Determining Whether YAP1 and POU2F3 Are Antineuroendocrine Factors

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Rudin et al.[1] reported that SCLCs can be subtyped into four groups (SCLC-A, SCLC-N, SCLC-Y, and SCLC-P) according to four molecules—ASCL1, NEUROD1, POU2F3, and YAP1—based on gene expression data of primary SCLC samples and cell lines. Initially, both YAP1 and POU2F3 were identified as markers of the non-neuroendocrine (non-NE) phenotype of SCLC; however, subsequent studies have gradually revealed that YAP1 and POU2F3 have different significance.

First, we consider YAP1. Several subsequent studies suggested that YAP1 expression alone cannot define a single group in primary SCLC.[2,3] Now, it is gradually becoming clear that the classification of SCLC-Y is a concept that was mainly established from the analysis of cell lines, and it should not be applied to the classification of primary SCLC. As noted in our review paper,[4] 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, from Rudin et al.[1]) reveals that there are only two cases of primary SCLC in the SCLC-Y group, and they mostly consisted of SCLC cell lines. Among 51 SCLC cell lines in the Cancer Cell Line Encyclopedia (https://sites.broadinstitute.org/ccle/), at least 18% (9 of 51) of the SCLC cell lines highly express the *YAP1* gene.[4] SCLC-Y is present in cell lines but not in primary tumors. Therefore, we need to consider how the SCLC-Y cell lines were established.

SCLC can be considered a heterogeneous tumor in which most cells have NE features and lack YAP1 expression, but a small number of cells have non-NE features and YAP1 expression. Pearsall et al.[5] reported that many of the SCLC CTC-derived tumors contained a small number of YAP1-positive and NE marker-negative cells as minor components. Furthermore, they 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. SCLC-Y may have been established from these YAP1-positive cells. Nevertheless, the possibility that SCLC-Y cell lines were derived from NSCLC mimicking SCLC has not been ruled out. Indeed, 97% of primary NSCLCs, excluding large cell neuroendocrine carcinomas (LCNECs), express YAP1.[2]

In 1985, Carney et al.[6] and Gazdar et al.[7] 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. Notably, "variant type" SCLC cell lines frequently originated from post-therapy tumors that recurred and were more resistant to radiation than the "classic type." Our previous studies[2,4] revealed that *YAP1*-low SCLC cell lines are the floating type and NE marker positive; thus, they correspond to the "classic type." In contrast, because *YAP1*-high SCLC cell lines are the adherent type and NE marker negative, they correspond to the "variant type." Note that the *POU2F3*-high SCLC cell line, according to our study, is a floating type and faintly expresses INSM1, indicating the "classic type."[4]

Here, we present the cluster analysis of 188 lung cancer cell lines, consisting of 137 NSCLC cell lines and 51 SCLC cell lines, on the basis of the gene expression of NE markers (*CHGA*, *SYP*, *NCAM1*, *NEUROD1*, *INSM1*, *TTF-1*, and *POU2F3*), Hippo pathway effectors (*YAP1* and *WWTR1*), and *RB1* (illustrated in Fig. 1). The cells are divided into the following two groups: NE type (right side) and non-NE type (left side). All non–NE-type cells exhibit high expression of *YAP1* and all NE-type cells

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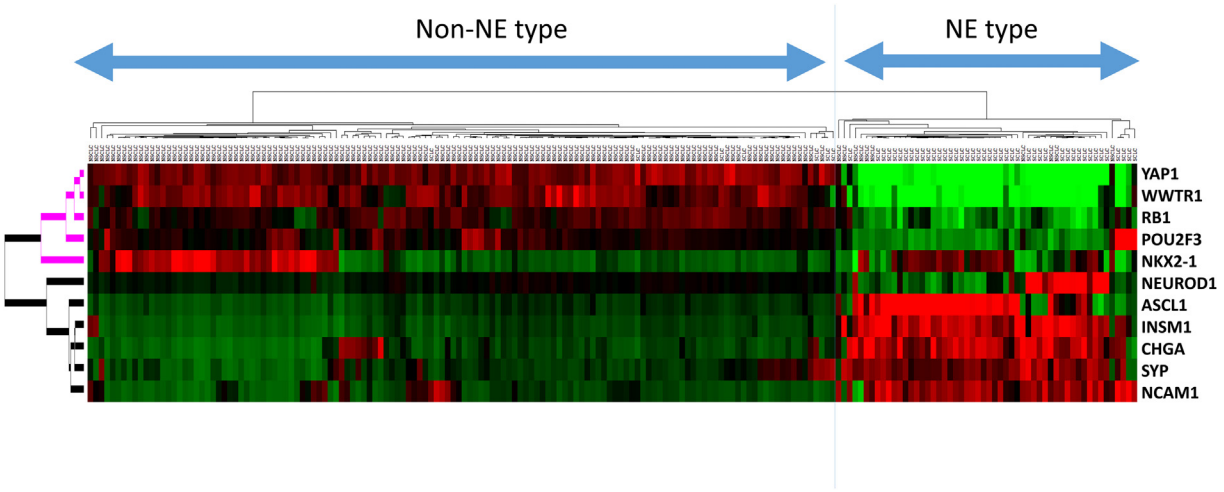

Figure 1. Cluster analysis of lung cancer cell lines, composed of 137 NSCLC and 51 SCLC cell lines from the Cancer Cell Line Encyclopedia (https://sites.broadinstitute.org/ccle/), on the basis of gene expression levels of *WWTR1*, *NKX2-1(TTF-1)*, *YAP1*, *INSM1*, *POU2F3*, *ASCL1*, *NEUROD1*, *SYP*, *CHGA*, *NCAM*, and *RB1*. We used a cluster program (http://rana.lbl.gov/EisenSoftware.htm, accessed on March 19, 2008) for the cluster analysis of the gene expression data of the cell lines and displayed the results with the aid of TreeView software (http://rana.lbl.gov/EisenSoftware.htm, accessed March 21, 2008) (Eisen Laboratory, Stanford University, Stanford, CA). The cell lines can be classified into the following two groups: NE type (right side) and non-NE type (left side). NE, neuroendocrine.

exhibit low expression of *YAP1*, indicating YAP1 is undoubtedly an anti-NE factor. Recently, YAP1 has been found to contribute to chemotherapy resistance[2,4,8] and sensitivity to immunotherapy.[9]

Next, we consider POU2F3. In Figure 1, although POU2F3 high- and low-expressing cells are found in the NE phenotype, POU2F3 high- and low-expressing cells are also found in the non-NE phenotype, which is similar to TTF-1 (NKX2-1). Nevertheless, POU2F3 high-expressing cells tend to have lower expression of other NE markers in the NE phenotype. The significance of POU2F3 in primary lung cancer needs to be clarified.

In this issue, Baine et al.[10] revealed that POU2F3 is specifically positive in a subset of basaloid squamous cell carcinomas (SCCs) that have morphologic similarities to SCLC and LCNEC. This new finding suggests a closer biological relationship between SCLC/LCNEC and basaloid SCC than is currently recognized and advances our understanding of the role of POU2F3 in tumor cells.

The expression of POU2F3 has been observed in a very limited number of tumors, including cutaneous basal cell carcinomas, SCCs, thymic tumors, and pulmonary SCLC.[11–13] POU2F3 is a transcription factor, and one of the roles of POU2F3 is the generation of rare, solitary chemosensory cells in the mucosal epithelium, called tuft cells, which are distributed in various parts of the body.[14,15] The expression pattern of POU2F3 is considered to be consistent with the distribution of tuft cells in bronchial epithelia (as found by Baine et al.[10]) and in thymic epithelia.[12] Tuft cells sense external stimuli by taste-like signaling pathways and generate epithelial cell-specific outputs, such as the cytokine IL-25, eicosanoids associated with allergic immunity, and the neurotransmitter acetylcholine.[15] Thus, we speculate that tuft cells can be viewed as "neuroendocrine cells" in the broad sense of the term.

Recent studies of POU2F3-positive tumors have proposed a group of tumors with a tuft cell signature.[12,13] Nevertheless, it is not clear whether POU2F3-positive tumors originate from tuft cells. As revealed by Baine et al.,[10] POU2F3-positive cells in the bronchial epithelium, which are thought to be tuft cells, are negative for p40, a sensitive marker for SCC, suggesting POU2F3-positive basaloid SCC may arise from nontuft cells and heterotopically express POU2F3. As Baine et al.[10] pointed out, basaloid SCC has been reported to exhibit up-regulated expression of various NE markers compared with conventional poorly differentiated SCC.[16] Therefore, POU2F3 expression in basaloid SCC may represent activation of chemosensory pathways, analogous to an increased rate of expression of NE markers in these tumors.

Another role of POU2F3 is the induction of epidermal differentiation. POU2F3 is primarily expressed in the epidermis and plays a critical role in keratinocyte proliferation and differentiation.[17] Figure 2 illustrates that POU2F3 is diffusely positive in the epidermis (Fig. 2A and B) but negative in the stratified squamous epithelium of the esophagus (Fig. 2C and D). Although this may be related to the fact that the epidermis is a sensory tissue, it can be inferred that POU2F3 also behaves like a marker of epidermal differentiation. We speculate that

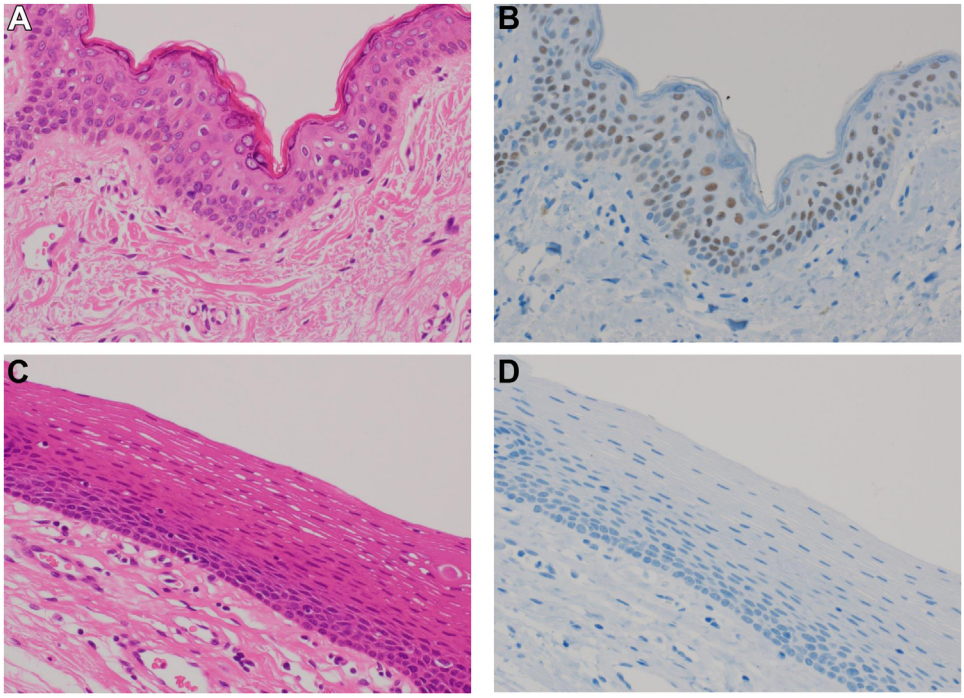

Figure 2. POU2F3 expression in the epidermis and esophageal squamous epithelium. (*A*) HE staining of skin tissue (×400). (*B*) Immunohistochemistry for POU2F3 in skin tissue. (*C*) HE staining of the esophagus (×400). (*D*) Immunohistochemistry for POU2F3 in the esophagus. HE, hematoxylin and eosin.

some tumors, similar to cutaneous basal cell carcinomas and SCCs, may retain POU2F3 expression as an epidermal-like differentiation trait.

We need to consider what POU2F3 expression indicates in cases of SCLC and LCNEC. Previous studies have revealed that POU2F3 expression and the expressions of ASCL1, NEUROD1, and other NE markers are mutually exclusive.[1,3] Nevertheless, as mentioned previously, tuft cells are a type of NE cell and POU2F3 is a transcription factor essential for their generation. We believe that POU2F3-positive SCLC is an NE tumor in nature, even though the typical NE markers are negative and that POU2F3 is, in a sense, an NE marker.

In this issue, Baine et al.[10] provided meaningful data on POU2F3 expression in primary lung tumors, but further genomic, transcriptomic, epigenetic, and proteomic analyses of POU2F3-expressing tumors with NE and non-NE features will be needed to understand the clinicopathologic significance of POU2F3.

CRediT Authorship Contribution Statement

Ryota Matsuoka, Hitomi Kawai, Takeshi Ito, and Daisuke Matsubara: responsible for writing the manuscript and generating the figures, and had final approval of the submitted and published versions.

Daisuke Matsubara: person with overall responsibility.

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