Expression of SATB2 in neuroendocrine carcinomas of the lung: frequent immunopositivity of large cell neuroendocrine carcinoma with a diagnostic pitfall

Introduction

According to the histological classification of the World Health Organization (WHO),[1] lung cancer has different histological types, such as adenocarcinoma and squamous cell carcinoma, neuroendocrine neoplasms, and metastatic tumors, for which various treatment options, including surgical resection and radiation therapy as well as anticancer agents, thymidine kinase inhibitors, and immune checkpoint inhibitors, are available. Because therapeutic choices and results differ greatly depending on the histological type and the related genotype, making accurate histopathological diagnosis is of primary importance, for which immunohistochemical marker studies are often useful and considered to be essential.

Neuroendocrine neoplasms of the lung are classified into small cell lung carcinoma (SCLC), large cell neuroendocrine carcinoma (LCNEC), carcinoid tumor, and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia.[1] SCLC and LCNEC are collectively referred to as neuroendocrine carcinoma (NEC), and noted for the poor prognosis of affected patients, with the positivity of some immunohistochemical neuroendocrine markers being a hallmark for their histopathological diagnosis.

Recent studies have found that some neuroendocrine neoplasms of various organs, including the lung, are immunohistochemically positive for special AT-rich sequence-binding protein 2 (SATB2) and caudal-type homeobox 2 (CDX2), both of which are known as positive markers for colorectal cancer.[2-4] Considering that the most frequent type of metastatic cancer of the lung is of colorectal origin,[5] the expression of STAB2 and/or CDX2 can be a misleading factor for histopathological differential diagnosis between pulmonary NEC and metastatic colorectal cancer. However, their expression in NEC of the lung has not been sufficiently elucidated, and there is no known previous report showing any differences in the positive rate of SATB2 between SCLC and LCNEC, nor any studies that examined the relationship between CDX2 and SATB2 expression in pulmonary NEC. In an attempt to clarify these points, the present investigation was conducted to evaluate the expression of SATB2 and CDX2 in a series of SCLC and LCNEC cases, also with some related immunohistochemical marker studies and some attention to the relationship to demographic and clinical factors of the patients.

Materials and Methods

Patients

The records of 47 cases of pure SCLC treated from January 1, 2016 to July 31, 2019 were obtained from the database of our university's branch hospital. All pathologic specimens associated with those were obtained by transbronchial biopsy. We reviewed all available histologic slides according to the WHO diagnostic criteria.[1] The samples in which the number of tumor cells was less than 100 or the cells were

significantly crushed were considered to be inappropriate for the analysis and excluded. As a result, 45 of those cases of SCLC were enrolled in this study.

Nine cases of pure LCNEC treated from January 1, 2015 to July 31, 2019 were also obtained from the same database. After reviewing all available slides obtained for histology, three cases were excluded because a diagnosis of small cell carcinoma was favored. Additionally, four cases of pure LCNEC treated during the same period were obtained from the database of our university hospital, the histopathologic diagnosis of which was confirmed by reviewing all available histologic slides. Thus 10 LCNEC cases in total were enrolled finally. All LCNEC specimens in those cases were obtained by surgery.

The demographic and clinical data were obtained from the medical record of each patient, including age, sex, smoking habit (Brinkman Index: BI), serum tumor marker values (CEA, carcinoembryonic antigen; CYFRA, cytokeratin fragment 19; NSE, neuron specific enolase; ProGRP, pro-gastrin releasing peptide), the number of times anticancer drugs was administered, ECOG Performance status, and UICC tumor stage.

Immunohistochemistry

All specimens were routinely fixed in 10% buffered formalin and embedded in paraffin. One representative slide was selected from each case, for which immunohistochemistry for SATB2, CDX2, thyroid transcription factor-1 (TTF-1), CD56, synaptophysin, chromogranin A, and cytokeratin 20 (CK20) was performed. The antibodies used were as follows: SATB2 (SANTA CRUZ, Dallas, TX, USA; clone SATBA4B10, 1:200 dilution), CDX2 (Dako, Santa Clara, CA, USA; clone DAK-CDX2, 1:50 dilution), CD56 (NICHIREI BIOSCIENCES INC., Tokyo, Japan; clone MRQ-42, ready to use), TTF-1 (Dako; clone 8G7G3/1, 1:100 dilution), Chromogranin A (Dako; clone DAK-A3, 1:200 dilution), Synaptophysin (JAPAN TANNER CORPORATION, Osaka, Japan; clone SP11, ready to use), and CK20 (Dako; clone Ks20.8, ready to use). Immunohistochemistry for CK20 was performed using Omnis (Dako), while Autostainer Link 48 (Dako) was utilized for the other antibodies, according to the instructions of each manufacturer.

Immunohistochemistry results were evaluated and the proportion of the positive extent was scored (proportion score) as 0 (negative), 1 (1-9%), 2 (10-49%), or 3 (>50%). Proportion scores of 2 and 3 were regarded as positive. The staining intensity was not considered because there existed no ambiguous intensity cases to evaluate.

Statistical analysis

For the immunohistochemistry with each antibody, the difference of the positivity based on the proportion score between SCLC and LCNEC was tested by Fisher's exact test on 2×2 tables.
For the demographic and clinical data of the SCLC cases, each variable was divided into two categories as shown in the following each parenthesis: age (< 65, ≥ 65), sex (male, female), BI (< 600, ≥ 600), CEA ($\leq$

5 ng/ml, >5 ng/ml), CYFRA (≤3.5 ng/ml, >3.5 ng/ml), NSE (≤16.3 ng/ml, >16.3 ng/ml), ProGRP (≤81 pg/ml, >81 pg/ml), the number of times anticancer drugs was administered (≥ 4, < 4), ECOG Performance status (0~1, 2~4), and UICC tumor stage (I~III, IV). The relationship between each variable divided into two categories and the SATB2 positivity based on the proportion score was also tested by Fisher's exact test on 2×2 tables for the SCLC cases. The comparison of overall survival between SATB2 positive-cases and negative cases in small cell carcinoma was evaluated by the Kaplan-Meier method with the log-rank test.

A P-value < 0.05 was considered to indicate the statistical significance. SPSS for Windows version 26 (IBM, Armonk, NY) was used for all statistical analyses.

Results

Demographics of the patients

The average age of the 45 SCLC patients at the time of diagnosis was 70.3 years (range 48-84 years), of whom 36 (80%) were male and nine (20%) were female. The 10 LCNEC patients, nine males and one female, had an average age of 73.5 years (range 65-84 years) at the time of diagnosis.

All SCLC patients were smokers with BI ≥ 600 in 37 out of 45 patients (82%). As for LCNEC, all patients were smokers with BI ≥ 600.

Histopathologic findings

The tumor cells of SCLC showed scant cytoplasm and finely granular and hyperchromatic nuclei, which formed irregular-shaped sheetlike nests (Fig. 1A). Those of LCNEC had abundant eosinophilic cytoplasm and vesicular nuclei with prominent nucleoli, which formed sheetlike nests characterized by the cell arrangement of rosette-like and/or peripheral palisading patterns (Fig. 1B).

Immunohistochemical profiles

Immunohistochemistry results with each antibody are summarized in Table 1. In the SCLC cases, the positive rate of TTF-1, CD56, synaptophysin, and chromogranin A was 64.4%, 95.6%, 71.1%, and 46.7%, respectively, while in the LCNEC cases, it was 50%, 100%, 47.9% and 100%, respectively. All except for two SCLC cases were positive for at least one of the three neuroendocrine markers (Fig. 1C, D).

17 out of 45 (37.8%) SCLC cases (Fig. 2A) and seven out of 10 (70%) LCNEC ones (Fig. 2B) were positive for SATB2. Although the positive rate seemed to be higher in the LCNEC cases than in the SCLC ones, no significant difference was achieved between them statistically (*P* = 0.084).

All SCLC cases were negative for CDX2 (Fig. 2C), whereas two LCNEC cases (20%) were CDX2 positive (Fig. 2D), the difference of which was statistically significant (*P* = 0.025). Furthermore, one LCNEC case was positive for CDX2 as well as SATB2 (Fig. 2B, D).

3

CK20 was negative in all SCLC and LCNEC cases.

Relationship between demographic/clinical variables and SATB2 expression

For the SCLC cases, the relationship between the demographic/clinical data and the SATB2 expression is shown in Table 2. Of the demographic and clinical variables examined, only high-BI ($\geq$ 600) was significantly related to the positivity of SATB2 (P = 0.017), in which the SATB2 positivity was noticed more frequently in the high-BI cases (17 out of 37, 45.9%) then in the low-BI ones (0 out of 8, 0%).

As for the comparison of overall survival between SATB2-positive cases and SATB2-negative cases in SCLC, no statistical difference was found (p=0.558, Fig.3).

Discussion

The present results confirmed the SATB2 expression in some pulmonary NECs, as noted in 17 out of 45 (37.8%) SCLC cases and seven out of 10 (70%) LCNEC ones. Although the difference was not statistically significant (P = 0.084), the LCNEC cases tended to have a higher positive rate compared with the SCLC cases. As for CDX2, two LCNEC cases were positive, while all SCLC cases were negative, and the difference of the two was statistically significant (P = 0.025) though the sensitivity of CDX2 as a positive marker in the LCNEC cases was low. Thus, the present results suggest that in pulmonary NECs, LCNECs express colorectal markers such as SATB2 and CDX2 more frequently as compared to SCLCs. The positive rates of immunohistochemical markers other than SATB2 and CDX2 in the present study were similar to those noted in previous reports,[6,7] which was considered to demonstrate that the specimens in this study were not extremely biased.

Along with the understanding of the molecular biological mechanism of the lung carcinogenesis in recent years, treatment strategies have become subdivided according to the histopathological diagnosis and the related genetic mutation status, making accurate histopathological diagnosis primarily important. In addition, the lungs represent the most frequent target of metastasis. Of 82,193 cases of chest surgery performed in 2016 in Japan, surgery for metastatic lung tumors accounted for 8,497 (10.3%), including colorectal cancer (47.7%), renal cancer (8.5%), and lung cancer metastasis (2.6%), in descending order of frequency.[5] Therefore, it is not an uncommon situation that histopathological differential diagnosis between primary pulmonary cancer and metastatic colorectal cancer is required, especially when the patient have a past history of colorectal cancer. This is also the case for patients with pulmonary NECs.

In general, a pulmonary NEC can be distinguished from a metastatic colorectal adenocarcinoma based on the morphological findings. However, the discrimination could be difficult when typical morphological features of neuroendocrine neoplasms, such as rosette-like and/or nuclear palisading patterns, cannot be recognized. Furthermore, cases with a past history of poorly differentiated colorectal adenocarcinoma present additional challenges. For these confusing situations, the examination of immunohistochemical

markers is mandatory. However, the positive staining of SATB2 and CDX2 in pulmonary NEC specimens can lead to a misdiagnosis that the tumor is of metastatic colorectal adenocarcinoma. Therefore, it is important to recognize that some pulmonary NECs, especially LCNECs, are frequently positive for SATB2 and less frequently CDX2, and to understand that the expression of neuroendocrine markers should be concurrently checked. The sufficient positivity of neuroendocrine markers will favor a diagnosis of NEC in spite of the expression of SATB2 and/or CDX2.

SATB2, a protein comprised of 733 amino acids and encoded by the SATB2 gene located on chromosome 2q33.1, binds to DNA, and transcription factors regulate its expression in the nucleus, which have important roles in the developmental process such as facial development, neocortical differentiation, skeletal development, and osteoblast differentiation.[8-11] In normal tissues, the protein is strongly expressed in the epithelium of the lower gastrointestinal tract, cerebral cortex, and hippocampus.[12] With regard to the expression in neoplasms, it was initially focused that SATB2 was immunohistochemically positive in a large majority of adenocarcinomas of the lower gastrointestinal tract (86-97%).[12-14] However, high positive rates of SATB2 have also been reported in rectosigmoid neuroendocrine tumor (NET) (96-100%), appendiceal NET (79-100%), and Merkel cell carcinoma (79%) recently.[2-4] Other studies analyzing pulmonary SCLC or pulmonary NEC (details not shown) have shown the positive rate of SATB2 ranging from 21 to 33%.[2,4] Besides, a most recent study has reported the expression of SATB2 in pulmonary and thymic neuroendocrine tumors (carcinoid tumor and NEC), with a higher percentage of expression in SCLC comparing to the previous reports.[15] In the present study, the positive rate of SATB2 in SCLC cases was similar to some previous studies,[2,4] while the present study has also revealed that LCNEC tends to have a higher positive rate of SATB2 than SCLC. Considering that SATB2 is expressed in the cerebral cortex and hippocampus tissues, it may be plausible that the SATB2 expression is involved in the neural or neuroendocrine differentiation of tumors derived from various organs, which, however, is to be elucidated by further studies.

CDX2 is a protein encoded by the CDX2 gene in chromosome 13q12.2, a homeobox gene that plays a role in differentiation of normal intestinal epithelium and neural tube closure in vertebrates.[15-17] In normal tissues, CDX2 is expressed in the pancreatic duct and pancreatic centroacinar cells, as well as the digestive tract, but not in other organs including the lungs,[18] while another role of CXD2, the suppression of tumor growth, has also been noted.[19] As for pulmonary NEC, previous studies have reported that the positive rates of CDX2 are 0-15% in SCLC and 31% in LCNEC,[20-23] with the same tendency observed in our results.

Although both SATB2 and CDX2 have been regarded as markers for colorectal cancers, the expression of SATB2 and/or CDX2 in pulmonary NEC does not seem to indicate simple intestinal differentiation as discussed above as well as because all cases in the present study were negative for CK20 that is a well-known intestinal epithelial marker.[24] Here, an interesting point to consider is that SATB2 and CDX2 expressions have been observed even in conventional pulmonary adenocarcinomas, with the reported frequency of 3-7.2% for SATB2,[12,13,25] and 0-11% for CDX2,[21,23,25] respectively. On the other hand, the

positive rates in pulmonary adenocarcinomas with an enteric morphology such as pulmonary enteric adenocarcinoma have been shown to be higher, that is, 13-15.4% for SATB2,[26,27] and 61.5-71.3% for CDX2,[26-29] respectively. These results imply divergent roles of SATB2 and CDX2 in regard to differentiation of pulmonary tumors.

It has been well known that lung carcinogenesis is strongly related to the habit of tabaco smoking, and that is especially the case for SCLC.[31-32] Interestingly, in the present study, all SATB2-positive SCLC cases had a history of heavy smoking (BI ≥ 600), which was significantly more frequent compared to SATB2-negative SCLC cases. Recently, the biological heterogeneity of SCLC has emergently been noted, and four subtypes of SCLC have been proposed based on the expression of transcription factors and co-transcription factors in the mRNA level (ASCL1, NEUROD1, YAP1, and POU2F3), which has also been evaluated in the protein level.[33-35] Of these factors, ASCL1 and NEUROD1 are related to the regulation of neuroendocrine differentiation. Considering the possibility that the expression of SATB2 is involved in the neuroendocrine differentiation, further investigation of SATB2 as a factor reflecting a subtype of SCLC in relation to smoking may be needed, although the expression of SATB2 does not seem to be directly related to the prognosis of patients.[15]

Conclusion

A limitation of the present study is the relatively small number of LCNEC cases, thus additional analysis with a larger cohort will be necessary. Nevertheless, our results showed that some pulmonary NECs, especially LCNEC, are positive for SATB2 in immunohistochemistry findings, which should be kept in mind when SATB2 is used as a marker for diagnosis of metastatic colorectal cancer. Besides, further investigation of the additional expected clinical significance of SATB2 expression in pulmonary NECs might be needed.

Conflict of Interest:None

References

1   Travis WD, International Agency for Research on C. WHO classification of tumours of the lung, pleura, thymus and heart. Lyon: International Agency for Research on Cancer; 2015.

2   Bellizzi AM. SATB2 in neuroendocrine neoplasms: strong expression is restricted to well-

differentiated tumours of lower gastrointestinal tract origin and is most frequent in Merkel cell carcinoma among poorly differentiated carcinomas. Histopathology 2020;76:251-64.

3   Fukuhara M, Agnarsdottir M, Edqvist PH, Coter A, Ponten F. SATB2 is expressed in Merkel cell carcinoma. Arch Dermatol Res 2016;308:449-54.

4   Hoskoppal D, Epstein JI, Gown AM, Arnold Egloff SA, Gordetsky JB, Shi CJ, et al. SATB2 protein expression by immunohistochemistry is a sensitive and specific marker of appendiceal and rectosigmoid well differentiated neuroendocrine tumours. Histopathology 2020;76:550-9.

5   Committee for Scientific Affairs TJAfTS, Shimizu H, Endo S, Natsugoe S, Doki Y, Hirata Y, et al. Thoracic and cardiovascular surgery in Japan in 2016 : Annual report by The Japanese Association for Thoracic Surgery. Gen Thorac Cardiovasc Surg 2019;67:377-411.

6   Bellizzi AM. Assigning site of origin in metastatic neuroendocrine neoplasms: a clinically significant application of diagnostic immunohistochemistry. Adv Anat Pathol 2013;20:285-314.

7   Yatabe Y, Dacic S, Borczuk AC, Warth A, Russell PA, Lantuejoul S, et al. Best Practices Recommendations for Diagnostic Immunohistochemistry in Lung Cancer. J Thorac Oncol 2019;14:377-407.

8   Britanova O, Akopov S, Lukyanov S, Gruss P, Tarabykin V. Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS. Eur J Neurosci 2005;21:658-68.

9   Gyorgy AB, Szemes M, de Juan Romero C, Tarabykin V, Agoston DV. SATB2 interacts with chromatin-remodeling molecules in differentiating cortical neurons. Eur J Neurosci 2008;27:865-73.

10   Dobreva G, Chahrour M, Dautzenberg M, Chirivella L, Kanzler B, Farinas I, et al. SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. Cell 2006;125:971-86.

11   Dobreva G, Dambacher J, Grosschedl R. SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin mu gene expression. Genes Dev 2003;17:3048-61.

12   Magnusson K, de Wit M, Brennan DJ, Johnson LB, McGee SF, Lundberg E, et al. SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. Am J Surg Pathol 2011;35:937-48.

13   Lin F, Shi J, Zhu S, Chen Z, Li A, Chen T, et al. Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. Arch Pathol Lab Med 2014;138:1015-26.

14   Dragomir A, de Wit M, Johansson C, Uhlen M, Ponten F. The role of SATB2 as a diagnostic marker for tumors of colorectal origin: Results of a pathology-based clinical prospective study. Am J Clin Pathol 2014;141:630-8.

15. Vrana, JA, Boland, JM, Terra, S, et al. SATB2 Is Expressed in a Subset of Pulmonary and Thymic Neuroendocrine Tumors. *Am J Clin Pathol*. May 12 2021;epub ahead of print

16 Suh E, Traber PG. An intestine-specific homeobox gene regulates proliferation and differentiation. Mol Cell Biol 1996;16:619-25.

17 Lorentz O, Duluc I, Arcangelis AD, Simon-Assmann P, Kedinger M, Freund JN. Key role of the Cdx2 homeobox gene in extracellular matrix-mediated intestinal cell differentiation. J Cell Biol 1997;139:1553-65.

18 Savory JG, Mansfield M, Rijli FM, Lohnes D. Cdx mediates neural tube closure through transcriptional regulation of the planar cell polarity gene Ptk7. Development 2011;138:1361-70.

19 Moskaluk CA, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF, Jr. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. Mod Pathol 2003;16:913-9.

20 Sivagnanasundaram S, Islam I, Talbot I, Drummond F, Walters JR, Edwards YH. The homeobox gene CDX2 in colorectal carcinoma: a genetic analysis. Br J Cancer 2001;84:218-25.

21 Bari MF, Brown H, Nicholson AG, Kerr KM, Gosney JR, Wallace WA, et al. BAI3, CDX2 and VIL1: a panel of three antibodies to distinguish small cell from large cell neuroendocrine lung carcinomas. Histopathology 2014;64:547-56.

22 Barbareschi M, Murer B, Colby TV, Chilosi M, Macri E, Loda M, et al. CDX-2 homeobox gene expression is a reliable marker of colorectal adenocarcinoma metastases to the lungs. Am J Surg Pathol 2003;27:141-9.

23 Lee H, Fu Z, Koo BH, Sheehan CE, Young GQ, Lin J, et al. The expression of TTF1, CDX2 and ISL1 in 74 poorly differentiated neuroendocrine carcinomas. Ann Diagn Pathol 2018;37:30-4.

24 Vidarsdottir H, Tran L, Nodin B, Jirstrom K, Planck M, Jonsson P, et al. Immunohistochemical profiles in primary lung cancers and epithelial pulmonary metastases. Hum Pathol 2019;84:221-30.

25 Moll R, Lowe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. Am J Pathol 1992;140:427-47.

26 Kriegsmann M, Harms A, Longuespee R, Muley T, Winter H, Kriegsmann K, et al. Role of conventional immunomarkers, HNF4-alpha and SATB2, in the differential diagnosis of pulmonary and colorectal adenocarcinomas. Histopathology 2018;72:997-1006.

27 Matsushima J, Yazawa T, Suzuki M, Takahashi Y, Ota S, Nakajima T, et al. Clinicopathological, immunohistochemical, and mutational analyses of pulmonary enteric adenocarcinoma: usefulness of SATB2 and beta-catenin immunostaining for differentiation from metastatic colorectal carcinoma. Hum Pathol 2017;64:179-85.

28 Bian T, Zhao J, Feng J, Zhang Q, Qian L, Liu J, et al. Combination of cadherin-17 and SATB homeobox 2 serves as potential optimal makers for the differential diagnosis of pulmonary enteric

adenocarcinoma and metastatic colorectal adenocarcinoma. Oncotarget 2017;8:63442-52.

29   Inamura K, Satoh Y, Okumura S, Nakagawa K, Tsuchiya E, Fukayama M, et al. Pulmonary adenocarcinomas with enteric differentiation: histologic and immunohistochemical characteristics compared with metastatic colorectal cancers and usual pulmonary adenocarcinomas. Am J Surg Pathol 2005;29:660-5.

30   Wang CX, Liu B, Wang YF, Zhang RS, Yu B, Lu ZF, et al. Pulmonary enteric adenocarcinoma: a study of the clinicopathologic and molecular status of nine cases. Int J Clin Exp Pathol 2014;7:1266-74.

31.   Bade, BC, Dela Cruz, CS. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. *Clin Chest Med*. Mar 2020;41(1):1-24.

32   Ou SH, Ziogas A, Zell JA. Prognostic factors for survival in extensive stage small cell lung cancer (ED-SCLC): the importance of smoking history, socioeconomic and marital statuses, and ethnicity. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2009;4:37–43.

33.   Baine, MK, Hsieh, MS, Lai, WV, et al. SCLC Subtypes Defined by ASCL1, NEUROD1, POU2F3, and YAP1: A Comprehensive Immunohistochemical and Histopathologic Characterization. *J Thorac Oncol*. Dec 2020;15(12):1823-1835.

34.   Lantuejoul, S, Fernandez-Cuesta, L, Damiola, F, Girard, N, McLeer, A. New molecular classification of large cell neuroendocrine carcinoma and small cell lung carcinoma with potential therapeutic impacts. *Transl Lung Cancer Res*. Oct 2020;9(5):2233-2244.

35.   Sato, Y, Okamoto, I, Kameyama, H, et al. Integrated Immunohistochemical Study on Small-Cell Carcinoma of the Lung Focusing on Transcription and Co-Transcription Factors. *Diagnostics (Basel)*. Nov 13 2020;10(11): 949