Editorial

Molecular basis of heterogeneity in small cell lung cancer

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Introduction

Small cell lung cancer (SCLC) is an aggressive neuroendocrine differentiated neoplasm (1). Despite a high response rate to first-line cytotoxic chemotherapy, almost all patients have an early relapse. Although immune checkpoint blockade therapies for SCLC have been established recently, survival rates have not yet surpassed those of non-SCLC patients (2). Thus, novel therapeutic modalities are desirable.

During the development and treatment of tumors, including SCLC, cancer cells show and increase intratumoral heterogeneity, and acquire the resistance to therapies (3). Analysis of tumor heterogeneity could help to resolve the disease relapse. Thus, accurate assessment of tumor heterogeneity at the molecular level is crucial for the development of effective therapies (1,4).

Molecular basis and subtypes of SCLC

SCLC is characterized by specific gene defects, such as the *TP53* and *RB1* mutations found in the majority of SCLC patients. Additionally, activation of certain signaling pathways (such as PI3K/AKT) and inactivation of other pathways (such as Notch) have been demonstrated in SCLC (5,6). Comprehensive genomic analyses of human SCLC samples and cell lines have also highlighted the importance of particular transcriptional networks, as well as the molecular function of SOX2 and Achaete-Scute complex homolog 1 (ASCL1), in SCLC (7,8).

Histologically, the morphological features of SCLC, including dense sheets of small cells and scant cytoplasm, are clearly defined. In addition, for diagnosis of SCLC, the demonstration of neuroendocrine differentiation using neuroendocrine markers, including insulinoma-associated protein 1 (INSM1), is needed. However, a minority of SCLCs do not show common neuroendocrine markers. Moreover, morphological heterogeneity is evident in the combined type of SCLC, in which non-SCLC components such as adenocarcinoma, squamous cell carcinoma, or large cell carcinoma are present to differing extents (9).

Although it is difficult to distinguish between the heterogeneous components of SCLC by histopathological methods, large-scale genomic analysis of SCLC has recently begun to reveal the molecular basis of SCLC heterogeneity. For example, Rudin *et al.* proposed a system of nomenclature to describe SCLC subtypes according to the dominant expression of four transcription factors considered to be the master regulators of SCLC. The four subtypes were designated as (I) SCLC-A, characterized by ASCL1; (II) SCLC-N, characterized by neurogenic differentiation factor 1 (NEUROD1); (III) SCLC-Y, characterized by yes-associated protein 1 (YAP1); and (IV) SCLC-P, characterized by POU class 2 homeobox 3 (POU2F3) (10). The SCLC-A subtype is a neuroendocrine-high form of SCLC associated with high levels of ASCL1, a member of the basic helix-loop-helix family of transcription factors (11). In contrast, the SCLC-Y subtype is a neuroendocrine-low subtype of SCLC associated with the activation of

NOTCH, Hippo, and RE-1 silencing transcription factor (*REST*) genes (12,13). ASCL1 plays a pivotal role in small cell carcinogenesis, and acts as a driver oncogene (11).

Significance of Notch signaling in SCLC

In a whole genome sequencing study, it is shown that about 25% of SCLC cases have mutations of Notch family genes (5), indicating the significance of Notch signaling in small cell lung carcinogenesis. Notch signaling is an essential cell signaling system that induces the expression of several genes, such as hairy and enhancer split-1 (*Hes1*) (14). Some gene transfection and knockdown experiments in SCLC cell lines demonstrated that Notch1 is involved in the suppression of cellular proliferation and neuroendocrine differentiation and enhancement of apoptosis (15,16). The Notch1-Hes1 pathway represses neuroendocrine differentiation by suppressing neuroendocrine-promoting transcription factors such as ASCL1 (17). Indeed, most classical SCLC cases and cell lines with neuroendocrine differentiation express ASCL1 and INSM1, and show an absence of NOTCH1 (18). Conversely, in non-SCLC cases, ASCL1 and/or INSM1 are negative but NOTCH1 is positive (18). Furthermore, in the combined type of SCLC, which is characterized by both SCLC and non-SCLC compartments, INSM1 is positive but NOTCH1 is negative in the SCLC compartment, while INSM1 is negative but NOTCH1 is positive in the non-SCLC compartment (19). Intriguigly, it is also reported that Notch signaling plays important roles in tumor heterogeneity of SCLC (12).

Notch-ASCL1-p53-RB axis in small cell lung carcinogenesis

A rare subset of epidermal growth factor receptor (*EGFR*) mutant adenocarcinomas showing resistance to treatment with EGFR tyrosine kinase inhibitors was reported to transform into SCLC (20). The transformation from adenocarcinoma to SCLC may have occurred via one of two proposed mechanisms. First, as an aspect of tumor heterogeneity, some tumor cells may have exhibited SCLC traits from the outset; second, SCLC traits may have been acquired during treatment with EGFR tyrosine kinase inhibitors. Bi-allelic inactivation of *TP53* and *RB1* is known to drive the formation of SCLC (5,7,10-13) and is understood to be a prerequisite for small cell lung carcinogenesis. Importantly, Niederst *et al.* reported that the transformation from adenocarcinoma to SCLC was always accompanied by the loss of *RB1* (20). In line with these findings, Meder *et al.* analyzed combined type SCLC from the point of view of the NOTCH-ASCL1-p53-RB axis (21) and suggested that non-SCLC tumor tissue harboring Notch abnormalities could transform into SCLC through the acquisition of *RB1* mutations. Conversely, combined SCLC could originate from pure SCLC; in this context, the non-SCLC component would be expected to show active Notch signaling and decreased levels of ASCL1/INSM1. Thus, we may speculate that the balance of Notch signaling activation and ASCL1 expression prior to mutation of *RB1* and *TP53* is an important factor in small cell carcinogenesis, including the formation of combined type tumors.

The role of SOX2 and Wnt signaling in SCLC

Amplification of *SOX2* occurs frequently in SCLC, while suppression of SOX2 using shRNA blocks proliferation in *SOX2*-amplified SCLC lines (7). In addition, SOX2 has distinct effects on transcriptional programs in SCLC-A and SCLC-Y subtypes. For example, in the SCLC-A subtype, ASCL1-recruited SOX2 regulates *INSM1* and *WNT11* expression, whereas, in the SCLC-Y subtype, SOX2 regulates *YAP1* expression (8), which, in turn, leads to the suppression of neuroendocrine differentiation (22). Furthermore, Wnt signaling also plays an important role in lung cancer cell biology. For example, WNT11 regulates neuroendocrine differentiation, cellular proliferation, and epithelial-mesenchymal transition in the SCLC-A subtype (23). ASCL1 is a key regulator of WNT11–WNT7b balance in lung cancer; SCLC cells strongly express WNT11, whereas WNT7b is frequently expressed in non-SCLC cells. In conclusion, the intratumoral heterogeneity of lung cancer could be explained partly by these distinct transcriptional programs and cell signaling systems (*Figure 1*).

A case of combined SCLC with enteric adenocarcinoma

Wang *et al.* have recently reported a case of combined SCLC with enteric adenocarcinoma (24). Their study included an assessment of the expression of two transcriptional factors, thyroid transcription factor-1 (TTF-1) and caudal type homeobox 2 transcription factor (CDX2) in this rare neoplasm. TTF-1 is known to be a useful marker for the diagnosis of thyroid and lung cancers, while CDX2 plays a critical role in intestinal development and has been widely used as a diagnostic marker of intestinal differentiation (25).

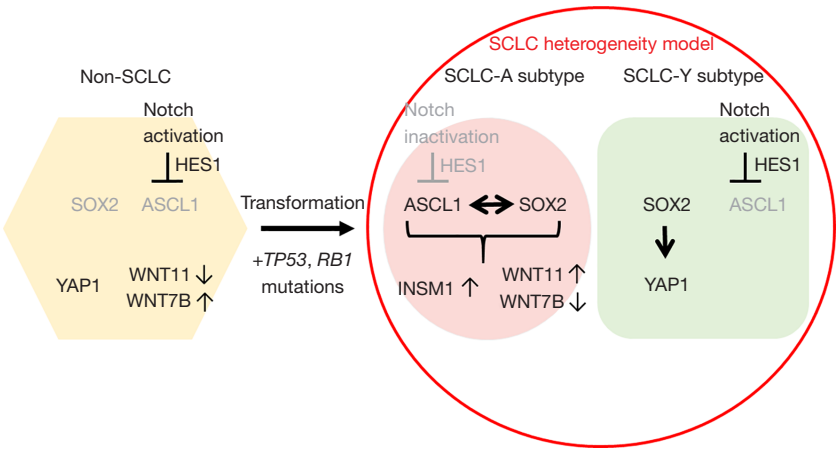

Figure 1 Hypothetical signaling pathways and transcriptional programs underlying carcinogenesis and intratumoral heterogeneity in small cell lung cancer (SCLC). SCLC may arise from lung epithelial cells with abnormalities in both TP53 and RB1, followed by inactivation of Notch signaling and expression of Achaete-Scute complex homolog 1 (ASCL1)/insulinoma-associated protein 1 (INSM1) in the SCLC-A subtype (neuroendocrine-high). Following activation of Notch signaling or yes-associated protein 1 (YAP1) expression, non-SCLC or SCLC-Y subtypes could emerge, resulting in the combined form of SCLC or other forms of intratumoral heterogeneity. SOX2 plays context-dependent roles in distinct lung cancer cells. Alternatively, SCLC may arise from a pre-existing non-SCLC tumor as a result of *RB1* mutation. Wnt signaling also affects lung cancer cell biology, while ASCL1 is a key regulator of WNT11–WNT7b balance.

These two markers have also been found to aid in the identification of tumor origin in cases where the primary lesion is unknown. Intriguingly, immunohistochemical analysis revealed expression of both TTF-1 and CDX2 proteins in the enteric adenocarcinoma component of the tumor. In addition, further defects, namely EGFR p.L861Q mutation and breast cancer susceptibility gene (*BRCA2*) deficiency, were detected in the biopsied tissue by next-generation sequencing. For further verification, sequencing of these genes in both SCLC and enteric adenocarcinoma components would be desirable. Detection of common gene mutations in both histological tumor types would enable us to conclude that the two components of this neoplasm originated from genetically identical cells and to hypothesize that the SCLC and enteric adenocarcinoma traits were acquired through a common process of carcinogenesis. Furthermore, analysis of the Notch pathway, as well as *ASCL1*, *SOX2*, *RB1*, and Wnt signaling-related genes could provide valuable information to verify the mechanism of intratumoral heterogeneity in this case.

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