma and squamous cell carcinoma of the lung. *Sci Rep.* 2018;8:134.

18. Amatya VJ, Kushitani K, Kai Y, et al. Glypican-1 immunohistochemistry is a novel marker to differentiate epithelioid mesothelioma from lung adenocarcinoma. *Mod Pathol.* 2018;31:809-815.

Figure Legends:

Figure 1. Gene expression analysis.

A. Supervised hierarchical clustering of 6 lung adenocarcinomas and 6 epithelioid mesotheliomas. A total of 6724 transcripts with at least two-fold differential expression between epithelioid mesothelioma and lung adenocarcinoma are shown. A total of 3278 transcripts were upregulated in epithelioid mesothelioma, and 3346 were upregulated in lung adenocarcinoma. Nine lines of SOX6s are shown in black arrows, with some lines overlapping.

B. Scatter plot of differential expression of 6724 transcripts, based on at least two-fold differential expression between epithelioid mesothelioma and lung adenocarcinoma. Nine transcripts of SOX6 are upregulated in epithelioid mesothelioma when compared to lung adenocarcinoma and are shown as black dots.

C. Line graph of processed raw signals of 6724 transcripts in 6 epithelioid mesotheliomas and 6 lung adenocarcinomas, with at least two-fold differential expression between epithelioid mesothelioma and lung adenocarcinoma. Purple lines indicate the raw signals of 6724 transcripts, and the 9 black lines represent SOX6.

Figure 2. Immunohistochemical expression of SOX6 in epithelioid mesothelioma.

A-F. Representative examples of epithelioid mesotheliomas with multiple histological patterns showing nuclear SOX6 expression.

Figure 3. Immunohistochemical expression of SOX6 in lung adenocarcinoma.

A-F. Representative examples of lung adenocarcinomas with multiple histological patterns showing no nuclear SOX6 expression.

Figure 1

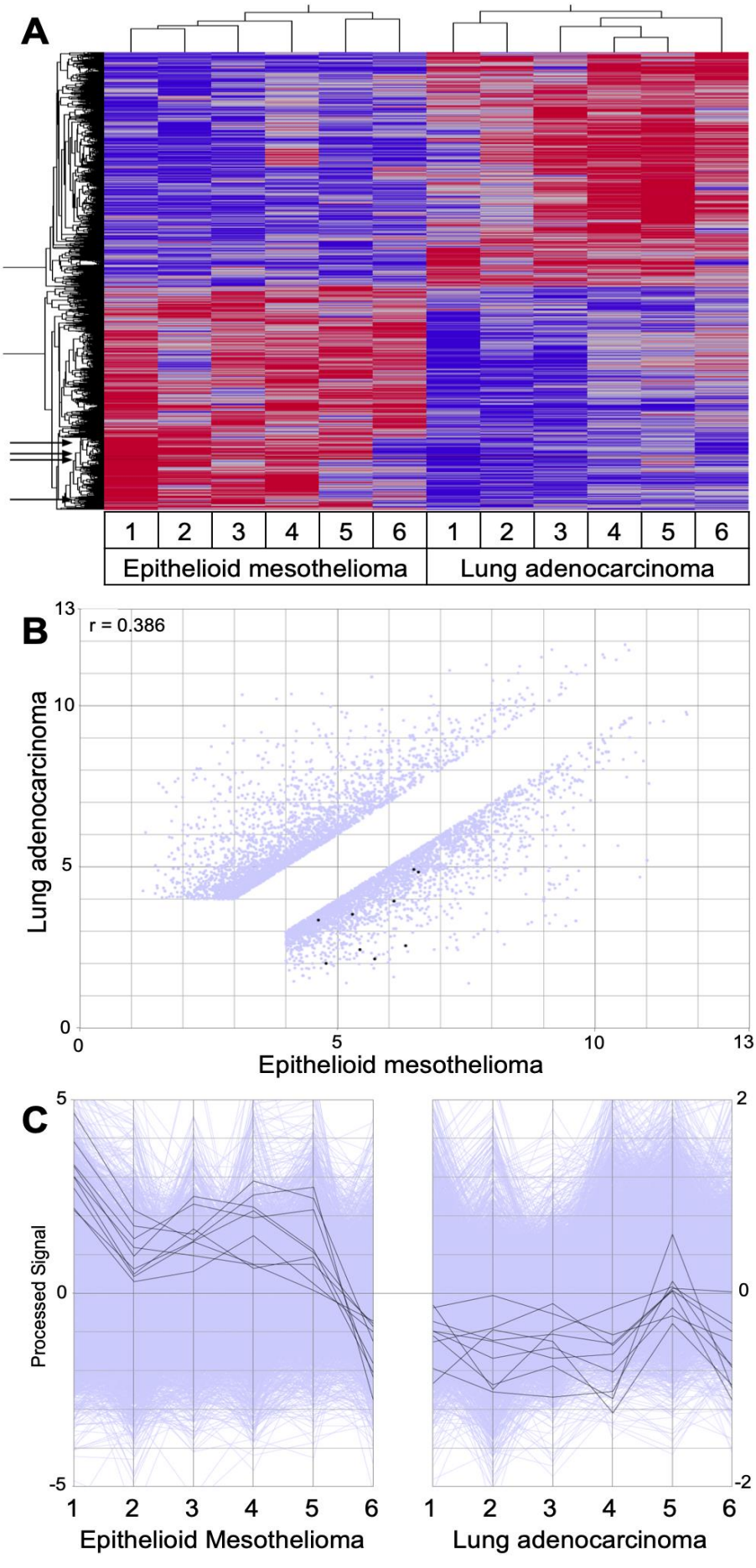
A
1 2 3 4 5 6 1 2 3 4 5 6
Epithelioid mesothelioma
Lung adenocarcinoma
B
r = 0.386
Lung adenocarcinoma
Epithelioid mesothelioma
0 5 10 13
C
Processed Signal
5 0 -5
2 0 -2
1 2 3 4 5 6
Epithelioid Mesothelioma
Lung adenocarcinoma

Figure 2

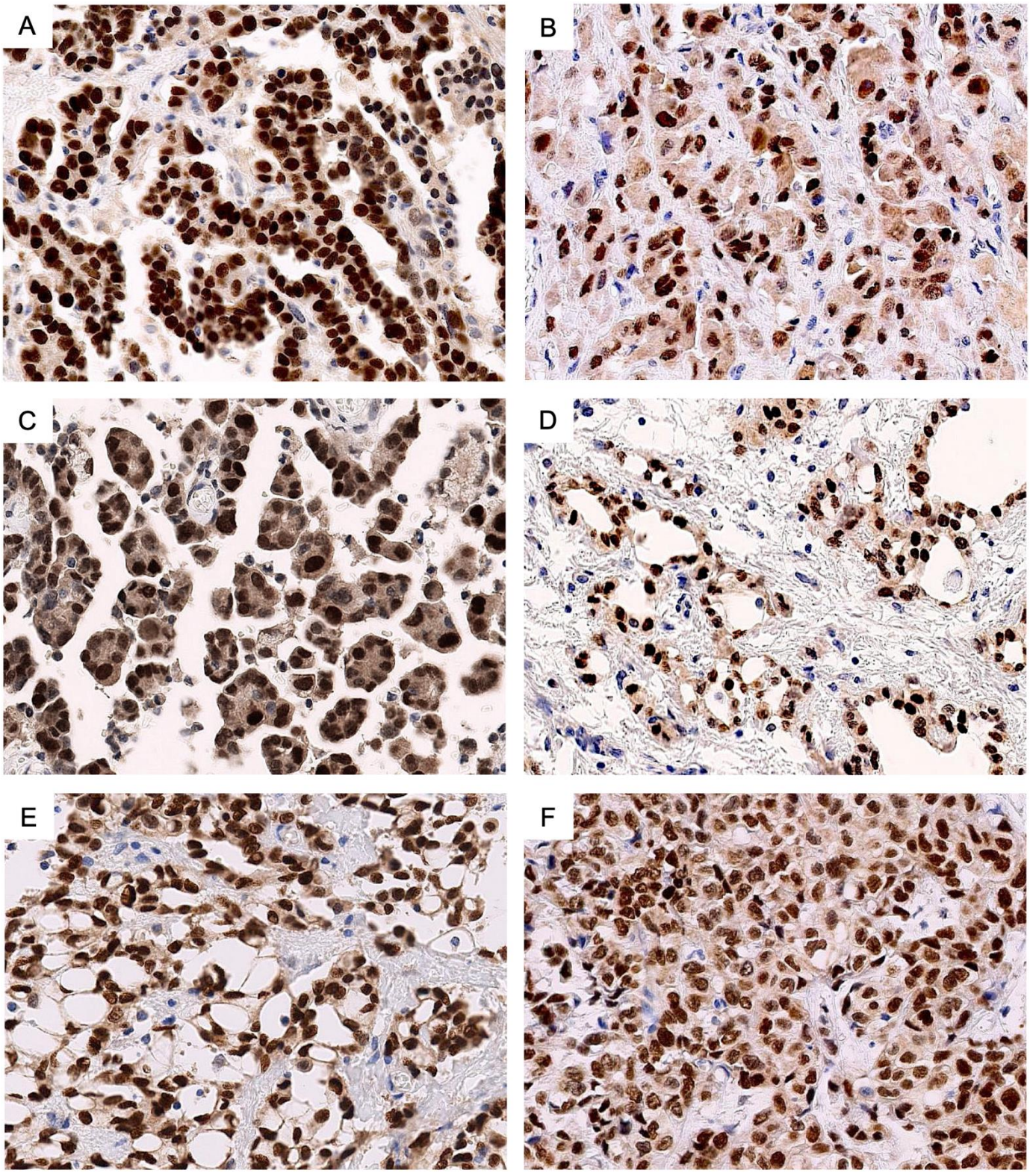

Figure 3

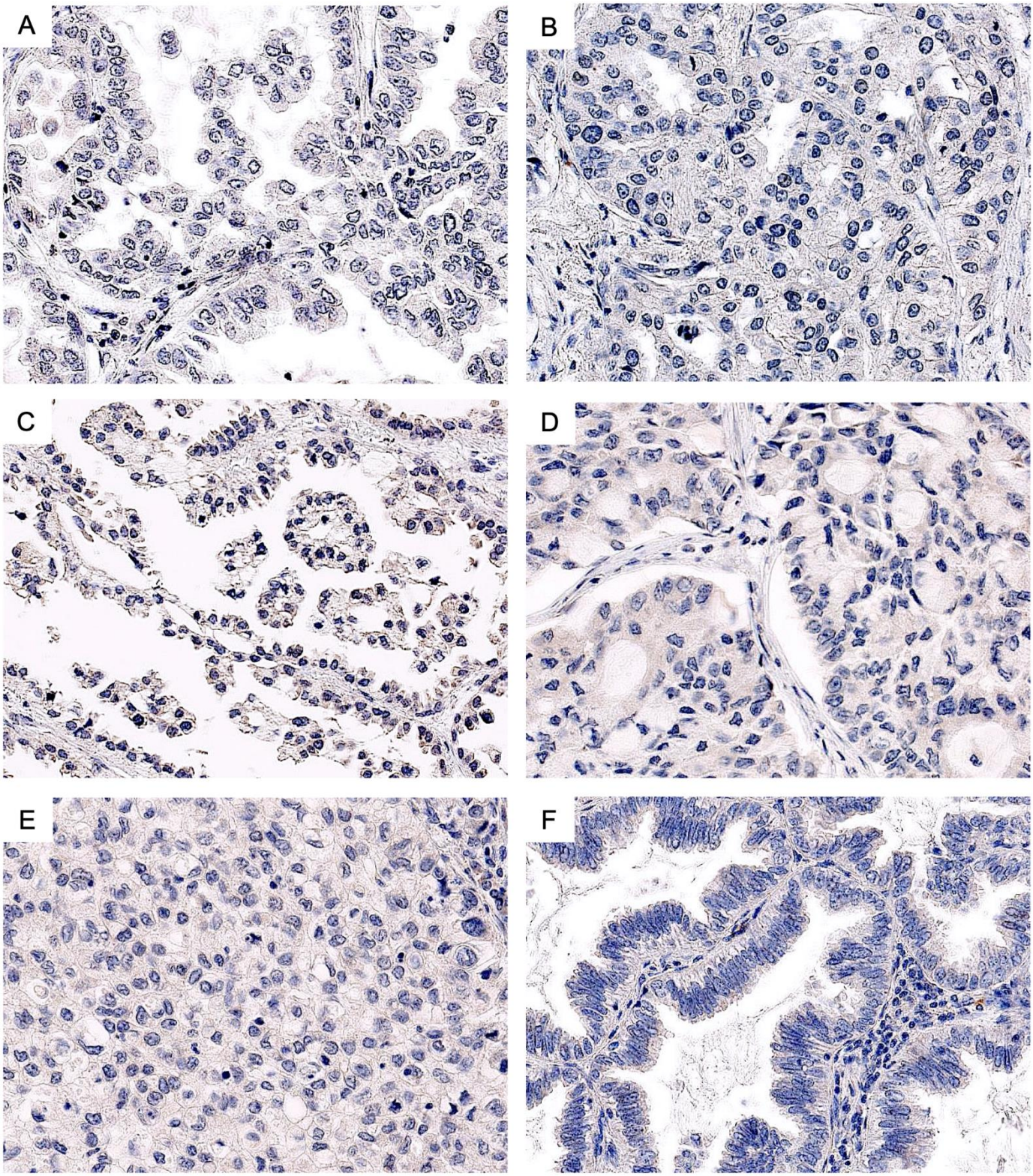

TABLE 1. Immunohistochemical findings for epithelioid mesothelioma and lung adenocarcinoma.

Epithelioid mesothelioma (54 cases)						Lung adenocarcinoma (69 cases)					
Marker	Number of positive cases	Immunohistochemical score*				Marker	Number of positive cases	Immunohistochemical score*			
		0	1+	2+	3+			0	1+	2+	3+
SOX6	53 (98%)	1	0	5	48	SOX6	5 (7%)	64	2	3	0
Calretinin	53 (98%)	1	4	0	49	Calretinin	15 (22%)	54	9	6	0
D2-40	53 (98%)	1	2	6	45	D2-40	7 (10%)	62	4	3	0
WT1	42 (78%)	12	10	3	29	WT1	0 (0%)	69	0	0	0

WT1: Wilms' tumor 1

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

TABLE 2. Sensitivity, specificity, positive predictive value, and negative predictive value 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%
Wilms Tumor 1	80%	100%	100%	85%
Intelectin-1*	76%	100%	97%	81%
DAB2*	80%	97%	100%	79%
Glypican-1**	100%	97%	96%	100%

PPV: positive predictive value; NPV: negative predictive value

*ref#9; **ref#18

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

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%

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

EM: epithelioid mesothelioma; LAC: lung adenocarcinoma