Immunohistochemical Differential Diagnosis Between Large Cell Neuroendocrine Carcinoma and Small Cell Carcinoma by Tissue Microarray Analysis With a Large Antibody Panel

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Abstract

*To elucidate additional phenotypic differences between large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC), we performed tissue microarray (TMA) analysis of surgically resected LCNEC and SCLC specimens. Immunostaining with 48 antibodies was scored based on staining intensity and the percentage of cells that stained positively. Four proteins were identified as significantly expressed in LCNEC as compared with SCLC: cytokeratin (CK)7, 113 vs 49 (*P < .0301*); CK18, 171 vs 60 (*P < .0008*); E-cadherin, 77 vs 9 (*P < .0073*); and β-catenin, 191 vs 120 (*P < .0286*). Immunostaining of cross-sections containing LCNEC and SCLC components revealed significant expression of CK7, CK18, and β-catenin in the LCNEC component compared with the SCLC component in 2 of 3 cases. Our results indicate that significant expression of CK7, CK18, E-cadherin, and β-catenin is more characteristic of LCNEC than of SCLC, and these findings provide further support that these tumor types are separate entities morphologically and immunophenotypically, if not biologically.*

Lung cancer is a major cancer throughout the world and the most common cause of cancer mortality. The revised World Health Organization (WHO) classification of lung cancer published in 1999 classifies neuroendocrine tumors into 4 major histologic categories: low-grade malignant "typical" carcinoid, intermediate-grade malignant "atypical" carcinoid, and 2 high-grade tumors, large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC).[1] In 1991, Travis et al[2] introduced the term *large cell neuroendocrine carcinoma* to describe a distinct category of high-grade neuroendocrine tumor with biologic and light microscopic characteristics different from those of high-grade SCLC. Morphologically, LCNEC is characterized by neuroendocrine morphologic features (rosette formation), large tumor cells (3 times larger in diameter than a small resting lymphocyte) with a low nuclear/cytoplasmic ratio, numerous nucleoli, a high mitotic rate (>10 in 10 high-power fields), a large degree of necrosis, and immunohistochemically staining positive for one or more neuroendocrine markers.

Some authors have reported that LCNEC has a poorer prognosis than SCLC,[3,4] whereas others have reported finding no significant difference in outcome between LCNEC and SCLC.[5-7] SCLC is sensitive to chemotherapy, but the optimal therapy for LCNEC has yet to be defined. Demetri et al[8] advocated that LCNEC be treated in a manner similar to SCLC but acknowledged that there may be a greater role for surgical resection in LCNEC. Nevertheless, it remains unclear how patients with LCNEC should be treated. Until now, few investigators have attempted to identify differences in molecular expression between LCNEC and SCLC. Sturm et al[9] reported a significantly higher frequency of thyroid transcription factor (TTF)-1 positivity with SCLCs, but no other biologic markers

with significantly different expression between LCNEC and SCLC have been reported.

Tissue microarray (TMA) analysis is becoming broadly accepted as an efficient and expeditious method in the field of proteomics,[10-12] and it provides a great deal of information that is equivalent to the information obtained from many tissue sections obtained from a large number of patients. It also is suitable for high-throughput molecular profiling of tumor specimens. In the present study, we used TMA with a large panel of antibodies to identify the phenotypic differences between LCNEC and SCLC.

Materials and Methods

Case Selection

During the period from January 1992 to December 2003, a total of 1,921 patients with primary lung carcinoma were treated at the National Cancer Center Hospital East, Chiba, Japan. All primary lung cancers with a pathologic diagnosis based on the classification schema of the third edition of the WHO classification[1] were reviewed, and 49 cases were diagnosed as LCNEC (2.6%). The 10 cases for which an adequate tissue specimen was not available for pathologic review were excluded from the study, leaving a total of 39 cases (2.0%) of LCNEC. TMA also was performed on specimens from 14 cases histologically diagnosed as pure SCLC (0.7%). In addition, 3 cases of SCLC combined with LCNEC were used to verify the results obtained by TMA.

Pathologic Studies

The specimens were fixed with 10% formalin and embedded in paraffin. Serial 4-μm sections were stained with H&E by the alcian blue–periodic acid–Schiff method for cytoplasmic mucin production and by the elastic van Gieson method for elastic fibers. Sections were reviewed by 3 pulmonary pathologists (J.N., G.I., and T.Y.) according to the histologic criteria described in the WHO classification criteria, and discrepancies were resolved by joint discussion of the slides viewed with a multiheaded microscope.

Construction of Tumor TMAs

The most representative tumor areas were selected carefully and marked on the H&E-stained slide for construction of microarrays. TMAs were assembled with a tissue-arraying instrument (Beecher Instruments, Silver Spring, MD).[10] The microarray system consists of thin-walled stainless steel needles approximately 2 mm in diameter and a stylet for transferring and removing the contents of the needle. The assembly is held in an x-y position guide that is manually adjusted with digital micrometers. Core samples are retrieved from selected regions of donor tissue and precisely arrayed in a new (recipient) paraffin block. Extra samples of the specimens were obtained routinely by collecting 2 replicate core samples of tumor in different areas. Specimens from the 39 cases of LCNEC **Image 1B** and **Image 1D** and 14 cases of SCLC **Image 1C** and **Image 1E** were punched, and core samples were mounted in the same donor blocks **Image 1A**.

Normal Control TMA

The normal control TMA was used as the positive control array for each staining. This slide was composed of esophagus, stomach, small intestine, large intestine, liver, pancreas, spleen, brain, heart, lung, skin, testis, kidney, prostate gland, breast, thyroid gland, and adrenal gland samples.

Antibodies and Immunohistochemical Staining

The 48 antibodies used in the study are listed in **Table 1**. Immunohistochemical staining was performed as follows: TMA donor blocks were cut into 4-μm sections and mounted on silane-coated slides. The sections were deparaffinized in xylene and dehydrated in a graded alcohol series, and endogenous peroxidase was blocked with 3% hydrogen peroxide in absolute methyl alcohol. Heat-induced epitope retrieval was performed for 20 minutes at 95°C with a 0.02-mol/L concentration of citrate buffer (pH 6.0). After the slides cooled at room temperature for 60 minutes, they were rinsed with deionized water and incubated overnight with primary antibodies. The slides then were washed 3 times with phosphate-buffered saline and incubated with the EnVision+ System-HRP (DAKO, Glostrup, Denmark). The reaction products were stained with diaminobenzidine and counterstained with hematoxylin. Some antibodies (Table 1) were used in an automated immunostainer (Ventana Medical Systems, Tucson, AZ) after antigen retrieval by microwave heating and citrate buffer.[13]

Identification of Positive Cases

The cases were evaluated in random order without knowledge of patient history. Each case in which more than 10% of the cancer cells reacted positively for an antibody were recorded as positive.

Calculation of Staining Scores

Immunostaining was scored based on the intensity of staining and the percentage of cells that stained positively. Whenever there was a disagreement, the slides were reviewed, and consensus was reached. Staining scores were calculated by multiplying the percentage of positive tumor cells per section (0% to 100%) by the immunohistochemical staining intensity. The sections were classified according to staining intensity as negative (total absence of staining), 1+ (weak staining), 2+ (moderate staining), or 3+ (strong staining), and the scores obtained ranged from 0 to 300. The staining scores obtained for 2 samples from the same specimen were calculated, and the result was recorded as the

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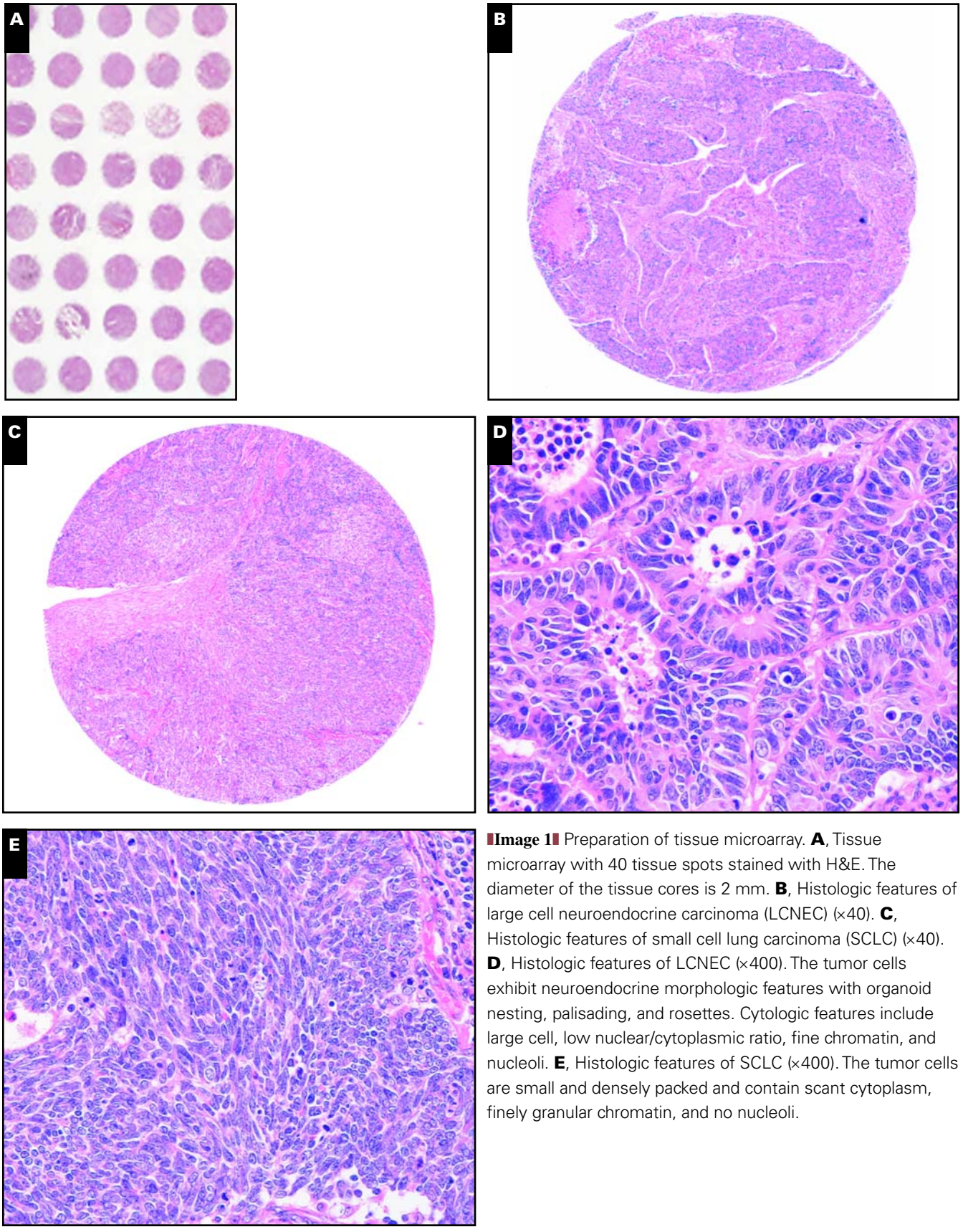

Image 1 Preparation of tissue microarray. **A**, Tissue microarray with 40 tissue spots stained with H&E. The diameter of the tissue cores is 2 mm. **B**, Histologic features of large cell neuroendocrine carcinoma (LCNEC) (×40). **C**, Histologic features of small cell lung carcinoma (SCLC) (×40). **D**, Histologic features of LCNEC (×400). The tumor cells exhibit neuroendocrine morphologic features with organoid nesting, palisading, and rosettes. Cytologic features include large cell, low nuclear/cytoplasmic ratio, fine chromatin, and nucleoli. **E**, Histologic features of SCLC (×400). The tumor cells are small and densely packed and contain scant cytoplasm, finely granular chromatin, and no nucleoli.

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Table 1
Antibodies Used

Classification/Antibody	Clone	Pretreatment	Dilution	Source
Cytokeratins				
CK1	34βB4	Microwave	1:20	Novocastra, Newcastle upon Tyne, England
CK4	6B10	Microwave	1:100	Novocastra
CK5/6	D5/16 B4	Microwave	1:50	DakoCytomation, Carpinteria, CA
CK7	OV-TL 12/30	Microwave	1:50	DakoCytomation
CK8	35βH11	Microwave	1:25	DakoCytomation
CK10	DE-K10	Microwave	1:50	DakoCytomation
CK13	KS-1A3	Microwave	1:100	Novocastra
CK14	LL002	Microwave	1:20	Novocastra
CK15	LHK15	Microwave	1:40	Novocastra
CK17	E3	Microwave	1:20	DakoCytomation
CK18	DC10	Microwave	1:25	DakoCytomation
CK19	RCK108	Microwave	1:50	DakoCytomation
CK20	Ks20.8	Microwave	1:25	DakoCytomation
Cytoskeletal filaments and markers				
Desmin	DE-R-11	Microwave	Prediluted	Ventana Medical Systems, Tucson, AZ
S-100	Polyclonal	None	Prediluted	Ventana Medical Systems
EMA	Mc5	None	Prediluted	Ventana Medical Systems
Vimentin	3B4	Microwave	Prediluted	Ventana Medical Systems
Drug resistant gene products and related markers				
Pgp	JSB-1	Microwave	1:20	Novocastra
MRP-1	MRPm6	Microwave	1:50	Sanbio, Uden, the Netherlands
MRP-2	M2III-6	Microwave	1:20	Sanbio
BCRP	BXP21	Microwave	1:20	Sanbio
Cox-1	Polyclonal	Microwave	1:50	IBL, Gunma, Japan
Cox-2	Polyclonal	Microwave	1:50	IBL
Apoptosis-associated proteins				
bcl-2	124	Microwave	1:40	DakoCytomation
bcl-x	Polyclonal	Microwave	1:500	Becton Dickinson Biosciences, San Jose, CA
bax	Polyclonal	Microwave	1:20	Oncogene Research Products, Cambridge, MA
bcl-1	P2D11F11	Microwave	Prediluted	Ventana Medical Systems
p 53	DO-7	Microwave	1:50	DakoCytomation
Growth factors and hormone receptors				
EGFR	EGFR.113	Microwave	1:10	Novocastra
c-erbB-2	CB11	Microwave	Prediluted	Ventana Medical Systems
IGFR	24-31	Microwave	1:100	Chemicon, Temecula, CA
c-kit	Polyclonal	Microwave	1:50	DakoCytomation
PgR	1A6	Microwave	Prediluted	Ventana Medical Systems
ER	6F11	Microwave	Prediluted	Ventana Medical Systems
Cellular adhesion molecules				
β-catenin	14	Microwave	1:200	Becton Dickinson Biosciences
E-cadherin	36	Microwave	1:100	Becton Dickinson Biosciences
NCAM	NCC-Lu-243	Microwave	1:25	Nippon Kayaku, Tokyo, Japan
CD29	7F10	Microwave	1:20	Novocastra
CD44	DF1485	Microwave	1:40	Novocastra
Cluster differential markers				
CD15	BY87	Microwave	Prediluted	Ventana Medical Systems
CD30	1G12	Microwave	Prediluted	Ventana Medical Systems
Mucin-related proteins				
Muc-1	Ma695	Microwave	1:100	Novocastra
Muc-2	Ccp58	Microwave	1:100	Novocastra
Muc-5AC	CLH2	Microwave	1:50	Novocastra
Muc-6	CLH5	Microwave	1:50	Novocastra
M-CCMC-1	HIK1083	Microwave	1:10	Kanto Chemical, Tokyo, Japan
Pneumocyte differential markers				
TTF-1	8G7G3/1	Microwave	1:50	DakoCytomation
SPPB	19H7	Microwave	1:25	Novocastra

BCRP, breast cancer resistance protein; EGFR, epidermal growth factor receptor; EMA, epithelial membrane antigen; ER, estrogen receptor; IGFR, insulin-like growth factor receptor; MRP, multidrug resistance protein; NCAM, neural cell adhesion molecule; PgP, P-glycoprotein; PgR, progesterone receptor; SPPB, surfactant precursor protein B; TTF, thyroid transcription factor.

score for that case. If one sample was lost, the staining score was calculated from the data for the remaining specimen alone. The staining scores for the specimens that contained SCLC combined with LCNEC were calculated using the intensity of staining and the percentage of each component stained on the entire slide.

Statistical Analysis

The staining score data are reported as means plus 95% confidence intervals. The Mann-Whitney *U* test was used to compare the staining scores of the LCNEC group and the SCLC group. All *P* values reported are 2-sided, and the significance

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level was set at less than .05. Differences between proportions were evaluated by using the Fisher exact test. All analyses were performed using Statview software (version 5.0 for Windows, SAS Institute, Cary, NC).

Results

Of the 5,406 core samples, 70 (1.3%) were lost on the TMA during processing of the slides for H&E preparation and immunostaining.

Positive Rates of LCNEC and SCLC

The percentages of LCNEC cases and SCLC cases that reacted positively for each antibody are summarized in **Table 2**. A positive reaction for cytokeratin (CK)18 was observed in 38 (97%) of 39 cases of LCNEC and 10 (71%) of 14 cases of SCLC, and the difference was significant ($P = .0143$). A positive reaction for E-cadherin was observed in 30 (77%) of 39 cases of LCNEC and 6 (43%) of 14 cases of SCLC, and the difference was significant ($P = .0419$).

Staining Scores for LCNEC and SCLC

The LCNEC and SCLC staining scores for each antibody are summarized in Table 2. Of the 13 cytokeratins tested, CK7 and CK18 had significantly higher staining scores in LCNEC. CK7 immunoreactivity was found in 30 (77%) of 39 cases of LCNEC and 7 (50%) of 14 cases of SCLC. The average staining score was 113 in LCNEC and 49 in SCLC; the difference was significant ($P = .0301$). **Image 2A** shows CK7 immunostaining of an LCNEC case with a staining score of 270. **Image 2B** shows CK7 immunostaining of an SCLC case with a staining score of 10. The average CK18 staining score was 171 in LCNEC and 60 in SCLC, and the difference was significant ($P = .0008$). **Image 2C** and **Image 2D** show CK18 immunostaining of an LCNEC case with a staining score of 240 and an SCLC case with a staining score of 40. No significant differences between LCNEC and SCLC were found in the expression of the other cytokeratins tested.

LCNEC had significantly higher staining scores for E-cadherin and β-catenin. E-cadherin expression was localized mainly on the membranes of the tumor cells. In some cases, E-cadherin expression was localized in the cytoplasm and nucleus, but results were recorded as negative. The average staining score for E-cadherin was 77 in LCNEC and 9 in SCLC. **Image 2E** and **Image 2F** show that the E-cadherin staining score was 80 in LCNEC and 10 in SCLC. Image 2F shows an E-cadherin staining score of 10 in SCLC. The expression of β-catenin was localized on the membranes and, in some cases, on the nucleus of the tumor cells. We classified the pattern of expression of β-catenin according to whether there was membranous or nuclear staining. Membranous β-catenin staining was found in 38 (97%) of 39 LCNEC cases and all 14 SCLC cases (100%). The average membranous β-catenin staining score was 191 in LCNEC and 120 in SCLC. **Image 2G** shows a membranous β-catenin staining score of 200 in LCNEC. **Image 2H** shows that the β-catenin staining score was 60 in SCLC. Nuclear β-catenin immunoreactivity was found in 5 (13%) of 39 LCNEC cases but in 0 (0%) of 14 SCLC cases. The average nuclear β-catenin staining score was 31 in LCNEC and 0 in SCLC, and the difference was not significant ($P = .4801$). There were no significant differences between LCNEC and SCLC in expression of the other cellular adhesion molecules.

We evaluated the expression of several other biologic markers, but no differences in expression were found between LCNEC and SCLC (Table 2).

Immunohistochemical Staining of CK7, CK18, E-Cadherin, and β-Catenin in Cross-Sections Containing LCNEC and SCLC Components

To determine whether the differences in expression of CK7, CK18, E-cadherin, and β-catenin in LCNEC and SCLC found as a result of the TMA analysis could be applied generally, their expression was evaluated in 3 cases of combined SCLC and LCNEC on slides that contained both components. The staining scores in these 3 cases are summarized in **Table 3**. In cases 1 and 2, expression of CK7, CK18, and β-catenin was clearly higher in the LCNEC components **Image 3**, and the results for these antibodies seemed similar to the results of TMA; however, E-cadherin expression was modestly higher in the LCNEC component in 1 case (case 1).

Discussion

The aim of the present study was to identify the distinct immunophenotypes of LCNEC and SCLC, and the technique used was based on large-scale analysis of protein expression detected by immunohistochemical analysis. Although it must be kept in mind that a potential limitation of TMA is that small core samples might not be representative of whole tumors, particularly in heterogeneous cancers,[14] the use of TMA has the advantage of enabling protein profiling, which probably more closely reflects the biologic characteristics of the tumor cells than does RNA detection. In the present study, we used the products of the staining intensity and distribution scores to assess immunoreactivity because they reveal phenotypic differences in greater detail. The TMA method identified 4 proteins as being overexpressed in LCNEC compared with SCLC: CK7 and CK18, which are involved in cytoskeleton organization, and β-catenin and E-cadherin, which are involved in cell adhesion. The results obtained were not surprising because the most striking morphologic differences between LCNEC and SCLC are cell shape and adhesiveness.

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Table 2
Positivity Rate, Staining Scores, and *P* Values for 39 Cases of LCNEC and 14 Cases of SCLS

	Positive Cases*			Staining Score		
Classification/Antibody	**LCNEC**	**SCLC**	***P***	**LCNEC**	**SCLC**	***P***
Cytokeratins						
CK1	1 (3)	0 (0)	.9999	0.1	0	.8877
CK4	9 (23)	2 (14)	.7062	2	0.5	.579
CK5/6	5 (13)	0 (0)	.3089	13	0	.4801
CK7	30 (77)	7 (50)	.9	113	49	.0301
CK8	26 (67)	8 (57)	.5238	51	39	.6211
CK10	0 (0)	0 (0)	.9999	0	0	.9999
CK13	4 (10)	2 (14)	.6489	0.5	1.8	.7699
CK14	1 (3)	1 (7)	.4623	0.2	0.4	.8087
CK15	0 (0)	0 (0)	.9999	0	0	.9999
CK17	6 (15)	0 (0)	.178	6.7	0	.3968
CK18	38 (97)	10 (71)	.0143	171	60	.0008
CK19	23 (59)	9 (64)	.7274	20	18	.9517
CK20	1 (3)	1 (7)	.4623	0.3	0.4	.9517
Cytoskeletal filaments and markers						
Desmin	0 (0)	0 (0)	.9999	0	0	.9999
S-100	8 (21)	6 (43)	.157	12	14	.3131
EMA	23 (59)	6 (43)	.2987	61	31	.2379
Vimentin	9 (23)	1 (7)	.2583	4	1	.3329
Drug-resistant gene products and related markers						
Pgp	0 (0)	1 (7)	.2642	0	1	.694
MRP-1	16 (41)	5 (36)	.7274	10	41	.9277
MRP-2	6 (15)	1 (7)	.6601	3	3.9	.6792
BCRP	24 (62)	9 (64)	.8557	39	23	.5056
Cox-1	0 (0)	0 (0)	.9999	0	0	.9999
Cox-2	0 (0)	0 (0)	.9999	0	0	.9999
Apoptosis-associated proteins						
bcl-2	30 (77)	13 (93)	.2583	118	107	.992
bcl-x	38 (97)	14 (100)	.9999	86	93	.364
bax	0 (0)	0 (0)	.9999	0	0	.9999
bcl-1	0 (0)	0 (0)	.9999	0	0	.9999
p53	30 (77)	8 (57)	.1819	170	120	.1609
Growth factors and hormone receptors						
EGFR	9 (23)	2 (14)	.7062	17	24	.4273
c-erbB-2	2 (5)	1 (7)	.9999	11	1	.9277
IGFR	25 (64)	6 (43)	.1664	32	12	.0845
c-kit	28 (72)	10 (71)	.9999	73	98	.6069
PgR	0 (0)	0 (0)	.9999	0	0	.9999
ER	0 (0)	0 (0)	.9999	0	0	.9999
Cellular adhesion molecules						
β-catenin						
Membranous	38 (97)	14 (100)	.9999	191	120	.0286
Nuclear	5 (13)	0 (0)	.3089	31	0	.4801
E-cadherin	30 (77)	6 (43)	.0419	77	9	.0073
NCAM	38 (97)	14 (100)	.9999	174	210	.1576
CD29	35 (90)	14 (100)	.5631	95	79	.9839
CD44	24 (62)	5 (36)	.0959	67	48	.2502
Cluster differential markers						
CD15	16 (41)	5 (36)	.7274	39	19	.709
CD30	0 (0)	0 (0)	.9999	2	0	.8877
Mucin-related proteins						
Muc-1	19 (49)	7 (50)	.9344	39	27	.8957
Muc-2	0 (0)	0 (0)	.9999	0	0	.9999
Muc-5AC	3 (8)	0 (0)	.5572	1.3	0	.6718
Muc-6	5 (13)	3 (21)	.4221	9	4	.7015
M-CCMC-1	0 (0)	0 (0)	.9999	0	0	.9999
Pneumocyte differential markers						
TTF-1	9 (23)	6 (43)	.1819	38	37	.4373
SPPB	5 (13)	2 (14)	.9999	21	1	.9839

BCRP, breast cancer resistance protein; EGFR, epidermal growth factor receptor; EMA, epithelial membrane antigen; ER, estrogen receptor; IGFR, insulin-like growth factor receptor; MRP, multidrug resistance protein; NCAM, neural cell adhesion molecule; PgP, P-glycoprotein; PgR, progesterone receptor; SPPB, surfactant precursor protein B; TTF, thyroid transcription factor.
* Data are given as number (percentage).

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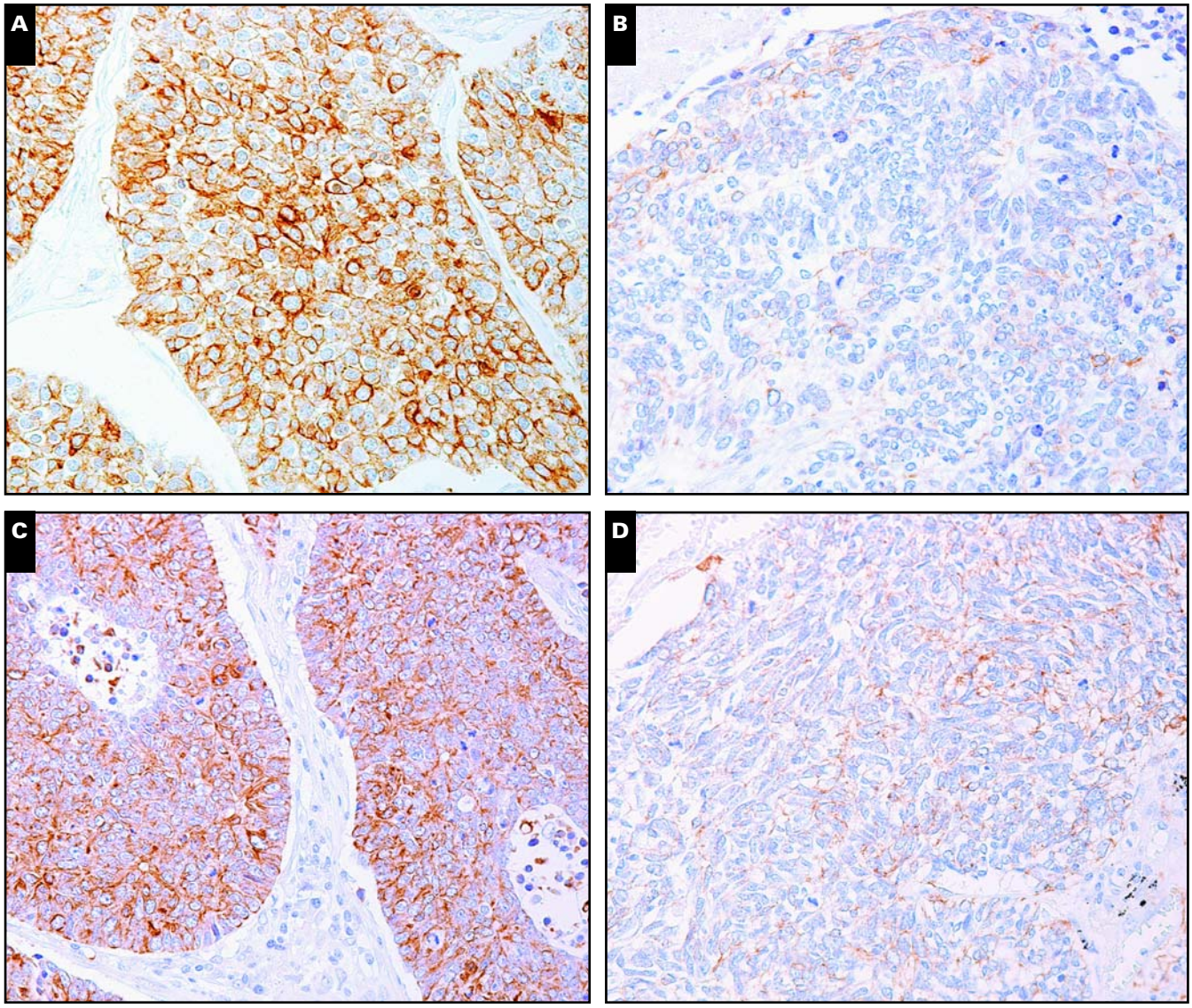

Image 2 Differences in immunostaining of cytokeratin (CK)7, CK18, E-cadherin, and β-catenin between large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC). **A**, CK7 immunostaining in LCNEC showing a staining score of 270 (positive cells, 90%; staining intensity, 3+) (×400). **B**, CK7 immunostaining in SCLC showing a staining score of 10 (positive cells, 10%; staining intensity, 1+) (×400). **C**, CK18 immunostaining in LCNEC showing a staining score of 240 (positive cells, 80%; staining intensity, 3+) (×400). **D**, CK18 immunostaining in SCLC showing a staining score of 40 (positive cells, 40%; staining intensity, 1+) (×400).

To our knowledge, this is the first study to identify significant differences in cytokeratin expression between LCNEC and SCLC. In normal adult lung tissue, CK7 and CK18 have been identified primarily in type II alveolar pneumocytes and in bronchial and bronchiolar epithelium. The results of testing for CK7 immunoreactivity in SCLC and LCNEC in previous studies yielded a wide variety of results. Lyda and Weiss[15] demonstrated immunoreactivity for CK7 in 2 (33%) of 6 cases of LCNEC and 2 (5%) of 38 cases of SCLC. By contrast, other studies of SCLC have reported CK7 expression in 4 (40%) of 10 cases,[16] 4 (80%) of 5 cases,[17] and 1 (9%) of 11 cases.[18] Wetzels et al[19] reported CK18 immunoreactivity in SCLC in 5 (83%) of 6 cases, and another study reported positivity in 8 (80%) of 10 cases.[16] However, CK18 expression had not been determined in LCNEC. The reasons for the discrepancies were unclear, but it should be kept in mind that the articles[16,18,19] were published before LCNEC had been defined and that LCNEC had been lumped together within SCLC in these studies. Most of the cases of LCNEC in our study showed diffuse, strong expression of CK7 and CK18, as opposed to focal and weak expression in SCLC, and significantly increased expression of CK7 (P = .0301) and CK18 (P = .0008) was observed in LCNEC.

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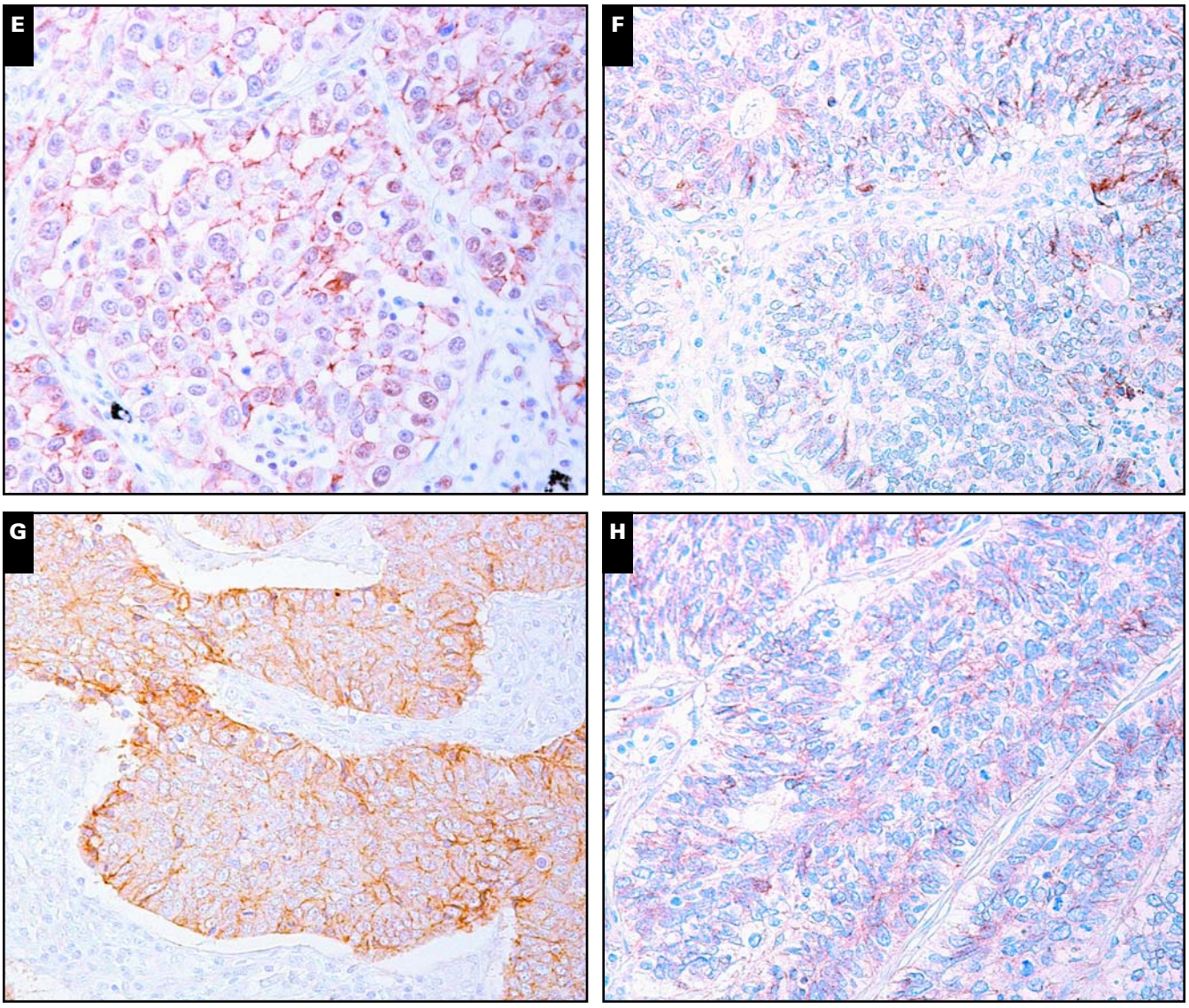

Image 2 (cont) **E**, E-cadherin immunostaining in LCNEC showing a staining score of 80 (positive cells, 80%; staining intensity, 1+) (×400). **F**, E-cadherin immunostaining in SCLC showing a staining score of 10 (positive cells, 10%; staining intensity, 1+) (×400). **G**, Membranous β-catenin immunostaining in LCNEC showing a staining score of 200 (positive cells, 100%; staining intensity, 2+) (×400). **H**, Membranous β-catenin immunostaining in SCLC showing a staining score of 60 (positive cells, 60%; staining intensity, 1+) (×400).

In normal adult lung tissue, E-cadherin and membranous β-catenin staining has been identified in bronchial and bronchiolar epithelium, but no nuclear β-catenin staining has been detected. In the study by Clavel et al,[20] E-cadherin–positive immunoreactivity was observed in 11 (73%) of 15 cases of LCNEC and membranous and nuclear β-catenin immunoreactivity was observed in 14 cases (93%) and 7 cases (47%), respectively, of LCNEC. Rodriguez-Salas et al[21] examined β-catenin expression in 50 pretreatment biopsy specimens of SCLC and reported that 14 (28%) of 50 cases were positive. The results of our own study showed E-cadherin and β-catenin expression in 77% and 97%, respectively, of the LCNEC cases and in 43% and 100%, respectively, of the SCLC cases.

Table 3
Staining Score of SCLC Cases Combined With LCNEC Cases

Case No./ Component	Staining Score			
	Cytokeratin 7	Cytokeratin 18	E-Cadherin	β-Catenin
1				
LCNEC	200	220	50	130
SCLC	10	10	0	20
2				
LCNEC	190	240	40	230
SCLC	10	10	50	60
3				
LCNEC	10	300	100	250
SCLC	0	300	100	240

LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma.

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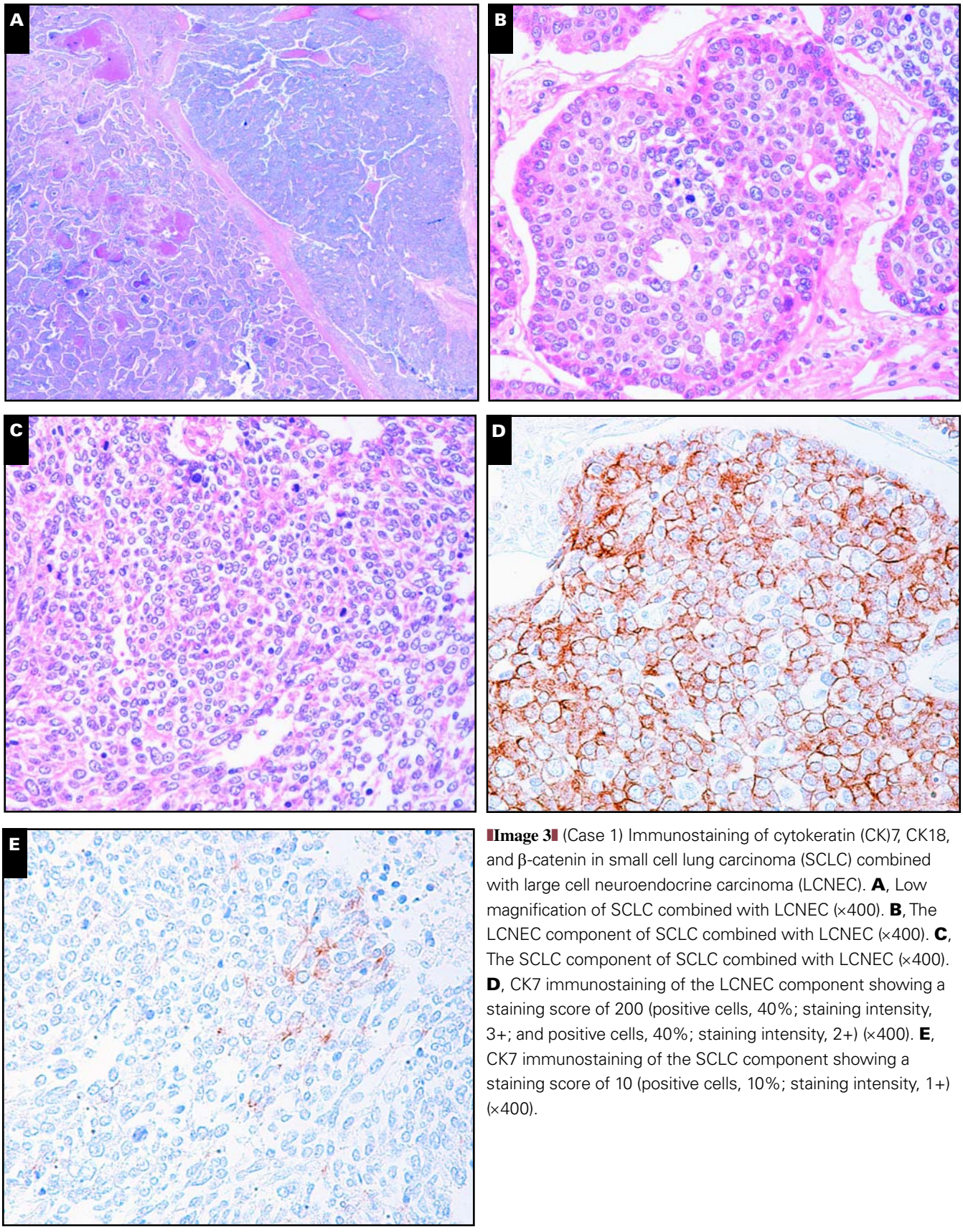

Image 3 (Case 1) Immunostaining of cytokeratin (CK)7, CK18, and β-catenin in small cell lung carcinoma (SCLC) combined with large cell neuroendocrine carcinoma (LCNEC). **A**, Low magnification of SCLC combined with LCNEC (×400). **B**, The LCNEC component of SCLC combined with LCNEC (×400). **C**, The SCLC component of SCLC combined with LCNEC (×400). **D**, CK7 immunostaining of the LCNEC component showing a staining score of 200 (positive cells, 40%; staining intensity, 3+; and positive cells, 40%; staining intensity, 2+) (×400). **E**, CK7 immunostaining of the SCLC component showing a staining score of 10 (positive cells, 10%; staining intensity, 1+) (×400).

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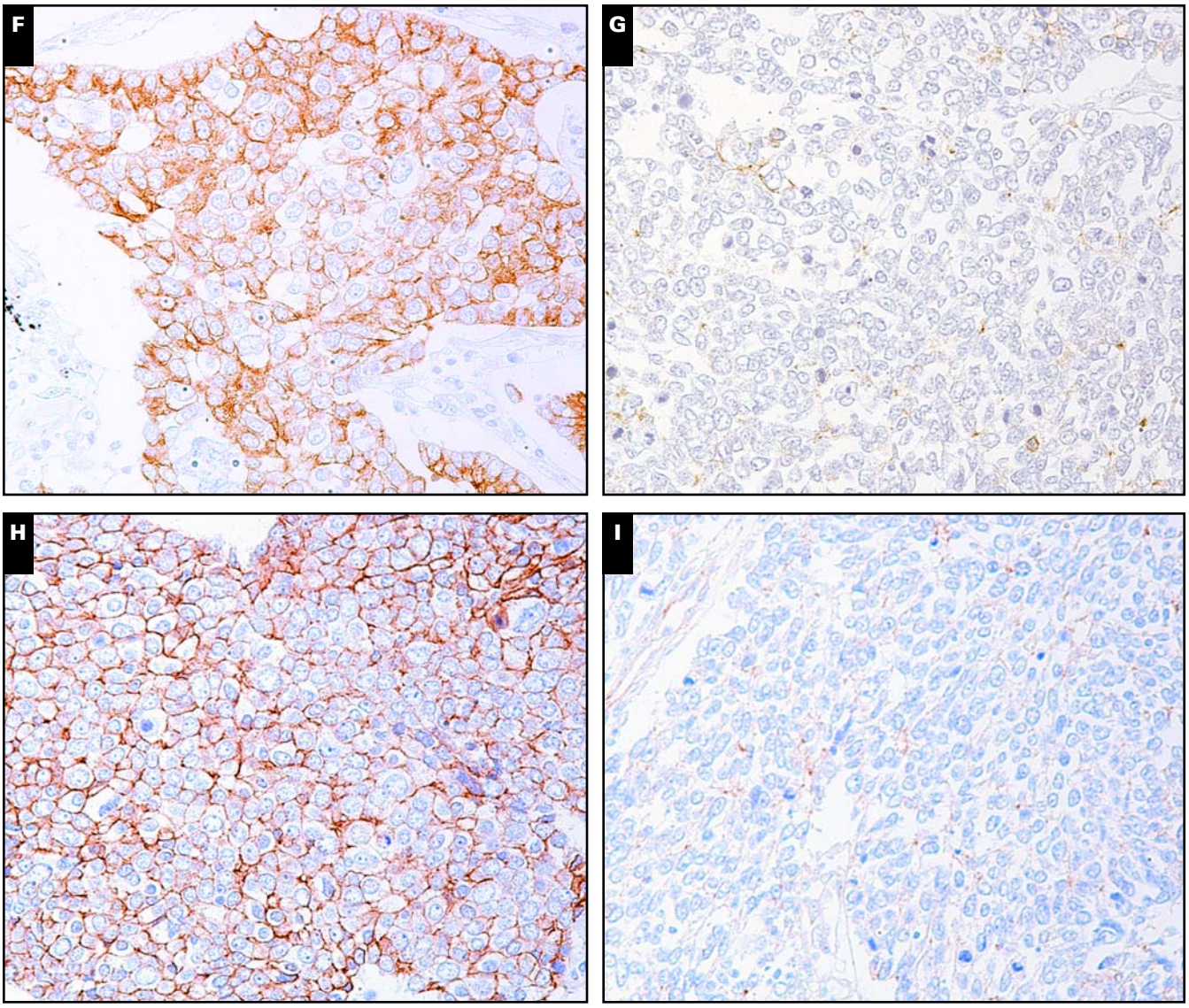

F, CK18 immunostaining of the LCNEC component showing a staining score of 220 (positive cells, 40%; staining intensity, 3+; and positive cells, 50%; staining intensity, 2+) (×400). **G**, CK18 immunostaining of the SCLC component showing a staining score of 10 (positive cells, 10%; staining intensity, 1+) (×400). **H** β-catenin immunostaining of the LCNEC component showing a staining score of 130 (positive cells, 10%; staining intensity, 3+; positive cells, 50%; staining intensity, 2+) (×400). **I**, β-catenin immunostaining of the SCLC component showing a staining score of 20 (positive cells, 20%; staining intensity, 1+) (×400).

However, the staining scores for E-cadherin and β-catenin in LCNEC and SCLC were very different, with both proteins being stained significantly in LCNEC. Moreover, nuclear β-catenin expression was 0% in SCLC, whereas 13% of the LCNEC cases were positive. Because E-cadherin and β-catenin have important roles in the pathogenesis of several human tumors, the E-cadherin cell adhesion system might have different roles in the pathogenesis of some cases of LCNEC.

Of the 3 cases of SCLC combined with LCNEC, 2 showed overexpression of CK7, CK18, and β-catenin in the LCNEC portion, indicating that the results obtained by TMA reliably predicted differences accentuated by morphologic features in these combined tumors. However, no overexpression of these proteins was found in the third case. The reason is unclear; however, the biologic characteristics of LCNEC and SCLC might not have been different despite the morphologic differences in this case.

Sturm and associates[9] reported positive immunostaining for TTF-1 in 85.5% of their SCLC cases and 49% of their LCNEC cases, and the percentage was significantly higher in SCLC. However, in our study, the positive rates and staining scores were lower in SCLC and LCNEC, and TTF-1 was not useful for distinguishing LCNEC from SCLC. Although the cause of the discrepancy is unclear, it has been suggested that

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the method of selection of the cases, the methods of fixation, or the interpretation of the results might have had a role in these differences.

Improved diagnostic criteria and prospective clinicopathologic studies are needed to validate the impression that patients with LCNEC have a clinical course different from that of patients with SCLC.[22] Our study clearly demonstrated that LCNEC and SCLC have a different biologic phenotype. We conclude that the 4 antibodies identified in our study might be useful for separating LCNEC from SCLC in biopsy specimens that have been crushed or are otherwise difficult to examine morphologically. Further studies are needed to define the expression of these molecules more precisely to enable pathologists to reliably distinguish between LCNEC and SCLC.

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