

mASH1 is Highly Specific for Neuroendocrine Carcinomas

An Immunohistochemical Evaluation on Normal and Various Neoplastic Tissues

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• **Context.**—High-grade neuroendocrine carcinomas and carcinoids can arise in different sites such as lung, gastrointestinal tract, prostate, and skin. Classic neuroendocrine markers such as CD56, synaptophysin, and chromogranin cannot distinguish carcinoids from high-grade neuroendocrine carcinomas. Recently, mouse monoclonal mASH1 has been shown to help discriminate carcinoids from high-grade neuroendocrine carcinomas in various neoplastic sites. To date, there have been no comprehensive immunohistochemistry studies with mASH1 on nonneuroendocrine neoplasms.

Objective.—To evaluate the specificity and sensitivity of mASH1 in various normal and neoplastic tissues, including lung cancers.

Design.—Formalin-fixed, paraffin-embedded tissue microarrays consisting of normal tissues and various neoplastic tissues were immunohistochemically evaluated with mASH1.

Results.—In normal tissues (n = 30), mASH1 (nuclear staining) was sparsely expressed in the molecular cell

layer, white matter, and granular cell layer of cerebellum; C cells in thyroid; and epithelial cells in thymus. In lung cancers, mASH1 stained 1.1% (1 of 93) of adenocarcinomas, 0.9% (1 of 111) of squamous cell carcinomas, 0% (0 of 30) of large cell carcinomas, 66.7% (6 of 9) of large cell neuroendocrine carcinomas, and 82.5% (94 of 114) of small cell carcinomas. In various other neoplastic tissues (n = 1114), mASH1 was expressed in thyroid medullary carcinomas, thymic carcinomas, and brain cancers; mASH1 was also expressed in a very low percentage of breast carcinomas, ovarian cancers, and pancreatic neuroendocrine tumors. All typical carcinoids of various sites were negative (0 of 11), however, in lung atypical carcinoids, mASH1 was expressed in 42.9% (9 of 21).

Conclusions.—Although not organ specific, mASH1 is highly specific for high-grade neuroendocrine carcinomas versus carcinoids and other nonneuroendocrine neoplasms.

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Neuroendocrine tumors comprise a family of neoplasms that exhibit a wide range of morphologic and functional characteristics. These neoplasms can be subclassified as low grade: carcinoids (typical and atypical) and high-grade neuroendocrine carcinomas (HGNECs): large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma; the latter 2 are associated with poor patient prognosis. Carcinoids and HGNECs can arise in different sites such as lung, gastrointestinal tract, prostate, and skin. Their diagnosis depends on the identification of morpho-

logic features and biomarkers indicative of neuroendocrine differentiation.¹ However, neuroendocrine neoplasms can show overlapping morphologic and immunohistochemical features independently of their site of origin, which can make identification of the primary location problematic.² In lung cancers, where both carcinoids and HGNECs can arise, the distinction between these neuroendocrine neoplasms and separation of them from nonneuroendocrine non-small cell lung carcinoma (NSCLC) can be difficult to interpret.³

Achaete-scute complex-like 1 (ASCL1), termed mASH1 in rodents and hASH1 in humans (m/hASH1), belongs to the basic helix-loop-helix family, and is a crucial transcription factor for neuroendocrine cell differentiation.^{2,4} It has been shown that mASH1 antibody (nuclear staining), which cross-reacts with hASH1, can help discriminate carcinoids from HGNECs in various neoplastic sites. La Rosa et al² demonstrated that although m/hASH1 is not a site-specific marker, it can be used as a diagnostic marker for HGNECs, thus helping to distinguish them from carcinoids. An additional study by Shida et al⁵ revealed that m/hASH1 was expressed in poorly differentiated neuroendocrine carcinoma, but was absent in gastroenteropancreatic carcinoids. Studies have also demonstrated that the mASH1 antibody can aid pathologists in distinguishing small cell lung

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Organ	mASH1 Expression
Adrenal	–
Bladder	–
Bone	–
Bone marrow	–
Cerebellum	+
Colon	–
Esophagus	–
Heart	–
Kidney	–
Larynx	–
Liver	–
Lung	–
Mesothelium	–
Parathyroid	–
Peripheral nerve	–
Pituitary	–
Placenta	–
Prostate	–
Salivary gland	–
Skeletal muscle	–
Skin	–
Small intestine	–
Spleen	–
Stomach	–
Thyroid	+ (thyroid C cells)
Testis	–
Tonsil	–
Thymus	+ (epithelial cells)
Uterine cervix	–
Uterus	–

Abbreviations: –, negative; +, positive.

cancer (SCLC) and LCNEC from NSCLC.^{2-4,6} Furthermore, m/hASH1 was also shown to be a useful marker in differentiating pulmonary SCLC from Merkel cell carcinoma.⁷

To date, there have been no comprehensive immunohistochemistry studies with mASH1 on nonneuroendocrine neoplasms, especially NSCLC, such as adenocarcinoma and squamous cell carcinoma. Additionally, classic neuroendocrine markers such as CD56, synaptophysin, and chromogranin cannot distinguish carcinoids from HGNECs.⁸⁻¹¹ Herein, we evaluate the specificity and sensitivity of mASH1 in various normal and neoplastic tissues, with emphasis on non-neuroendocrine carcinoma/neuroendocrine tumor cancers and demonstrate the specificity of mASH1 in SCLC, and LCNEC versus nonneuroendocrine NSCLC, including

adenocarcinoma, squamous cell, classic large cell, and carcinoids (neuroendocrine tumors).

DESIGN

Formalin-fixed, paraffin-embedded tissue microarrays (TMAs) (in-house tissue microarrays and commercially purchased tissue microarrays, US Biomax, Rockland, Maryland) were used, consisting of normal tissues (n = 30), various neoplastic tissues (n = 1114), NSCLC (n = 268), and SCLC (n = 114). Tissue sections were cut at 4 to 5 μ m and placed on positive-charged slides; then, they were deparaffinized in a xylene substitute, hydrated in a series of graded alcohols down to water, placed in a modified citrate buffer solution, and heated in a pressure cooker at 110°C for 15 minutes. The titer for mASH1 (for in vitro diagnostic use; 24B72D11.1, Biocare Medical, Concord, California) was optimized at 1:200, followed by a 2-step immunohistochemistry horseradish peroxidase micropolymer detection system and visualized with 3,3'-diaminobenzidine chromogen. A case was considered positive if 1% or more of tumor cells were positive for m/hASH1. Statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software, La Jolla, California). *P* values were determined using the χ^2 test and confirmed with a Fisher exact test. Values of .05 or less were considered statistically significant.

RESULTS

Results are summarized in Tables 1 through 4. In normal tissues, m/hASH1 (nuclear staining) was sparsely expressed in the molecular cell layer, white matter, and granular cell layer of cerebellum (cerebrum was negative), C cells in thyroid, and epithelial cells in thymus (Table 1). All other normal tissues were negative, including adrenal gland, pancreas, and argentaffin cells found in the gastrointestinal tract.

In NSCLC, mASH1 stained 1.1% (1 of 93) of adenocarcinomas, 0.9% (1 of 111) of squamous cell carcinomas, and 0% (0 of 30) of large cell carcinomas, and LCNEC stained 66.7% (6 of 9) (Figure 1, A; Table 2). In SCLC, mASH1 stained 82.5% (94 of 114) (Figure 1, B; Table 3). In various other neoplastic tissues, m/hASH1 was expressed in thyroid medullary carcinomas (Figure 2, A), but not in thyroid papillary and follicular carcinomas, thymic carcinomas (Figure 2, B), or brain cancers (Figure 2, C and D; Table 4). m/hASH1 was expressed focally in a low number of

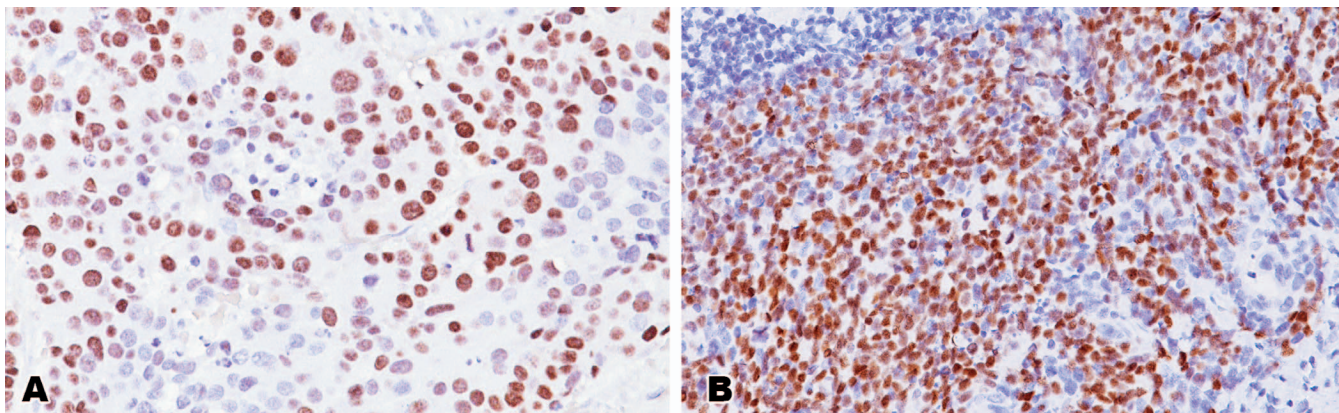


Figure 1. mASH1 staining in lung cancers. A, Large cell neuroendocrine carcinoma. B, Stage 4 small cell lung cancer (original magnification $\times 20$).

Table 2. mASH1 Staining in Various Neoplastic Tissues (n = 1114)

Tissue Type	No. Positive/ Total	% Positive
Adrenal cortical adenocarcinoma	0/10	0
Bladder (urothelial carcinoma)	0/38	0
Brain tumors (n = 159)		
Astrocytoma (grade I)	6/21	28.6
Astrocytoma (grade II–III)	33/40	82.5
Glioblastoma (grade IV)	19/35	54.3
Oligodendroglioma	11/13	84.6
Ependymoma	3/13	23.1
Medulloblastoma	0/15	0
Meningioma	0/2	0
Various benign brain tumors	0/20	0
Breast carcinomas (n = 162)		
Invasive ductal carcinoma	2/127	1.5
Invasive lobular carcinoma	1/18	5.6
Cholangiocarcinoma	0/9	0
Colon adenocarcinoma	0/94	0
Embryonal tumors (multiple)	0/6	0
Esophageal neuroendocrine carcinoma	3/3	100
Gastrointestinal carcinoids	0/5	0
Hepatocellular carcinoma	0/40	0
Lymphoma	0/17	0
Melanoma	0/22	0
Ovarian tumors (n = 88)		
Serous adenocarcinoma	1/62	1.6
Clear cell carcinoma	1/9	11.1
Dysgerminoma	1/5	20
Endodermal sinus carcinoma	0/3	0
Endometrioid	0/3	0
Mucinous	0/4	0
Granular cell tumor	0/2	0
Pancreatic tumors (n = 92)		
Neuroendocrine tumors	2/46	4.3
Pancreatic ductal carcinoma	0/21	0
Pancreatic adenocarcinoma	0/25	0
Pheochromocytoma	0/5	0
Prostate adenocarcinoma	0/38	0
Renal cell carcinoma	0/37	0
Bone and soft tissue tumors (n = 84)		
Bone (giant cell tumor)	0/11	0
Osteosarcoma	0/5	0
Liposarcoma	0/15	0
Fibrosarcoma	0/20	0
Dermatofibroma protuberans	0/3	0
Sarcoma unclassified	0/4	0
Rhabdomyosarcoma	0/10	0
Leiomyosarcoma	0/9	0
Epithelioid sarcoma	0/2	0
Synovial sarcoma	0/3	0
Carcinosarcoma	0/1	0
Angiosarcoma	0/1	0
Seminoma	0/70	0
Thymic tumors (n = 14)		
Thymic carcinoma	5/12	41.7
Thymic carcinoid	0/2	0
Thyroid carcinomas (n = 41)		
Medullary carcinoma	15/31	48.4%
Thyroid papillary carcinoma	0/9	0
Follicular carcinoma	0/1	0
Uterine carcinomas (n = 80)		
Squamous cell carcinoma	0/40	0
Adenocarcinoma	0/30	0
Adenosquamous cell carcinoma	0/10	0

Table 3. mASH1 Staining in Non–Small Cell Lung Carcinoma (n = 268)

Lung Cancer	No. Positive/ Total	% Positive
Adenocarcinoma	1/93	1.1
Squamous cell	1/111	0.9
Classic large cell	0/30	0.0
Large cell neuroendocrine carcinoma	6/9	66.7
Typical carcinoid	0/4	0.0
Atypical carcinoid	9/21	42.9

infiltrating ductal breast carcinoma (Figure 3, A), ovarian serous adenocarcinoma, and pancreatic neuroendocrine tumors (Table 2). All typical carcinoids of various sites were negative (0 of 12); however, in lung atypical carcinoids (Figure 3, B), m/hASH1 was expressed in 42.9% (9 of 21).

DISCUSSION

Past studies have shown CD56, chromogranin A, and synaptophysin to be the most reliable immunohistochemical markers to detect neuroendocrine differentiation in tumors of various sites, including neuroendocrine lung tumors.^{8–11} However, this panel of neuroendocrine markers cannot distinguish carcinoid from HGNEC. Proliferation rate (Ki-67) and mitotic index can aid in diagnosis, but this requires extra stains and time-consuming quantitative analysis. Also, in cases of small biopsies with limited tumor cells and/or frequently seen crush artifact, diagnosis can be particularly difficult.² Therefore, a more specific marker for distinguishing carcinoid and HGNEC may be of utmost importance in questionable cases.

In this study, various normal and nonneuroendocrine neoplastic tissues were tested for sensitivity and specificity with mASH1. In normal tissues, m/hASH1 was observed only in cerebellum, normal thyroid C cells, and epithelial cells in thymus (Table 1). However, survey of entire normal brain and other normal tissues is needed to further confirm the lack of m/hASH1 expression, as small punched tissue microarray spots may not be representative.

Excluding brain, thymic, and medullary carcinomas, positive-staining cases in neoplasms were observed in only 6 of 910 cases (0.66%; Table 2). m/hASH1 has been shown to be highly expressed in thyroid medullary cancer, which is a neuroendocrine cancer derived from the thyroid C cells.¹² In our results, m/hASH1 was expressed in 48.4% (15 of 31) and expression was also observed in 41.7% (5 of 12) of thymic carcinoma (Table 2). Thymic carcinoma with neuroendocrine differentiation has been previously reported; additionally, thymic epithelial cells have been shown to express neuroendocrine markers such as CD56, synaptophysin, and chromogranin.^{13,14} In contrast, although soft tissue tumors, such as rhabdomyosarcoma, carcinosarcoma, leiomyosarcoma, and synovial sarcoma, have been shown to express the same neuroendocrine markers,^{15–18} all 86 cases

Table 4. mASH1 Staining in Small Cell Lung Cancer (n = 114)

Stage	No. Positive/ Total	% Positive
1–4	94/114	82.5
1–3 (limited stage)	63/79	79.7
4 (extended stage)	31/35	88.6

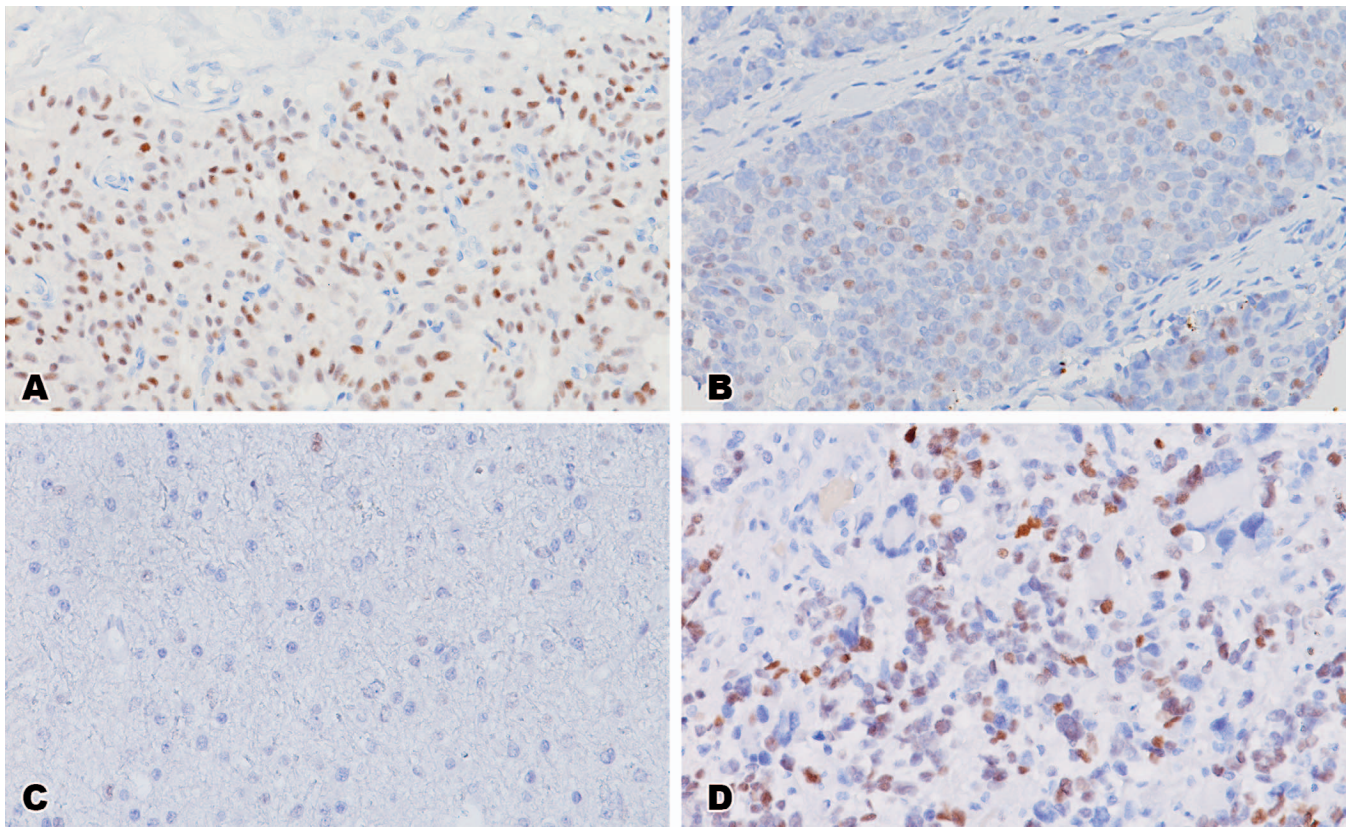


Figure 2. *m/hASH1* expression patterns in various neoplastic tissues. A, Medullary thyroid carcinoma. B, Undifferentiated thymic carcinoma. C, Low-grade astrocytoma. D, Grade IV glioblastoma (original magnification $\times 20$).

of bone and soft tissue tumors were negative for *m/hASH1* expression.

In brain tumors, *m/hASH1* was expressed in astrocytoma, glioblastoma, oligodendroglioma, and ependymoma tumors (Table 2). These data are supported by Somasundaram et al,¹⁹ as their study revealed that *m/hASH1* was overexpressed in progressive astrocytoma and glioblastomas and levels were increased in higher grades. In our study, we also observed progressive staining percentages in grade I versus grades II through IV (Figure 2, B and C; Table 2). In total, *m/hASH1* was expressed in 51.8% (72 of 139) of malignant brain cancers.

In breast cancers, only 3 cases (high grade, T4 stage) were focally positive for *m/hASH1* ($<10\%$; Table 2). Bogina et al²⁰ observed neuroendocrine differentiation in approximately 10% of breast cancers and showed significant association with T4 stage. Wachter et al²¹ also observed neuroendocrine differentiation in 20% of luminal B-like carcinomas using current World Health Organization criteria (at least 50% of tumor cells positive for synaptophysin or chromogranin A). Both studies used classic neuroendocrine markers, but did not use *mASH1*. Therefore, in tumors of unknown origin, breast cancer must be considered, especially when using chromogranin and synaptophysin as diagnostic markers.

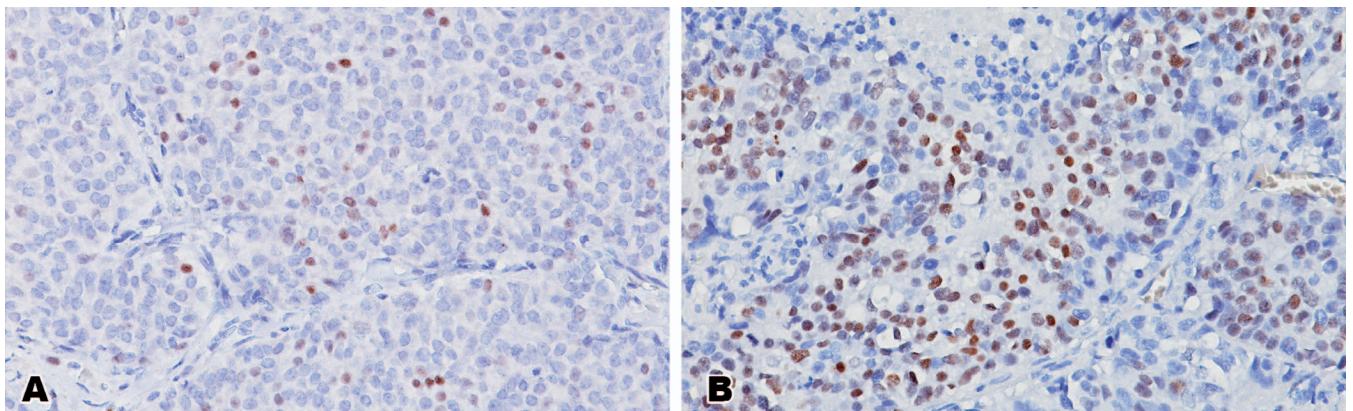


Figure 3. *m/hASH1* expression in (A) breast carcinomas, and (B) atypical lung carcinoids (original magnification $\times 20$).

Overall, m/hASH1 expression was highly specific to neuroendocrine tumors, with the exception of pancreatic neuroendocrine tumors, where merely 4.3% of specimens were positive (Table 2), and typical carcinoids in lung, which were all negative (0 of 4; Table 3). m/hASH1 expression was especially specific to SCLC versus nonneuroendocrine NSCLC ($P < .001$; Tables 3 and 4). Although NSCLC atypical carcinoids were positive (42.9%; cases had >10% positive cells) for mASH1, they stained less frequently than LCNEC (66.7%, $P < .001$) (Table 3) and SCLC, consistent with previous reports.^{3,6} Each LCNEC case was stained with synaptophysin and 9 of 9 cases were positive. Low expression of synaptophysin was observed in 3 of 9 positive cases of LCNEC that corresponded to the same mASH1-negative cases, and in 2 of those negative cases, a few tumor cells were positive for mASH1 (<1%). Our results are consistent with Hiroshima et al³ and Jiang et al,⁶ where m/hASH1 expression was observed in 58.9% (10 of 17) and 71.8% (56 of 78) of LCNECs, respectively, whereas expression was absent in classic large cell carcinoma.

mASH1 stained only 1.1% (1 of 93) and 0.9% (1 of 114) of lung adenocarcinoma and lung squamous cell carcinoma cases, respectively (Table 3). The lung adenocarcinoma was confirmed by immunohistochemistry to be TTF-1/napsin A positive and the lung squamous cell carcinoma was negative for TTF-1/napsin A and positive for p40. This kind of observation was also seen by Ionescu et al²²; however, the authors concluded that NSCLC with neuroendocrine differentiation should not be a subclass distinct from the other NSCLCs because the disease-specific characteristics and overall patient survival were not influenced by neuroendocrine differentiation.

On a final note, TTF-1 has been used to stain the majority of lung adenocarcinomas and has also been shown to stain SCLC; however, multiple studies have shown TTF-1 expression in lung carcinoids and LCNEC.^{21,23,24} Therefore, the ubiquity of TTF-1 expression in pulmonary carcinoids and HGNECs argues against its use in their subclassification. Ultimately, studies will be required for the rare types of neuroendocrine cancers not presented in this study. However, although not organ specific, mASH1 was highly specific for HGNECs versus carcinoids and other non-neuroendocrine neoplasms including NSCLC in the lung.

References

1. DeLellis RA. The neuroendocrine system and its tumors: an overview. *Am J Clin Pathol*. 2001;115(suppl):S5–S16.
2. La Rosa S, Marando A, Gatti G, et al. Achaete-scute homolog 1 as a marker of poorly differentiated neuroendocrine carcinomas of different sites: a validation study using immunohistochemistry and quantitative real-time polymerase chain reaction on 335 cases. *Hum Pathol*. 2013;44(7):1391–1399.

3. Hiroshima K, Iyoda A, Shida T, et al. Distinction of pulmonary large cell neuroendocrine carcinoma from small cell lung carcinoma: a morphological, immunohistochemical, and molecular analysis. *Mod Pathol*. 2006;19(10):1358–1368.
4. Ball DW, Azzoli CG, Baylin SB, et al. Identification of a human achaete-scute homolog highly expressed in neuroendocrine tumors. *Proc Natl Acad Sci U S A*. 1993;90(12):5648–5652.
5. Shida T, Furuya M, Kishimoto T, et al. The expression of NeuroD and mASH1 in the gastroenteropancreatic neuroendocrine tumors. *Mod Pathol*. 2008;21(11):1363–1370.
6. Jiang SX, Kameya T, Asamura H, et al. hASH1 expression is closely correlated with endocrine phenotype and differentiation extent in pulmonary neuroendocrine tumors. *Mod Pathol*. 2004;17(2):222–229.
7. Ralston J, Chiriboga L, Nonaka D. MASH1: a useful marker in differentiating pulmonary small cell carcinoma from Merkel cell carcinoma. *Mod Pathol*. 2008;21(11):1357–1362.
8. Gould VE, Wiedenmann B, Lee I, et al. Synaptophysin expression in neuroendocrine neoplasms as determined by immunocytochemistry. *Am J Pathol*. 1987;126(2):243–257.
9. Wilson BS, Lloyd RV. Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. *Am J Pathol*. 1984;115(3):458–468.
10. Yun JP, Xiang J, Hou JH, Tian QH, Fu J. Expression of CD56, as a potential diagnostic marker, in small cell carcinoma [in Chinese]. *Ai Zheng*. 2005;24(9):1140–1143.
11. Zheng G, Ettinger DS, Maleki Z. Utility of the quantitative Ki-67 proliferation index and CD56 together in the cytologic diagnosis of small cell lung carcinoma and other lung neuroendocrine tumors. *Acta Cytol*. 2013;57(3):281–290.
12. Sippel RS, Carpenter JE, Kunnimalaiyaan M, Chen H. The role of human achaete-scute homolog-1 in medullary thyroid cancer cells. *Surgery*. 2003;134(6):866–871; discussion 871–873.
13. Brelinska R, Ostalska D, Zabel M. Subtypes of thymic epithelial cells defined by neuroendocrine markers. *Histochem Cell Biol*. 2000;114(3):239–244.
14. Kuo TT. Frequent presence of neuroendocrine small cells in thymic carcinoma: a light microscopic and immunohistochemical study. *Histopathology*. 2000;37(1):19–26.
15. Miettinen M, Cupo W. Neural cell adhesion molecule distribution in soft tissue tumors. *Hum Pathol*. 1993;24(1):62–66.
16. Kim DH, Sohn JH, Lee MC, et al. Primary synovial sarcoma of the kidney. *Am J Surg Pathol*. 2000;24(8):1097–1104.
17. Teramachi K, Kanomata N, Hasebe T, Ishii G, Sugito M, Ochiai A. Carcinosarcoma (pure endocrine cell carcinoma with sarcoma components) of the stomach. *Pathol Int*. 2003;53(8):552–556.
18. Bahrami A, Gown AM, Baird GS, Hicks MJ, Folpe AL. Aberrant expression of epithelial and neuroendocrine markers in alveolar rhabdomyosarcoma: a potentially serious diagnostic pitfall. *Mod Pathol*. 2008;21(7):795–806.
19. Somasundaram K, Reddy SP, Vinnakota K, et al. Upregulation of ASCL1 and inhibition of Notch signaling pathway characterize progressive astrocytoma. *Oncogene*. 2005;24(47):7073–7083.
20. Bogina G, Munari E, Brunelli M, et al. Neuroendocrine differentiation in breast carcinoma: clinicopathological features and outcome. *Histopathology*. 2016;68(3):422–432.
21. Wachter DL, Hartmann A, Beckmann MW, et al. Expression of neuroendocrine markers in different molecular subtypes of breast carcinoma. *Biomed Res Int*. 2014;2014:408459.
22. Ionescu DN, Treaba D, Gilks CB, et al. Nonsmall cell lung carcinoma with neuroendocrine differentiation—an entity of no clinical or prognostic significance. *Am J Surg Pathol*. 2007;31(1):26–32.
23. Folpe AL, Gown AM, Lamps LW, et al. Thyroid transcription factor-1: immunohistochemical evaluation in pulmonary neuroendocrine tumors. *Mod Pathol*. 1999;12(1):5–8.
24. La Rosa S, Chiaravalli AM, Placidi C, Papanikolaou N, Cerati M, Capella C. TTF1 expression in normal lung neuroendocrine cells and related tumors: immunohistochemical study comparing two different monoclonal antibodies. *Virchows Arch*. 2010;457(4):497–507.