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Review

Molecular Subtypes of High-Grade Neuroendocrine Carcinoma (HGNEC): What is YAP1-Positive HGNEC?

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Abstract

Small cell lung cancer (SCLC) subtype classification, based on high-level expression of key transcriptional regulators; *ASCL1* (SCLC-A), *NEUROD1* (SCLC-N), *POU2F3* (SCLC-P), and *YAP1* (SCLC-Y), has recently been proposed. YAP1 (and POU2F3) has attracted attention as an important factor for non-neuroendocrine (non-NE) phenotypic subtyping of SCLC. However, subsequent studies reported that YAP1 expression alone cannot define a single group in primary SCLC, which makes it difficult to understand what SCLC-Y is by focusing only on SCLC. In this review, we concluded that YAP1 is an essential anti-neuroendocrine factor in both SCLC and non-small cell lung cancer (NSCLC) based on previous studies, including our own analysis of the cell lines and primary tumors of SCLC and NSCLC. The classification of SCLC-Y is a concept mainly established from the analysis of cell lines, and SCLC-Y cell lines correspond to "variant type" SCLC cell lines. Primary SCLC and large cell neuroendocrine carcinoma (LCNEC) are typically heterogeneous tumors composed mostly of NE-type cells, but they contain a small number of non-NE-type cells. Importantly, individual cells with NE features exhibit YAP1 loss, whereas the non-NE-type cells exhibit YAP1 expression. Although rare in primary SCLC, some cases of primary LCNEC have many YAP1-positive cells, which is correlated with chemotherapy resistance. YAP1 staining may be useful in the exclusion diagnosis of SCLC or in the selection of treatment for LCNEC.

Keywords: review; YAP1; SCLC; LCNEC; HGNEC; SCLC-Y; SCLC-I; chemo-sensitivity

1. Introduction

The four major histological subtypes of lung cancer have been adenocarcinoma, squamous cell carcinoma, neuroendocrine tumor (NET), and large cell carcinoma since the publication of the 4th edition of the World Health Organization (WHO) classification of tumors of the lungs, pleura, thymus and heart [1]. Large cell neuroendocrine carcinoma (LCNEC), previously a subtype of large cell carcinoma, was incorporated into NET. In NET, high-grade neuroendocrine cancer (HGNEC), which includes small cell lung cancer (SCLC) and LCENC, is differentiated from atypical carcinoid tumor (intermediate-grade) and typical carcinoid tumor (low-grade), and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) has been positioned as a pre-invasive lesion.

In recent years, molecular biological analysis of HGNEC has progressed rapidly, and it has become clear that SCLC and LCNEC can be divided into several subtypes according to gene mutation and expression patterns. YAP1 attracted attention as an important factor for non-neuroendocrine (non-NE) phenotypic subtyping of SCLC; however, several subsequent studies also suggested that YAP1 expression alone cannot define a single group in primary SCLC. At present, YAP1-positive SCLC remains unclear. We explain the meaning of YAP1 expression in SCLC and LCNEC from a pathological point of view based on recent literature and our own studies. Previous major studies on the classification of SCLC and LCNEC correlated with YAP1 expression are shown in Table 1 (Ref. [2–10]).

2. YAP1 and the Hippo Pathway

The Hippo pathway, a signaling pathways, plays an important role in controlling the size of organs through the coordinated regulation of cell fate [11]. Two serine/threonine kinases (MST1/2 and LATS1/2) that form the core components of the Hippo pathway inactivate the transcriptional coactivator YAP1 by phosphorylation and shut down cell proliferation signaling [12,13]. MST1/2—LATS1/2—YAP1 signaling is activated as a result of the cellular response to physical changes in the extracellular environment (cell adhesion, extracellular matrix hardness) [14]. Several signaling pathways (PI3K, TGFβ/BMP, SHH, WNT, and Notch) intersect with the Hippo pathway and ultimately control YAP1 activity [15]. When the Hippo pathway is activated, LATS1/2 phosphorylates serine residues in YAP1 (S61, S109, S127, S164, and S381) and phosphorylated S127 binds 14–3–3, causing cytoplasmic retention

Table 1. Classification of HGNEC correlated with YAP1 expression in previous reports.

SCLC Cell lines classification based on *YAP1* expression levels			
Authors	*YAP1*-high	*YAP1*-low	PMID
Ito, *et al.* [2]	*YAP1*-high SCLC cell lines: Adherent type, non-NE type, Resistant to cisplatin	YAP1-low SCLC cell lines: Floating type, NE-type, Sensitive to cisplatin	27418196
McColl, *et al.* [3]	*YAP1*-high SCLC cell lines: *INSM1*-low	*YAP1*-low SCLC cell lines: *INSM1*-high	29088741
Owonikoko, *et al.* [4]	*YAP1*-high SCLC: High expression of interferon-γ response genes, highest weighted score on a validated 18-gene T-cell–inflamed gene expression profile score, and high expression of HLA and T-cell receptor genes	-	33248321
Immunohistochemical analysis of YAP1 in HGNEC cases			
Authors	Results		PMID
Ito, *et al.* [2]	In primary SCLC cases, YAP1 was negative in 40 of 41 (98%). In primary LCNEC cases, YAP1 was negative in 18 of 30 (60%). In primary NSCLC cases except LCNEC cases, YAP1 was positive in 183 of 189 (97%). YAP1 expression in HGNEC correlated with chemo-resistance.		27418196
Baine, *et al.* [5]	In primary SCLC cases, there was no predominantly YAP1-positive case (0/159: 0%). YAP1 was expressed at low levels, primarily in combined SCLC, and was not exclusive of other (ASCL1, NEUROD1, POU2F3) subtypes.		30926931
Pearsall, *et al.* [6]	Although there was no predominantly YAP1-positive case among 39 CTC-derived SCLC tumors, more than 0.2% cancer cells were positive for YAP1 in 10 of 39, and more than 1% were positive for YAP1 in 2 of them.		32721553
SCLC Cell lines classification possibly correlated with *YAP1* expression levels			
Authors	*YAP1*-high	*YAP1*-low	PMID
Carney, *et al.* [7], Gazdar, *et al.* [8]	Variant type: Adherent type, non-NE type, Resistant to radiation	Classic type: Floating type, NE-type	2985257 2985258
Calbo *et al.* [9]	Mesenchymal phenotype: Adherent type, non-NE type (mouse SCLC)	Neuroendocrine type: Floating type, NE-type (mouse SCLC)	21316603
LCNEC classification related to *YAP1* expression levels			
Authors	*YAP1*-high	*YAP1*-low	PMID
George *et al.* [10]	Type II: *RB1* mutations, with *ASCL1*-low/*DLL1*-low/*NOTCH*-high/*YAP1*-high/immune related genes-high	Type I: *STK11/KEAP1* mutations, with *ASCL1*-high/*DLL1*-high/*NOTCH*-low	29535388

[13]. In addition, phosphorylated S381 binds CK1δ/ε, and further phosphorylation of serine (S384, S387) promotes the interaction between YAP1 and the SCFbTRCP ubiquitin ligase complex. This series of signaling results in the ubiquitination of YAP1 and proteasome-dependent degradation [16]. When the Hippo pathway is inactivated, YAP1 translocates to the nucleus and binds primarily to the transcription factor TEAD, inducing the upregulation of the genes involved in cell proliferation and inhibition of apoptosis [17]. Importantly, several transcription factors other than TEAD have been demonstrated to bind to YAP1 (e.g., TTF-1, SMADs, ß-catenin, Fos, and E2F) [17–19].

3. YAP1 is Involved in the Regulation of Pulmonary Differentiation

YAP1 and the Hippo pathway are involved in the regulation of alveolar epithelial differentiation. Core hippo pathway molecules, such as MOB1A/1B or MST1/2, were previously reported to regulate the differentiation of alveolar epithelium through the regulation of YAP1 expression [20,21]. In 2021, Little *et al.* [22] reported that TTF-1 promotes cell differentiation into alveolar type 1 or alveolar type 2 by binding chromatin in a cell-type-specific manner, and that YAP1/TAZ direct TTF-1 to its alveolar type 1-specific sites and prevent its binding to alveolar type 2-specific sites.

4. Role of YAP1 in Cancer

The strong expression of YAP1 is frequently observed in tumors such as hepatocellular carcinoma, ovarian cancer, and NSCLC [23–27]. The overexpression of YAP1 overcomes cell contact inhibition, induces epithelial–mesenchymal transition, and promotes cancer cell proliferation and invasion [23,24,28]. YAP1 is considered a key determinant of the resistance of tumors to platinum, including NSCLC, oral cancer, cervical cancer, thyroid cancer, and ovarian cancer [29–31]. Cheng *et al.* [32] reported that the downregulation of YAP1 by verteporfin (a YAP1 inhibitor) sensitized cells to DNA-damaging agents.

In addition to its oncogenic role, YAP1 functions as a key regulator of differentiation in lung tumors. Yijun *et al.* [33] reported that YAP1 inhibits the squamous differentiation of LKB1-deficient lung adenocarcinomas. Overexpression of nuclear YAP1 was reported to correlate with a poor prognosis in NSCLC [24]; however, nuclear YAP1 expression is also frequently found in non-mucinous adenocarcinoma in situ (non-mucinous AIS, a well differentiated subtype) and reactive alveolar epitheliums [34]. Ito *et al.* [34] reported that co-expression of CADM1 (tumor suppressor) and hippo pathway core kinases at the cell membrane, which is observed in non-mucinous AIS and normal alveolar epithelium, is important for suppressing the oncogenic role of nuclear YAP1.

5. Loss of YAP1 Defines NE Differentiation of Lung Tumors

In 2016, we first reported the importance of the loss of YAP1 in NE differentiation of lung tumors [2]. We demonstrated that SCLC cell lines can be classified into two groups: *YAP1*-low, NE marker-high, and floating type, and *YAP1*-high, NE marker-low, and adherent type. However, among primary tumors, YAP1 expression was rare in SCLC cases (2%), but common in NSCLC cases excluding LCNEC (97%). The typical pattern of YAP1 and ASCL1 expression in primary SCLC cases is shown in Fig. 1; most cells are YAP1-negative and ASLC1-positive. YAP1 was negative in carcinoid tumors in this report [2] and we concluded that the loss of YAP1 defines NE differentiation of lung tumors. Moreover, the VMRC-LCD cell line, established as an adenocarcinoma cell line, was confirmed to be an LCNEC cell line with loss of YAP1 expression in this study.

6. Molecular Subtyping of SCLC by Rudin *et al.* [35]

In 2019, Rudin *et al.* [35] reported that SCLC can be subtyped into 4 groups by four molecules; *ASCL1*, *NEUROD1*, *POU2F3*, and *YAP1*, based on genomic data of primary SCLC samples and cell lines. ASCL1, NEUROD1, and POU2F3 are transcriptional factors. ASCL1 and NEUROD1 are implicated in the neuroendocrine differentiation (NE differentiation) of cells [36–41]; therefore, *ASCL1*-positive SCLC (SCLC-A) and *NEUROD1*-positive SCLC (SCLC-N) strongly express NE markers. POU2F3 is an important master regulator for chemosensory cells [42–46], which are slightly present in the tongue, respiratory epithelium, trachea, urethra, and digestive organs. *POU2F3*-positive SCLC (SCLC-P) is considered a non-NE phenotype.

On the other hand, the triple-negative subtype (*ASCI1-/NEUROD1-/POU2F3-*) had higher expression of *YAP1* and was classified as *YAP1*-positive SCLC (SCLC-Y). SCLC-Y is also considered a non-NE phenotype. SCLC-Y was reported to be characteristically sensitive to CDK4/6 inhibitors, HSP90 inhibitors, and Aurora kinase inhibitors, but insensitive to BCL2 inhibitors [3,47,48].

6.1 SCLC-Y cannot be Identified in Primary SCLC

The classification of SCLC by Rudin *et al.* [35] is a perspective opinion model and requires further validation. In the subsequent reports, SCLC-A, N, and P were identified in primary SCLC cases, but not SCLC-Y. When Baine *et al.* [5] immunostained primary SCLC cases (n = 159) with these four markers, SCLC was divided into mainly two groups: one in which both or either of ASCL1 and NEUROD1 were positive, and another in which both ASCL1 and NEUROD1 were negative. The POU2F3-positive SCLC cases were included in the latter, but YAP1-positive SCLC cases were absent. Pearsall *et al.*

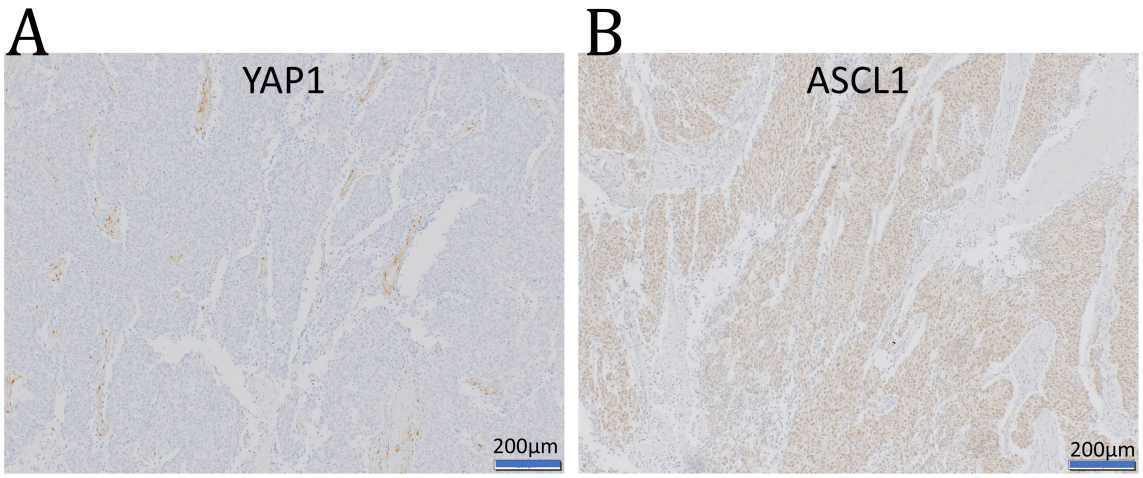

Fig. 1. Typical immunohistochemical expression patterns of YAP1 and ASCL1 of SCLC. Most of the cells are (A) YAP1-negative and (B) ASCL1-positive.

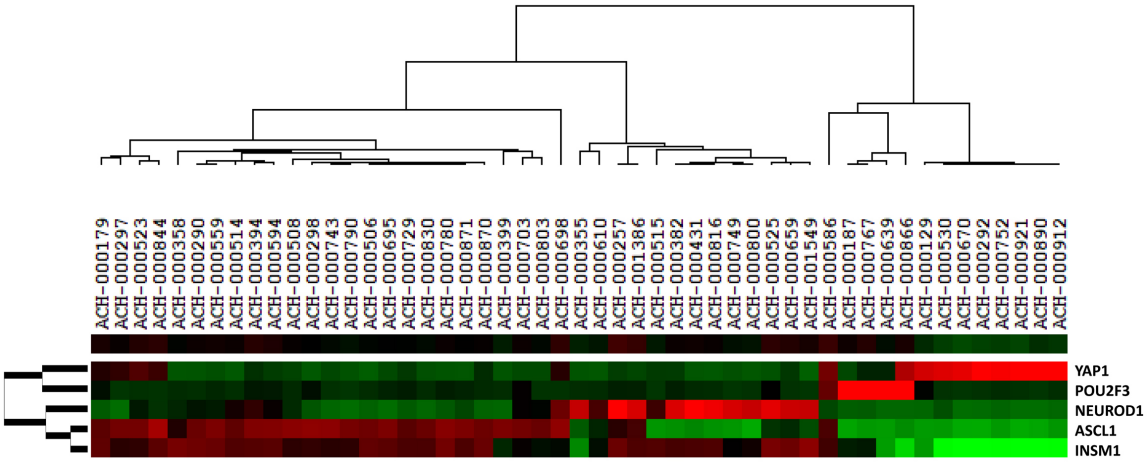

Fig. 2. Cluster analysis of 51 SCLC cell lines in Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle/) based on gene expression levels of *YAP1*, *INSM1*, *POU2F3*, *ASCL1* and *NEUROD1*. We used the cluster program (http://rana.lbl.gov/EisenSoftware.htm, accessed March 19, 2008) for a cluster analysis of the gene expression data of cell lines. In brief, we carried out average linkage hierarchical clustering of the 51 SCLC cell lines using the mean centering and normalization of genes. We then displayed the results obtained with the aid of TreeView software (Eisen Lab in Stanford University, Stanford, CA, USA) (http://rana.lbl.gov/EisenSoftware.htm, accessed March 21, 2008). The image used a color code to represent relative expression levels. Red represents expression levels greater than the mean for a given gene across all samples. Green represents expression levels less than the mean across samples. Fig. 2 shows that at least 18% (9/51) SCLC cell lines highly express the *YAP1* gene, and that none of them show high-expressions of *INSM1* gene, while high expressions of *INSM1* gene can be found in the cells highly expressing *ASCL1*, *NEUROD1*, or *POU2F3* genes.

[6] performed immunohistochemical analysis using 39 patients' serum-derived circulating tumor cell (CTC)-derived xenograft (CDX: CTC-derived explants) models of SCLC. However, there were no cases in which YAP1 was predominantly positive. Thus, what is SCLC-Y?

6.2 SCLC-Y is a Concept Mainly Established from SCLC Cell Lines

Close observation of the figure of "Molecular subtypes of SCLC defined by expression of key transcription regulators" (https://www.nature.com/articles/s41568-019-0133-9/figures/2) by Rudin *et al.* [35] reveals that in the SCLC-Y group, there are only two cases of primary SCLC and they are mostly composed of SCLC cell lines.

Cluster analysis of 51 SCLC cell lines in Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle/) based on gene expression data of *ASCL1*, *NEUROD1*, *POU2F3*, *YAP1*, and *INSM1* is shown in Fig. 2, and at least 18% (9/51) of SCLC cell lines highly express the *YAP1* gene.

Fig. 3. Relative gene expression levels of *YAP1*, *INSM1*, *POU2F3*, *ASCL1* and *NEUROD1* of 17 cell lines. Gene expression analysis of the 17 cell lines (①–⑪: floating type SCLC cell lines, ⑫–⑭: adherent type SCLC cell lines, ⑮–⑰: adherent type NSCLC cell lines) was carried out per mRNA-Seq using an Illumina GAIIx sequencer (Illumina, San Diego, CA, USA). Details were shown in our previous report (PMID: 27418196). Names of cell lines are as follows: ① H69 ② N417 ③ H146 ④ Lu135 ⑤ Lu139 ⑥ 510A ⑦ 526 ⑧ 2081 ⑨ H889 ⑩ Lu130 ⑪ H446 ⑫ SBC3 ⑬ SBC5 ⑭ LCMA ⑮ Lu99 ⑯ H460 ⑰ Lu65A.

As such, it can be concluded that the classification of SCLC-Y is a concept mainly established from the analysis of cell lines and should not be applied to primary SCLC cases.

6.3 SCLC-Y Cell Lines Correspond to "Variant Type" SCLC Cell Lines

In 1985, Carney, Gazdar *et al.* [7,8] reported that there are two types of SCLC cell lines: "classic type", which is floating with the NE phenotype, and "variant type", which is adherent with a non-NE phenotype. Of note, "variant type" SCLC cell lines frequently originated from post-therapy tumors that recurred and were more resistant to radiation than the "classic type".

The expression levels of *YAP1*, *INSM1*, *POU2F3*, *NEUROD1*, and *ASCL1* of 14 SCLC cell lines (11 floating type and 3 adherent type) and 3 NSCLC cell lines (3 adherent type) in our lab are shown in Fig. 3. *YAP1*-low SCLC cell lines are the floating type and NE marker-positive, thus they correspond to "classic type". On the other hand, as *YAP1*-high SCLC cell lines are the adherent type and NE marker-negative, they correspond to "variant type" (Fig. 3).

6.4 Two Possibilities for the Establishment of SCLC-Y Cell Lines

There are two possibilities for the establishment of SCLC-Y cell lines, as shown in Fig. 4.

6.4.1 SCLC-Y Cell Lines Possibly Derived from Minor Components of YAP1-Positive Cells in Pure SCLC

Histologically, SCLC looks uniform at first glance, but it is actually composed of a heterogeneous cell population.

In 2011, Calbo *et al.* [9] established two types of cell lines from the same SCLC tumor from mice: an adhesive cell line with the EMT phenotype (≈ "variant type") and a floating cell line with the NE phenotype (≈ "classic type").

In 2020, Pearsall *et al.* [6] reported that many of the CTC-derived tumors contained a small number of YAP1-positive and NE marker-negative cells as minor components. Furthermore, Pearsall *et al.* [6] established two types of cell lines, a YAP1-positive and NE marker-negative cell line, and a YAP1-negative and NE marker-positive cell line, from partially YAP1-positive CTC-derived tumors.

Ireland *et al.* [49] reported that MYC activates NOTCH signaling and transiently reprograms ASCL1-positive cells into NEUROD1-positive cells, and further into YAP1-positive and non-NE-type cells.

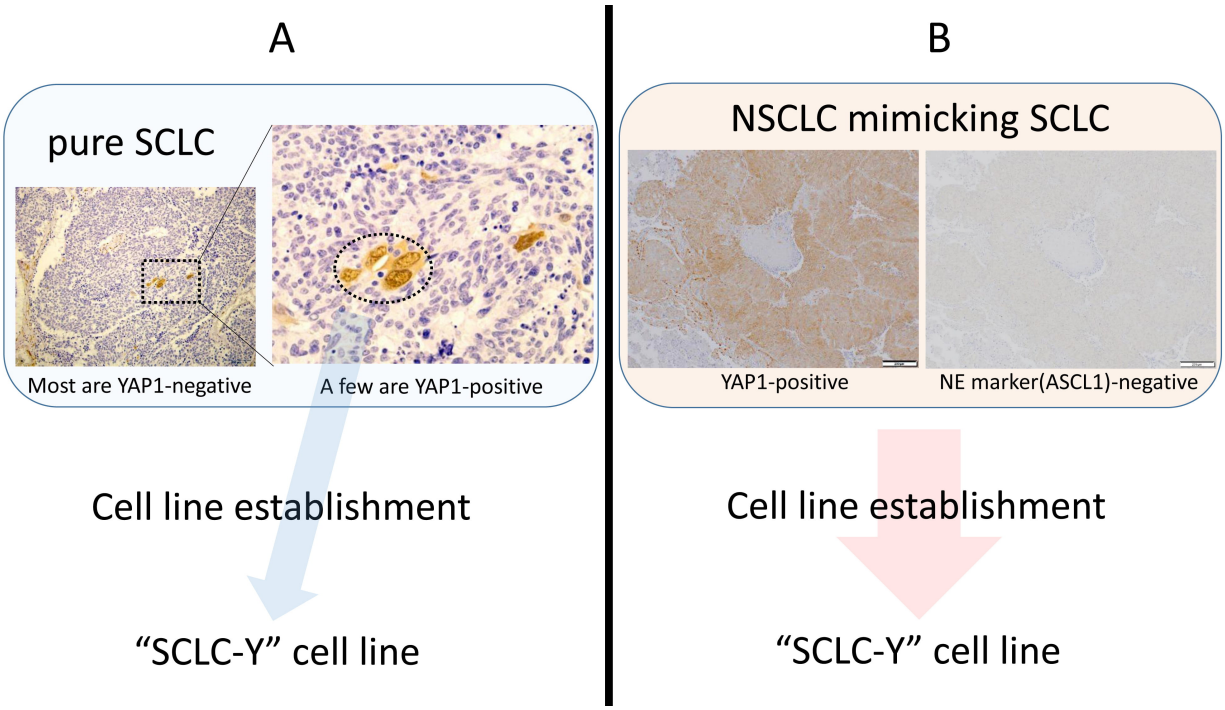

Fig. 4. Two possibilities for the establishment of “SCLC-Y” cell line. (A) SCLC contains minor component of YAP1 positive cells. “SCLC-Y” cell line might have been established from these minor component of YAP1-positive cells. (B) “SCLC-Y” cell line may have been established from NSCLC mimicking SCLC.

Based on the above reports, the following can be inferred: SCLC is essentially YAP1-negative and exhibits NE differentiation, but a few cells in the tumor mass are transiently reprogrammed into YAP1-positive and non-NE-type cells. This reprogramming may be promoted through the process of the acquisition of resistance to chemotherapy and radiation therapy. SCLC-Y cell lines may be irreversibly established from these minor components in SCLC (Fig. 4A).

6.4.2 SCLC-Y Cell Lines Possibly Derived from NSCLC Mimicking SCLC

The other possibility is that cell lines derived from NSCLC were misdiagnosed as YAP1-positive SCLC cell lines (Fig. 4B). The pathological diagnosis of SCLC only requires histological morphology, not immunostaining of NE markers. However, it is well known that some NSCLC (basaloid squamous cell carcinoma) histologically mimic SCLC. If we focus only on SCLC, YAP1-positive cases are rare, but if we extend the scope to NSCLC, many cases are YAP1-positive. In our study, 97% of NSCLC cases, excluding LCNEC, were YAP1 positive. As shown in Fig. 3, the characteristics of *YAP1*-high SCLC cell lines were more similar to those of NSCLC cell lines than to those of *YAP1*-low SCLC cell lines. Thus, whether SCLC-Y cell lines are NSCLC cell lines cannot be concluded.

6.5 Relationship between SCLC-I and SCLC-Y

Recently, Gay *et al.* [50,51] demonstrated that according to the gene signature analysis of SCLC cases that were triple-negative for *ASCL1*, *NEUROD1*, and *POU2F3*, the triple-negative subtype uniquely expressed numerous immune-related genes and was designated as SCLC-inflamed (SCLC-I). SCLC-I highly expresses genes associated with the EMT phenotype and immune-related genes such as *HLA*, IFN activity, and immune checkpoint genes. Of note, SCLC-I is highly sensitive to immunotherapy compared with the other three subtypes. Moreover, administration of cisplatin to xenografts derived from SCLC-A patients results in tissue migration to SCLC-I. This suggests that resistance to platinum drugs is acquired by switching subtypes. They also reported that the expression of *YAP1* was higher in both SCLC-P and SCLC-I than in SCLC-A and SCLC-N, although *YAP1* expression did not exclusively define a subtype [51]. Owonikoko *et al.* [4] reported that SCLC-Y is associated with the high expression of interferon-γ response genes, T-cell inflammatory genes, HLA, and T-cell receptor genes.

Thus, there is significant phenotypic overlap between SCLC-I and SCLC-Y, and it is possible that we are looking at different aspects of the same tumor. However, as mentioned above, SCLC-Y is a concept established from cell lines and does not necessarily represent the full nature of the primary tumor. Further analysis of the relationship between SCLC-I and SCLC-Y is needed.

7. Molecular Subtyping of LCNEC

In SCLC, inactivating mutations in *TP53* and *RB1* are almost inevitable. Comprehensive next-generation sequence analysis of LCNEC in recent years revealed that inactivating mutations in *RB1* and *TP53* are also frequent in LCNEC. According to the genome profile, LCNEC was mainly divided into two groups: "SCLC-type" and "NSCLC-type" or "type I" and "type II".

7.1 Classification of LCNEC by Rekhtman et al. [52]

In 2016, Rekhtman *et al.* [52] reported next-generation sequencing analysis of 241 cancer-associated genes (oncogene and tumor suppressor genes) for 45 cases of pure-LCNEC and compared the results with those of lung adenocarcinoma (151 cases), squamous cell carcinoma (36 cases), small cell carcinoma (42 cases), and carcinoid (13 cases). In this report, LCNEC was mainly classified into two types: "SCLC-type" (18 cases) with joint inactivation of *RB1* and *TP53*, and "NSCLC-type" (25 cases) with genetic abnormalities or decreased immunohistochemical expression of *STK/KRAS/KEAP1/NFE2L2*, which are both characteristic of NSCLC. However, they noted that genetic abnormalities of *KEAP1* and *NFE2L2* in "SCLC-type" LCNEC were more frequent than in SCLC.

7.2 Classification of LCNEC by George et al. [10]

In 2018, George *et al.* [10] analyzed 75 cases of LCNEC by whole-exome sequencing (WES) and whole-genome sequencing (WGS). *TP53*, *RB1*, *STK11*, *KEAP1*, *ADAMTS12*, *ADAMTS2*, *GAS7*, and *NTM8* were detected in LCNEC with a high mutation rate. *TP53*-inactivating mutation was detected in 92% of LCNEC cases and mutations in *STK11/KEAP1/RB1* were detected in 82%. Mutations in *RB1* and *STK11/KEAP1* were mutually exclusive, and LCNEC was mainly divided into two groups: LCNEC with *STK11/KEAP1* alteration ("type I" LCNEC) exhibiting high expression of NE genes (*ASCL1* and *DLL1*) and low expression of *NOTCH* signaling-related genes *(ASCL1*-high/*DLL1*-high/*NOTCH*-low), whereas LCNEC with *RB1* loss ("type II" LCNEC) exhibited the low expression of NE genes and upregulation of *NOTCH* signaling-related genes (*ASCL1*-low/*DLL1*-low/*NOTCH*-high).

Recently, Derks *et al.* [53] reported that overall survival is superior if RB1-positive (*RB1* wild type) LCNEC is treated using NSCLC-type chemotherapy (platinum-gemcitabine or -taxanes) instead of SCLC-type chemotherapy (platinum-etoposide), but there was no difference in outcome for RB1-negative (*RB1*-mutated) LCNEC. The genetic status of *RB1* may be an important factor in LCNEC classification and treatment selection. Focusing on *RB1* mutations, "type II" in George's report corresponds to "SCLC-type" in Rehktoman's report, and "type I" corresponds to "NSCLC-type", although the expression patterns of NE markers and *NOTCH* signaling-related genes in "type I" and "type II" are different from those in typical NSCLC and SCLC, respectively.

George *et al.* [10] also reported that "type II" LCNEC characteristically highly expresses *YAP1* and immune-related genes; therefore, "type II" LCNEC may be the counterpart of YAP1-positive SCLC. However, there are few studies that immunohistochemically examined the expression of YAP1 in LCNEC cases, except for our previous report.

7.3 Immunohistochemical Expression Pattern of YAP1 in LCNEC Cases

We previously examined the staining patterns of YAP1 and NE markers in 30 LCNEC cases [2]. Of 30 LCNEC cases, 60% (18/30) were YAP1-negative and exhibited a similar pattern to typical SCLC cases, in which most cells were YAP1-negative (as shown in Fig. 5A,B), whereas 40% (12/30) were YAP1-positive LCNEC. YAP1-positive LCNEC exhibited a conspicuous mixture of YAP1-positive and YAP1-negative cells (Fig. 5C,D) or diffusely YAP1-positive pattern (Fig. 5E,F), in which YAP1-positive cells were NE marker-negative or weakly positive (Fig. 5C–F). This suggested that YAP1 staining is useful for detecting cell components with the loss or suppression of NE differentiation in high-grade neuroendocrine tumors. In addition, our previous study revealed that YAP1 positivity can predict resistance to platinum-based chemotherapy in LCNEC cases [2].

8. YAP1 Expression Correlates with Chemo-Resistance in HGNEC

Several studies reported that *YAP1* expression in SCLC cell lines is significantly correlated with resistance to chemotherapy and radiation therapy [2,54,55]; however, the mechanism remained unclear. In 2021, Qingzhe *et al.* [56] demonstrated that YAP1 is not only a predictive marker, but also a key molecule that causes loss of NE differentiation and determines chemotherapy resistance. Thus, YAP1 signaling plays an essential role in the establishment of intratumoral heterogeneity, promoting the fate conversion of SCLC from NE to non-NE tumor cells by inducing REST expression, and YAP1 suppresses GSDME expression in SCLC cells and is associated with acquired resistance to chemotherapy in SCLC [56].

9. Loss of YAP1 is the Essence of NE Differentiation in SCLC and NSCLC Cell Lines.

In 2021, Pearson *et al.* [57] reported the possibility that the role of YAP1 is different between tumors characterized by *RB1*-inactivating mutations, such as SCLC and retinoblastoma, and solid tumors characterized by wild type *RB1* such as adenocarcinoma and squamous cell carcinoma. In the former, YAP1 may act as a tumor suppressor, whereas in the latter, it may act as an oncogene. Focusing only on SCLC, it is difficult to understand the true role of YAP1 or

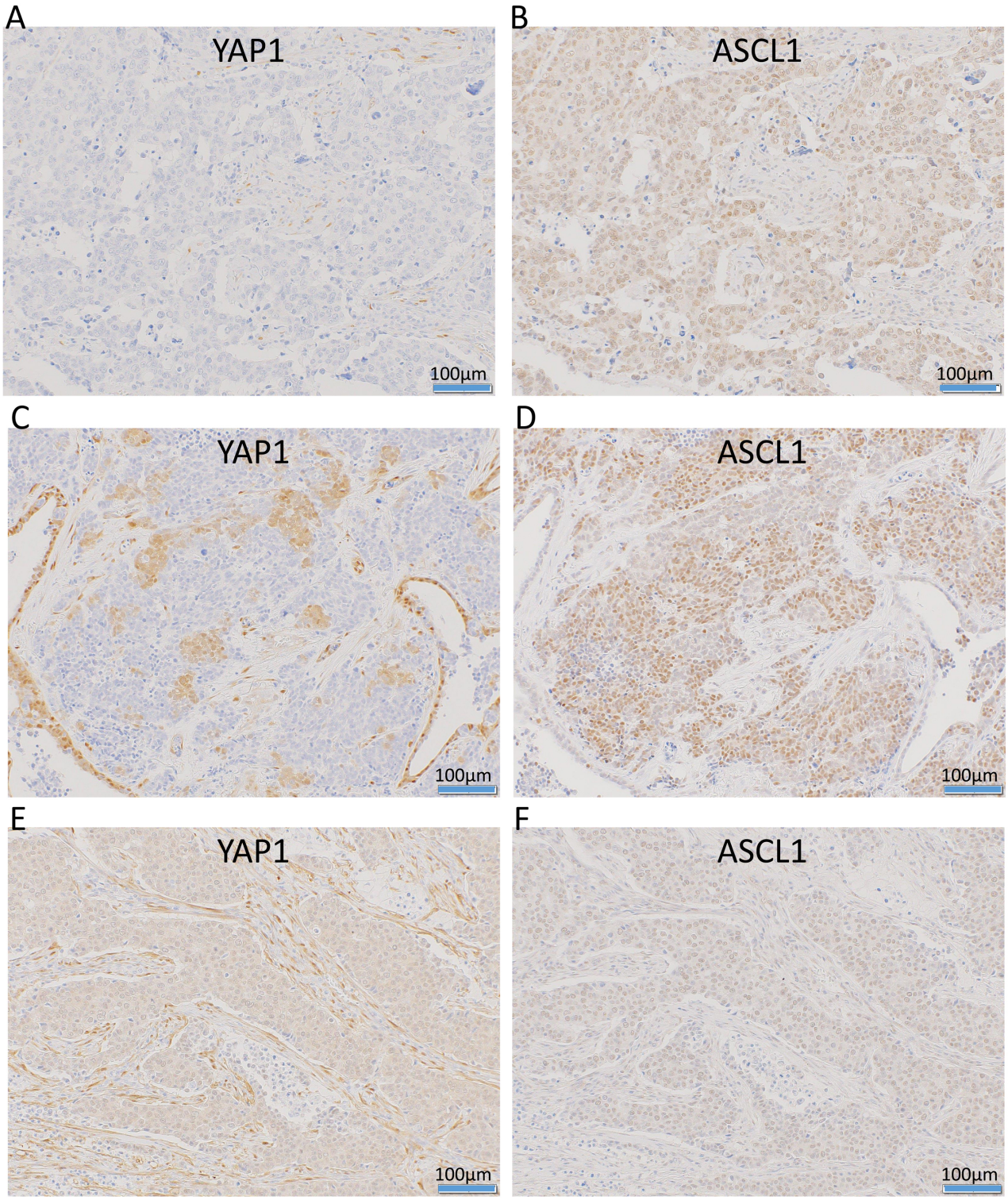

Fig. 5. Immunohistochemical expression patterns of YAP1 and ASCL1 of LCNEC, using Serial sections. Top figures: YAP1-negative case, in which most of the cells are (A) YAP1-negative and (B) ASLC-1-positve. Middle figures: YAP1-positive case, which contains (C) conspicuous mixture of YAP1-positive and YAP1-negative cells. (D) YAP1-positive cell components are negative for ASCL1, and YAP1-negative cell components are positive for ASCL1. Bottom figures: YAP1-positive case, which is (E) diffusely positive for YAP1 and (F) weakly positive for ASCL1. Details of immunohistochemistry and evaluation are shown in our previous report (PMID: 27418196).

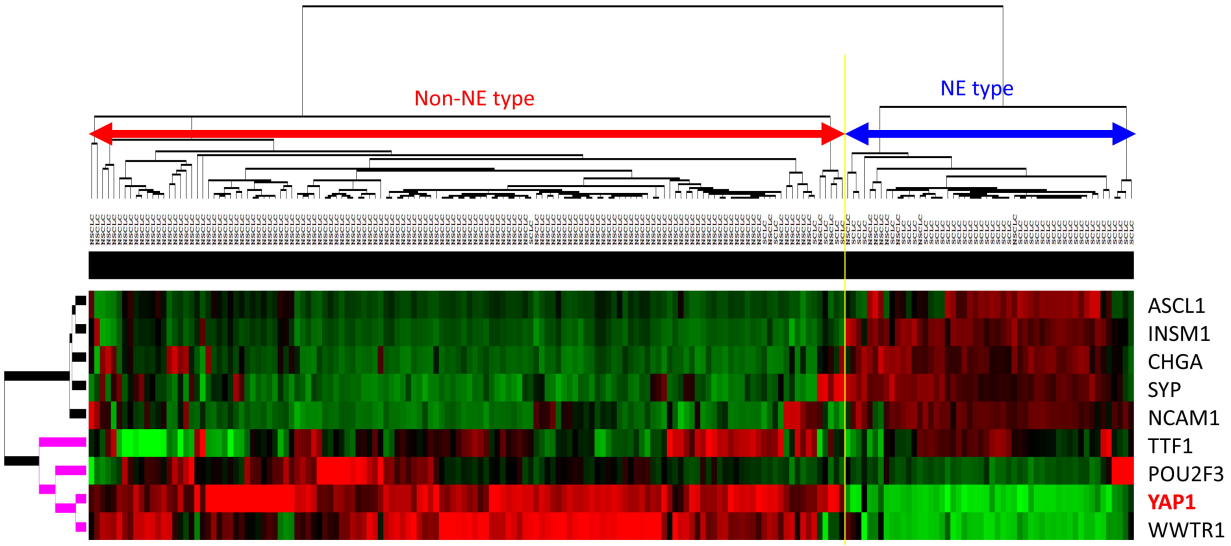

Fig. 6. Cluster analysis of lung cancer cell lines including 137 NSCLC and 51 SCLC cell lines in Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle/) based on gene expression levels of *WWTR1*, *TTF-1*, *YAP1*, *INSM1*, *POU2F3*, *ASCL1* and *NEUROD1*. We used the cluster program (http://rana.lbl.gov/EisenSoftware.htm, accessed March 19, 2008) for a cluster analysis of the gene expression data of cell lines, and displayed the results obtained with the aid of TreeView software (http://rana.lbl.gov/EisenSoftware.htm, accessed March 21, 2008) (Eisen Lab in Stanford University, Stanford, CA, USA), as mentioned in Fig. 2 legends. Fig. 6 shows that cell lines can be classified into two groups: NE type (right side) and non-NE type (left side).

other markers. However, by expanding the field of view to NSCLC or other tumors, such as retinoblastoma, the significance of these molecules may become clearer.

We present the cluster analysis of 188 lung cancer cell lines, including 137 NSCLC cell lines and 51 SCLC cell lines, based on the gene expression of NE markers (*CHGA*, *SYP*, *NCAM1*, *INSM1*, *TTF-1*, and *POU2F3*) and Hippo pathway effectors (*YAP1* and *WWTR1*) (shown in Fig. 6). Cells are divided into two groups: NE type (right side) and non-NE type (left side), and all of the non-NE-type cells exhibit high expression of *YAP1* and all of the NE-type cells exhibit low expression of *YAP1*. On the other hand, high expression of *POU2F3* was observed in both NE and non-NE types. The high expression of *POU2F3* is not specific to NE tumors (like *TTF-1*). We can therefore conclude that the loss of *YAP1* is the essence of NE differentiation of cancer cell lines regardless of whether they originated from primary SCLC or NSCLC.

10. Conclusions and perspectives

We briefly explained the meaning of YAP1 expression in HGNEC based on recent literature and our own studies. Focusing only on SCLC, it is difficult to understand the true meaning of YAP1. Only by expanding the field of view to NSCLC can we see that the loss of YAP1 is the essence of neuroendocrine differentiation of individual cells.

SCLC-Y is a concept mainly established from SCLC cell lines. SCLC is characterized by NE features, but it is a heterogeneous tumor in which most cells exhibit NE features and lack YAP1 expression. However, a small number of cells that have lost the characteristics of NE features exhibit YAP1 expression. As SCLC-Y is thought to have been established from these YAP1-positive cells, it should not be applied to the classification of primary SCLC. Conversely, if we encounter a case diagnosed as pure SCLC with diffuse and strong YAP1 positivity, we should doubt the diagnosis and consider the possibility of NSCLC.

In our study, more than half of the LCNEC cases were YAP1-negative, but the remaining exhibited a mixture of YAP1-positive and NE-marker negative (or weak) cells, and YAP1 expression correlated with chemoresistance. At present where there is no gold-standard chemotherapy for advanced or metastatic LCNEC, immunostaining for YAP1 may help predict susceptibility to platinum-based chemotherapy. For researchers, focusing on this heterogeneity of LCNEC and analyzing YAP1-positive and -negative components may help elucidate the mechanism of NE differentiation or loss of NE features. Furthermore, new therapeutic methods may be developed based on the control of NE differentiation. "Type II" LCNEC with RB1 loss (*RB1* mutations) and low expression of NE genes may be the counterpart of SCLC-Y, but there is no conclusive evidence. Further analysis of the relationship between the YAP1 expression pattern in LCNEC and its genetic background and susceptibility to therapies, including immunotherapy, is required.

Author contributions

HK—contributed to the manuscript preparation of Chapters 1, 2, and 3 and to the analysis of gene expression data. RM—contributed to the manuscript preparation of Chapters 4, 5, and 6 and to preparing the images of cancer tissue specimens. TI—contributed to the manuscript preparation of Chapters 7, 8, 9, and 10 and to the extraction of gene expression data of cancer cells in CCLE. DM—interpreted the data, designed the outline of this paper, and provided instructions to HK, RM, and TI.

Ethics approval and consent to participate

In this review article, we used pictures of histology of SCLC and LCNEC cases in our previous study. Informed consent was obtained from all patients, and the study was approved by the Institutional Ethics Review Committee (R03-258 in the university of Tsukuba).

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Conflict of interest

The authors declare no conflict of interest.

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