Pulmonary Neuroendocrine Cells and Small Cell Lung Carcinoma: Immunohistochemical Study Focusing on Mechanisms of Neuroendocrine Differentiation

Takaaki Ito1,2, Shinji Kudoh2, Kosuke Fujino2,3, Mune Sanada2,3, Yuki Tenjin2,4, Haruki Saito2,5, Yuko Nakaishi-Fukuchi1,2, Hiroki Kameyama1, Takaya Ichimura6, Naoko Udaka7, Noritaka Kudo2, Akira Matsuo2 and Younosuke Sato2

1Department of Medical Technology, Faculty of Health Science Kumamoto Health Science University, Kumamoto, Japan, 2Department of Pathology and Experimental Medicine, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan, 3Department of Respiratory Surgery, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan, 4Department of Respiratory Medicine, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan, 5Department of Otorhinolaryngology and Head and Neck Surgery, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan, 6Department of Pathology, Saitama Medical School, Saitama, Japan and 7Division of Surgical Pathology, Yokohama City University Hospital, Yokohama, Japan

Received March 28, 2022; accepted April 12, 2022; published online May 24, 2022

Neuroendocrine (NE) differentiation has been histochemically detected in normal and cancer tissues and cells. Immunohistochemical analyses have provided a more detailed understanding of NE biology and pathology. Pulmonary NE cells are a rare lung epithelial type, and small cell carcinoma of the lung (SCLC) is a high-grade NE tumor. Pulmonary NE and SCLC cells share common mechanisms for NE differentiation. Neural or NE cell lineage-specific transcription factors, such as achaete-scute homologue 1 (Ascl1) and insulinoma-associated protein 1 (INSM1), are crucial for the development of pulmonary NE cells, and NE differentiation is influenced by the balance between Ascl1 and the suppressive neural transcription factor, hairy-enhancer of split 1, a representative target molecule of the Notch signaling pathway.

In this review, we discuss the importance of Ascl1 and INSM1 in identifying pulmonary NE and SCLC cells and introduce Ascl1-related molecules detected by comparative RNA-sequence analyses. The molecular classification of SCLC based on the expression of lineage-specific transcription or co-transcription factors, including ASCL1, NEUROD1, POU2F3, and YAP1, was recently proposed. We attempted to characterize these 4 SCLC subtypes using integrated immunohistochemical studies, which will provide insights into the molecular characteristics of these subtypes and clarify the inter- and intratumor heterogeneities of SCLC.

Key words: pulmonary neuroendocrine cells, Ascl1, small cell lung carcinoma, molecular classification, immunohistochemistry

I. Introduction

The lung is an organ with the primary role of facilitating gas exchange between inspired air and circulating blood, and other functions include air conduction, the clearance of inhaled foreign materials, hormonal regulation, and the metabolism of xenobiotics. A well-organized, but complex, epithelial system is constructed in the airways and
alveoli to maintain these functions. At least eight principal types of airway epithelial cells have been identified in mammals: ciliated cells, basal cells, neuroendocrine (NE) cells, mucous goblet cells, club cells, serous cells, small mucous granule cells, and brush (tuft) cells [27]. Pulmonary NE cells (PNEC) are a rare and distinct cell type that is located throughout the airways and alveolar duct epithelium. Historically, PNEC were detected as clear cells (Helle Zellen) and considered to belong to a widespread endocrine system [17]. These cells were subsequently found to have an argyrophilic cytoplasm [18, 19]. The development of advances in ultrastructural research made a significant contribution to our understanding of PNEC. Bensch et al. [6] reported the presence of neurosecretory granules in human PNEC. Lauweryns et al. [40–42] investigated the PNEC of animals, described the innervation of clustered PNEC, and called the innervated structure “neuroepithelial bodies”. The unified classification of “Feyrter bodies” was subsequently developed, and the amine precursor uptake and decarboxylation (APUD) concept was proposed based on the common ultrastructural features of these cells and their ability to take up the active peptides present in neurons [49, 50]. The APUD family was suggested to include cells of the anterior pituitary gland and hypothalamus, pinealocytes, carotid body chief cells, thyroid C cells, lung and gastrointestinal NE cells, pancreatic islet cells, Merkel cells, melanocytes, endocrine cells of the placenta and thymus, and sympathetic ganglia cells, such as cells of the adrenal medulla. Since some of these cells lack APUD properties and the neural crest hypothesis of the APUD family was not always true, APUD was replaced with the term “diffuse NE system” [51]. With advances in immunohistochemical methods, more NE markers have been proposed, and a large number appear to be related to organelles that are specifically involved in NE differentiation and physiological functions, including cell membrane-specific proteins, dense core granule-related proteins, cytoplasmic enzymes, amine and peptide hormones, and cytoskeletal proteins [12, 15, 26, 30].

II. Immunohistochemical Features of Small Cell Lung Carcinoma

After the establishment of the APUD concept, the diffuse NE system concept in normal organs and tissues, was extended to neoplasms in the diffuse NE system. The NE differentiation of lung neoplasms was initially investigated using ultrastructural and biochemical techniques. One of the ultrastructural features of NE differentiation is dense core granules, which are synaptic vesicle-like organelles for the transportation of secretory products. The NE differentiation of pulmonary carcinoids and small cell lung cancer (SCLC) was examined ultrastructurally based on the presence of dense core granules [7, 8, 25, 28]. Pulmonary NE tumors are a distinct family of lung cancers with shared morphological, ultrastructural, immunohistochemical, and molecular characteristics, and include various histological subtypes, such as classical and atypical carcinoids, large cell NE carcinoma, and SCLC [11]. SCLC is the most malignant type among this lung cancer family, and is characterized by rapid growth and metastasis as well as NE differentiation [11, 13, 52]. Immunohistochemistry is a very useful method for accurately diagnosing small cell carcinoma, and is performed to identify NE differentiation in tumors. Chromogranin A (CHGA), synaptophysin (SYP), and neural cell adhesion molecule (NCAM) have been used as reliable NE molecules for the diagnosis of small cell carcinoma [11, 59, 60]. We identified insulinoma-associated protein 1 (INSM1) as a crucial transcription factor for the NE differentiation of SCLC, and showed that it is an excellent immunohistochemical marker for NE differentiation in tissue samples [20]. The roles of proneural transcription factors, such as achaete-scute complex homologue 1 (Ascl1) and its regulator, the Notch signaling pathway, in the regulation of NE differentiation have been examined [5, 9, 35, 36, 45]. Ascl1 is a master regulator of NE differentiation [9] and a lineage-specific oncogene of SCLC [10, 23]. Recent studies proposed that SCLC may be subclassified into 4 molecular subtypes based on the expression of lineage-specific transcription and co-transcription factors, which include ASCL1, neurogenic differentiation factor 1 (NeuroD1), POU class 2 homeobox 3 (POU2F3), and yes-associated protein 1 (YAP1) [55], with ASCL1 being the most common subtype [4, 53, 55, 56].

The findings of immunohistochemical studies on PNEC and SCLC are discussed herein with a focus on ASCL1, a representative NE differentiation-regulating molecule.

III. Histochemical, Immunohistochemical, and Ultrastructural Features of PNEC

Prior to the development of immunohistochemistry, various histochemical methods were used to identify and characterize NE cells. Silver stains were applied to display argyrophil or argentaffin reactions. Various argyrophilic stains were formulated, including the Bodian, Grimelius silver nitrate and Pascual stains. These stains appeared to work well for gastrointestinal and pancreatic NE cells; however, difficulties were associated with obtaining reproducible data on airway NE cells. In our experience, the modified Pascual’s argyrophil stain [14] has been useful for identifying NE cells in the human fetal lungs (Fig. 1A) [31].

Various immunohistochemical markers for the detection of PNEC have been examined and applied to clinical settings. Until the 21st century, most of these markers have been discovered with regard to molecules associated with neural phenotypes; including cell membrane components, such as NCAM, somatostatin receptors, O2-sensor enzyme and G-protein oα (Fig. 1B) [33], cytoplasmic components, such as neuron-specific enolase, PGP9.5, and calcium-
binding proteins and neurofilaments, and secretory vesicle components, such as SYP, synaptobrevin, CHGA, calcitonin gene-related peptide, serotonin and gastrin-releasing peptide [12, 15, 26, 30]. On the other hand, nuclear markers for NE transcription factors, such as Ascl1 and INSM1, have more recently been used [9, 15, 34].

An ultrastructural analysis is a powerful tool for identifying PNEC because the detection of neurosecretory (dense core) granules is direct evidence of the cell type. Since brush (tuft) cells may be the origin of SCLC with low NE differentiation [29], the ultrastructures of PNEC and brush cells are presented for comparison in Figure 2 [32]. The morphological and physiological characterization of brush cells has not yet been performed in detail [54], and differences and similarities in the morphologies of PNEC and brush cells remain unclear; however, both may be the origin of SCLC. The size of the microvilli of these cells markedly differ, and some brush cells have sometimes small vesicular granules as PNECs though contents of the granules are unknown (Fig. 2).
IV. NE Differentiation Regulated by the Balance between Ascl1 and Hairy-enhancer of Split 1 (Hes1) in the Lung

The molecular mechanisms underlying the NE differentiation of lung epithelial cells have not yet been elucidated in detail. Nevertheless, the balance between basic helix-loop-helix (bHLH) transcription factors, such as Ascl1 and Hes1, is important for predicting the PNEC lineage fate in the lungs [9, 34]. The activation of Ascl1 is crucial for NE differentiation based on the finding showing that mice deficient for the Ascl1 gene lacked PNEC [9, 34]. In contrast, PNEC differentiation was promoted in Hes1 gene-deficient mice [34]. These findings indicate that Ascl1 induces PNEC in concert with Notch signaling pathways, including Hes1, thereby influencing the PNEC cell lineage (Fig. 3). However, the balance of these two bHLH factors alone does not affect NE differentiation in the lung epithelial system because the regulatory mechanisms involved in the differentiation and proliferation of PNEC are complex and include interactions between a number of intrinsic and extrinsic factors.

V. Significance of ASCL1 in the NE Differentiation of Lung Neoplasms

The significance of ASCL1 in the NE differentiation of the mouse fetal lung epithelium and human SCLC was initially reported by Borges et al. [9]. A mouse experimental study showed that the overexpression of Ascl1 in lung epithelial cells induced lung neoplasms with NE features [44]. ASCL1 gene transfection has been reported in human lung adenocarcinoma cell lines, and NE differentiation was detected in ASCL1 gene-transfected adenocarcinoma cells [20, 39, 45, 48]. After the transfection of the ASCL1 gene, SYP, CHGB, and secretogranin 2 were expressed by A549 adenocarcinoma cells [39, 48], CHGA, SYP, and NCAM by H1975 and H358 adenocarcinoma cells [20], and NCAM by PC9 adenocarcinoma cells [45]. In addition to NE differentiation, ASCL1 has been suggested to play various roles in cell growth, survival, migration, tumor initiation, and epithelial-mesenchymal transition [16, 36, 38, 39, 43, 47, 48, 57, 58]. ASCL1 is a lineage-specific oncogene of SCLC [10, 23]. SCLC has recently been subdivided into 4 main subtypes based on the expression of lineage-specific transcription and co-transcription factors [55], and SCLC with the dominant expression of ASCL1 is the most common subtype [4, 53, 55, 56]. Transcriptional targets of ASCL1 were discovered using chromatin immunoprecipitation sequencing (ChIP-seq) [3, 10], which revealed many target molecules of ASCL1. These target molecules include genes related to NE differentiation, cell survival [3], and tumorigenesis [10], which suggests that ASCL1 is a lineage-specific oncogene of SCLC. A ChIP-seq analysis of ASCL1 confirmed neural and NE differentiation-related target molecules, such as gastrin-releasing peptide, INSM1,
and potassium voltage-gated channel subfamily members [3, 10]. Zhang et al. [61] examined SCLC tumors and cell lines using a 50-gene expression-based NE score, developed based on expression array and RNA-seq data. SCLC cell lines may be subdivided into two groups: classical and variant. Classical SCLC cell lines are characterized by floating cell growth in medium and high NE differentiation, and variant SCLC cell lines by adherent growth and low NE differentiation [22, 61]. Notch activity and the loss of ASCL1 expression have been reported in variant SCLC with low NE differentiation [36, 61].

To clarify ASCL1-associated molecules, we generated ASCL1 gene-transfected A549 adenocarcinoma cell lines, which exhibited various biological phenotypes, in addition to the induction of NE differentiation [36, 39, 58]. These cells lost the adhesive growth pattern and grew with a floating growth pattern (Fig. 4A). They showed EMT phenotypes with changes in the morphology and expression of EMT-related molecules [36]. Furthermore, when xenotransplanted into skin on the backs of immunocompromised mice, these cells lost the glandular structure to gain an undifferentiated morphology (Fig. 4B). An RNA sequencing analysis of ASCL1 gene-transfected adenocarcinoma cell lines revealed a large number of ASCL1-induced molecules related to cell proliferation, enzymes, the extracellular matrix, cell membrane, and transcription factors as well as neural or neuroendocrine differentiation by Ascl1 transfection. B: For example, Potassium voltage-gated channel modifier subfamily F member 1 (KCNF1) and insulinoma-associated protein 1 (INSM1), ASCL1-related molecules, are applied to immunohistochemistry on tumor tissues from mock A549 adenocarcinoma cells and ASCL1 gene-transfected A549 cells (Ascl1-induced pulmonary neuroendocrine tumor) grown in immunocompromised mice, as well as surgically resected small cell lung carcinoma (SCLC) samples. Immunohistochemically, KCNF1 mainly localized to the cell membrane of ASCL1-induced pulmonary neuroendocrine tumors and SCLC samples, and INSM1 to their nuclei. Bar = 100 μm. C: INSM1 immunostaining is available for cytological samples. Bar = 100 μm.

Fig. 4. A: Ascl1-induced neuroendocrine differentiation is established by the transfection of the ASCL1 gene into an adenocarcinoma cell line (A549). Ascl1-induced pulmonary neuroendocrine tumors show neuroendocrine differentiation, as shown in RT-PCR for synaptophysin (SYP) and chromogranin A (CHGA). In phase-contrast photographs, ASCL1-transfected A549 adenocarcinoma cells show changes from an adhesive to floating morphology. Bar = 50 μm. RNA sequence analyses revealed many genes related to cell proliferation, signaling, enzymes, the extracellular matrix, cell membrane, and transcription factors as well as neural or neuroendocrine differentiation by Ascl1 transfection. B: For example, Potassium voltage-gated channel modifier subfamily F member 1 (KCNF1) and insulinoma-associated protein 1 (INSM1), ASCL1-related molecules, are applied to immunohistochemistry on tumor tissues from mock A549 adenocarcinoma cells and ASCL1 gene-transfected A549 cells (Ascl1-induced pulmonary neuroendocrine tumor) grown in immunocompromised mice, as well as surgically resected small cell lung carcinoma (SCLC) samples. Immunohistochemically, KCNF1 mainly localized to the cell membrane of ASCL1-induced pulmonary neuroendocrine tumors and SCLC samples, and INSM1 to their nuclei. Bar = 100 μm. C: INSM1 immunostaining is available for cytological samples. Bar = 100 μm.
and the 2 ASCL1-tansfected A549 cell lines but down-regulated in the 3 ASCL1-negative SCLC cell lines and the 2 mock A549 cell lines. These ASCL1-related molecules included INSM1, Islet1, synaptotagmin 4, Potassium channel tetramerization domain-containing protein 16, Seizure-related gene 6, Membrane-spanning 4-domain family, subfamily A8, and cordon-bleu WH2 repeat protein, and their involvement in the pathobiology of PNEC will be examined in the future [39].

VI. Immunohistochemical Analysis of Molecular Subtypes of SCLC

SCLC is a high-grade NE cancer that is characterized by rapid growth, early metastasis, high sensitivity to radiochemotherapy, the easy acquisition of chemoresistance after chemotherapy, and mutations in the TP53 and RB1 genes [2, 23, 24]. The development of more effective molecular targeted therapies is expected. Rudin et al. [55]
proposed molecular subtypes of SCLC based on the expression of four transcription or co-transcription factors: ASCL1, NEUROD1, POU2F3, and YAP1. According to Rudin et al., ASCL1-positive SCLC (SCLC-A) and NEUROD1-positive SCLC (SCLC-N) subtypes belong to the high NE differentiation group, and POU2F3-positive SCLC (SCLC-P) and YAP1-positive SCLC (SCLC-Y) to the low NE differentiation group. However, the expression of these molecules in SCLC is not mutually exclusive, and a few SCLC cases have only one molecule. In our immunohistochemical analysis using surgically resected samples [56], more than 50% of SCLC-A cases showed positive immunostaining for NEUROD1, POU2F3, or YAP1, while the other subtypes of SCLC rarely showed a single molecule (Fig. 5A). This combined expression of the four molecules has been supported by recent findings [4, 53]. Using the transcriptome data of Asian SCLC tissue samples from the GSE60052 (n = 79) RNA sequence dataset [37], we examined the relationships between the four molecules and some transcription and signal molecules. A summary of the correlation diagram of these molecules is shown in Figure 5B. SCLC-A cases were more likely to express INSM1, DLL3, WNT11, SOX2, and EZH2, but not NOTCH receptors, REST, or YAP-related molecules. SCLC-N cases showed the positive expression of INSM1 and WNT11, similar to SCLC-A, but were more likely to express MYC and IGF1R. SCLC-P cases expressed NOTCH receptors, GF11, and YAP-related molecules. In contrast to SCLC-A cases, SCLC-Y cases expressed NOTCH receptors, REST, and YAP-related molecules, but not INSM1, DLL3, SOX2, or EZH2 (Fig. 5B). Figure 5C shows the immunohistochemical findings of each molecular subtype. These findings often correspond with the public dataset analysis, but are not always accurate because positive staining for INSM1 has been reported in cases of the low-NE SCLC subtype (Fig. 5C). NE-lineage transcription factors, such as ASCL1 and INSM1, may be positively expressed in SCLC-P and SCLC-Y cases with NE differentiation. Although each subtype exhibits distinct vulnerability to therapeutic chemicals [21], the molecular subclassification of SCLC is not practically useful for treatment selection. In the near future, with the development of molecular target therapies, the immunohistochemical application of crucial molecules in tissue samples will provide valuable information for the assessment of cell biological and therapeutic issues in the diagnosis of SCLC.

VII. Declarations

Conflict of interests

The authors have no conflicts of interest to declare.

Ethical approval

All studies using human pathological samples followed the guidelines of the Ethics Committee of Kumamoto University (No. 342). All animal experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of Kumamoto University.

VIII. Acknowledgments

We thank Ms. Takako Maeda for her technical assistance and Mr. Wang G from Shandon University for his assistance with immunohistochemical techniques. The studies described in the present review were supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (20H03691, 18K19489, 16590318, 25460439), by a Grant from the Smoking Research Foundation, and by endowments from Dr. Yukoh Aihara of Aihara Allergy and Pediatric Clinic and from Prof. Kimitaka Itoh, Chubu University.

IX. References

1. Abe, H., Naito, Y., Sadashima, E., Fukushima, C., Takase, Y., Murata, K., et al. (2019) Insulinoma-Associated Protein 1 (INSM1) is a novel diagnostic marker of small cell lung cancer in bronchial brushing and pleural effusion cytology cell blocks: Reliability and cut-off value of INSM1. Cancer Cytopathol. 127; 598–605.
2. Alexandrov, L., Ju, Y., Haase, K., Van Loo, P., Martincorena, I., Nik-Zainal, S., et al. (2016) Mutational signatures associated with tobacco smoking in human cancer. Science 354; 618–622.
3. Augustyn, A., Borromeo, M., Wang, T., Fujimoto, J., Shao, C., Dospy, P. D., et al. (2014) ASCL1 is a lineage oncogene providing therapeutic targets for high-grade neuroendocrine lung cancers. Proc. Natl. Acad. Sci. U S A 111; 14788–14793.
4. Baine, M. K., Hsieh, M. S., Lai, W. V., Egger, J. V., Jungbluth, A. A., Daneshbod, Y., et al. (2022) SCLC subtypes defined by ASCL1, NEUROD1, POU2F3, and YAP1: A comprehensive immunohistochemical and histopathological characterization. J. Thorac. Oncol. 15; 1823–1835.
5. Ball, D. W. (2004) Achaete-scute homolog-1 and Notch in lung neuroendocrine development and cancer. Cancer Lett. 204; 159–169.
6. Bensch, K. G., Gordon, G. B. and Miller, L. R. (1965) Studies on bronchial counterpart of the Kulitschitzky (argentaffin) cell and innervation of bronchial gland. J. Ultrastruct. Res. 12; 668–686.
7. Bensch, K. G., Gordon, G. B. and Miller, L. R. (1965) Electron microscopic and biochemical studies on the bronchial carcinoid tumour. Cancer 18; 592–602.
8. Bensch, K. G., Corrin, B., Pariente, R. and Spencer, H. (1968) Oat cell carcinoma of the lung: its origin and relationship to bronchial carcinoid. Cancer 22; 1163–1172.
9. Borges, M., Linniola, R. I., Van de Velde, H. J., Chen, H., Nelkin, B. D., Mabry, M., et al. (1997) An Achaete-Scute homologue essential for neuroendocrine differentiation in the lung. Nature 386; 852–855.
10. Borromeo, M. D., Savage, T. K., Kolipara, R. K., He, M., Augustyn, A., Osborne, J. K., et al. (2016) ASCL1 and NEUROD1 reveal heterogeneity in pulmonary neuroendocrine tumors and regulate distinct genetic programs. Cell Rep. 16; 1259–1272.
11. Brambilla, E., Beasley, M. B., Austin, J. H. M., Capelozzi, V. L., Chirieac, L. R., Devesa, S. S., et al. (2015) Neuroendocrine tumours. Small cell carcinoma. In “WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. 4th ed.”, ed. by

Immunohistochemistry of PNEC and SCLC 81
11. W. D. Travis, E. Brambilla, A. P. Burke, A. Marx and A. G. Nicholson, IARC, Lyon, pp. 63–68.
12. Brouns, I., Verckli, L., Pintelon, I., Timmermans, J.-P. and Adriaensen, D. (2021) The pulmonary neuromorphological body microenvironment: a multifunctional unit in the airway epithelium. Adv. Anat. Embryol. Cell Biol. 233: 1–99.
13. Bunn, P. A. Jr, Minna, J. D., Augustyn, A., Gazdar, A. F., Ouadah, Y., Krasnow, M. A., et al. (2016) Small cell lung cancer: Can recent advances in biology and molecular biology be translated into improved outcomes? J. Thorac. Oncol. 11; 453–474.
14. Chunckian, C. J. and Schenk, E. A. (1979) A modification of Pascal's argyrophil method. J. Histotechnol. 2: 102–103.
15. Cutz, E., Yeger, H., Pan, J. and Ito, T. (2008) Pulmonary neuroendocrine cell system in health and disease. Curr. Respir. Med. Rev. 4; 174–186.
16. Demelash, A., Rudrabhatla, P., Pant, H. C., Wang, X., Amin, N., et al. (2017) Constitutive achaete-scute complex homologue 1 (ASCL1) regulates migration of lung cancer cells through Cdk5/p35 pathway. Mol. Biol. Cell 23; 2856–2866.
17. Feyerter, F. (1938) Uber die Arygyrophile des Helle Zellen-Systems in Bronchialbaum des Menschen. A. Mikrosk. Anat. Forsch. 76; 73–81.
18. Feyerter, F. (1949) Die “Helle-Zelle” der Bronchial-Schleimhaut und ihre Beziehungen zum Problem der Chemorezeptoren. Frankfurt Z. Pathol. 60; 517–559.
19. Fujino, K., Motooka, Y., Hassan, W. A., Ali Abdalla, M. O., Sato, Y., Kudoh, S., et al. (2015) Insulinoma-associated protein 1 is a crucial regulator of neuroendocrine differentiation in lung cancer. Am. J. Pathol. 185; 3164–3177.
20. Gazdar, A. F., Carney, D. N., Nau, M. M. and Minna, J. D. (1985) Characterization of variant subclasses of cell lines derived from small cell lung cancer having distinctive biochemical, morphological, and growth properties. Cancer Res. 45; 2924–2930.
21. Gazdar, A. F., Bunn, P. A. and Minna, J. D. (2017) Small-cell lung cancer: what we know, what we need to know and the path forward. Nat. Rev. Cancer 17; 725–737.
22. George, J., Lim, S. J., Seng, J. S., Cun, Y., Ozretic, L., Kong, G., et al. (2015) Comprehensive genomic profiles of small cell lung cancer. Nature 524; 47–53.
23. Gmelich, J. T., Bensch, K. G. and Liebow, A. A. (1967) Cells of Kulitschitzky type in bronchioles and their relationship to the origin of peripheral carcinoid tumor. Lab. Invest. 17; 88–98.
24. Gosney, J. R. (1992) Pulmonary Endocrine Pathology. Butworth-Heinemann, Oxford.
25. Harkema, J. R., Mariassy, A., St Goerge, J., Hyde, D. M. and Plopper, C. G. (1999) Epithelial cells of the conducting airways. In “The Airway Epithelium. Physiology, Pathophysiology and Pharmacology”, ed. by S. G. Farmer and D. W. Hay, Dekker, New York, pp. 3–39.
26. Hattori, S., Matsuda, M., Tateishi, R., Tatsunami, N. and Terazawa, T. (1968) Oat-cell carcinoma of the lung containing serotonin granules. Gan 59; 123–129.
27. Huang, Y. H., Klingbeil, O., He, X. Y., Wu, X. S., Arum, G., Lu, B., et al. (2018) POU2F3 is a master regulator of a tuft cell-like variant of small cell lung cancer. Genes Dev. 32; 915–928.
28. Ito, T. (1999) Differentiation and proliferation of pulmonary neuroendocrine cells. Prog. Histochem. Cytochem. 34; 245–324.
29. Ito, T., Nakatani, Y., Nagahara, N., Ogawa, T., Shibagaki, T. and Kanisawa, M. (1987) Quantitative study of pulmonary endocrine cells in anencephaly. Lung 165; 297–304.
30. Ito, T. and Kaniswa, M. (1990) Endocrine cells and brush cells at the bronchiole-alveolar junctions of neonatal Syrian golden hamster lungs. J. Morphol. 206; 217–223.
31. Ito, T., Udaka, N., Kawano, N. and Kitamura, H. (1999) Ontogeny of pulmonary neuroendocrine cells which express alpha subunit of guanine-binding protein Go. Histochem. Cell Biol. 111; 389–395.
32. Ito, T., Udaka, N., Okudela, K., Yazawa, T. and Kitamura, H. (2003) Mechanisms of neuroendocrine differentiation in pulmonary neuroendocrine cells and small cell carcinoma. Endocr. Pathol. 14; 133–139.
33. Ito, T., Kudoh, S., Ichimura, T., Fujino, K., Hassan, W. A. and Udaka, N. (2017) Small cell lung cancer, an epithelial to mesenchymal transition (EMT)-like cancer: significance of inactive Notch signaling and expression of achaete-scute complex homologue 1. Hum. Cell 30; 1–10.
34. Jiang, L., Huang, J., Higgs, B. W., Hu, Z., Xiao, Z., Yao, X., et al. (2016) Genomic landscape survey identifies SRSF1 as a key onco-driver in small cell lung cancer. PLoS Genet. 12; e1005895.
35. Jiang, T., Collins, B. J., Jin, N., Watkins, D. N., Brock, M. V., Matsui, W., et al. (2009) Achaete-scute complex homologue 1 regulates tumor-initiating capacity in human small cell lung cancer. Cancer Res. 69; 845–854.
36. Kudoh, S., Tenjin, Y., Kameyama, H., Ichimura, T., Yamada, T., Matsuo, A., et al. (2020) Significance of Achaete-Scute Complex Homologue 1 (ASCL1) in pulmonary neuroendocrine carcinomas; RNA sequence analyses using small cell lung cancer cells and ASCL1-induced neuroendocrine carcinoma cells. Histochem. Cell Biol. 153; 443–456.
37. Lauweryns, J. M. and Peuskins, J. C. (1968) Arygyrophil (kinin and amine producing?) cells in human infant airway epithelium. Life Sci. 8; 577–585.
38. Lauweryns, J. M. and Peuskins, J. C. (1972) Neuroepithelial bodies (neurosecretory or sensory organ?) in human infant bronchial epithelium. Anat. Rec. 172; 471–482.
39. Lauweryns, J. M., Cokelaere, M. and Theunynck, P. (1973) Serotonin producing neuroepithelial bodies in rabbit respiratory mucosa. Science 180; 410–413.
40. Li, Y. and Linnoila, R. I. (2012) Multidirectional differentiation of Achaete-Scute homologue-1-defined progenitors in lung development and injury repair. Am. J. Respir. Cell Mol. Biol. 47; 768–775.
41. Linnoila, R. I., Zhao, B., DeMayo, J. L., Nelkin, B. D., Baylin, S. B., DeMayo, F. J., et al. (2000) Constitutive achaete-scute homologue-1 promotes airway dysplasia and lung neuroendocrine tumors in transgenic mice. Cancer Res. 60; 4005–4009.
42. Meder, L., Konig, K., Ozretic, L., Schultheiss, A. M., Ueckerth, F., Ade, C. P., et al. (2016) NOTCH, ASCL1, p53 and RB alterations define an alternative pathway driving neuroendocrine and small cell lung carcinomas. Int. J. Cancer 138; 927–938.
43. Mirski, S. E., Gerlach, J. H. and Cole, S. P. (1987) Multidrug resistance in a human small cell lung cancer cell line selected in adriamycin. Cancer Res. 47; 2594–2598.
44. Osada, H., Tatamatsu, Y., Yatabe, Y., Horio, Y. and Takahashi, T. (2005) ASH1 gene is a specific therapeutic target for lung cancers with neuroendocrine features. Cancer Res. 65; 10680–
48. Osada, H., Tomida, S., Yatabe, Y., Tatematsu, Y., Takeuchi, T., Murakami, H., et al. (2008) Roles of achaete-scute homologue 1 in DKK1 and E-cadherin repression and neuroendocrine differentiation in lung cancer. *Cancer Res.* 68; 1647–1655.

49. Pearse, A. G. (1968) Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. *Proc. R. Soc. Lond. B Biol. Sci.* 170; 71–80.

50. Pearse, A. G. (1969) The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryonic, physiologic and pathologic implication of the concept. *J. Histochem. Cytochem.* 17; 305–313.

51. Pearse, A. G. and Takor, T. (1979) Embryology of the diffuse neuroendocrine system and its relationship to the common peptides. *Fed. Proc.* 38; 2288–2294.

52. Pietanza, M. C., Byers, L. A., Minna, J. D. and Rudin, C. M. (2015) Small cell lung cancer: will recent progress lead to improved outcomes? *Clin. Cancer Res.* 21; 2244–2255.

53. Qu, S., Fetsch, P., Thomas, A., Pommier, Y., Schrump, D., Miettinen, M. M., et al. (2022) Molecular subtypes of primary SCLC tumors and their associations with neuroendocrine and therapeutic markers. *J. Thorac. Oncol.* 17; 141–153.

54. Reid, L., Meyrick, B., Antony, V. B., Chang, L. Y., Crapo, J. D. and Reynolds, H. Y. (2005) The mysterious pulmonary brush cell: A cell in search of a function. *Am. J. Respir. Crit. Care Med.* 172; 136–139.

55. Rudin, C. M., Poirier, J. T., Byers, L. A., Dive, C., Dowlati, A., George, J., et al. (2019) Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nat. Rev. Cancer* 19; 289–297.

56. Sato, Y., Okamoto, I., Kameyama, H., Kudoh, S., Saito, H., Sanada, M., et al. (2020) Integrated immunohistochemical study of small cell carcinoma of the lung with focusing on transcription and co-transcription factors. *Diagnostics* 10; e949

57. Sriuranpong, V. I., Borges, M. W., Ravi, R. K., Arnold, D. R., Nelkin, B. D., Baylin, S. B., et al. (2001) Notch signaling induces cell cycle arrest in small cell lung cancer cells. *Cancer Res.* 61; 3200–3205.

58. Tenjin, Y., Kudoh, S., Kubota, S., Yamada, T., Matsuo, A., Sato, Y., et al. (2019) Ascl1-induced Wnt11 regulates neuroendocrine differentiation, cell proliferation, and E-cadherin expression in small-cell lung cancer and Wnt11 regulates small-cell lung cancer biology. *Lab. Invest.* 99; 1622–1635.

59. Thunnissen, E., Boreczuk, A., Flieder, D. B., Witte, B., Beasley, M. B., Chung, J.-H., et al. (2017) The use of immunohistochemistry improves the diagnosis of small cell lung cancer and its differential diagnosis. An international reproducibility study in a demanding set of cases. *J. Thorac. Oncol.* 12; 334–346.

60. Travis, W. D. (2012) Update on small cell carcinoma and its differentiation from squamous cell carcinoma and other non-small cell carcinomas. *Mod. Pathol.* 25 Supp1; S18–30.

61. Zhang, W., Girard, L., Zhang, Y. A., Haruki, T., Papari-Zareei, M., Stastny, V., et al. (2018) Small cell lung cancer tumors and preclinical models display heterogeneity of neuroendocrine phenotypes. *Transl. Lung Cancer Res.* 7; 32–49.

This is an open access article distributed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC-BY-NC), which permits use, distribution and reproduction of the articles in any medium provided that the original work is properly cited and is not used for commercial purposes.