



# 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 *RBI* 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). Intriguingly, 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

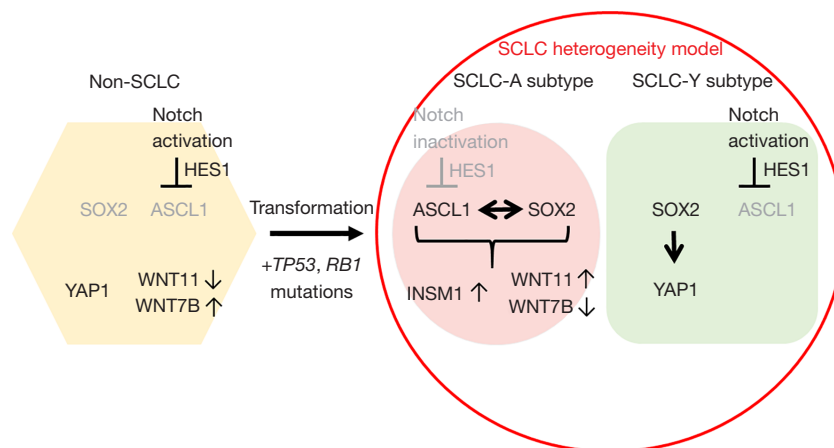
accompanied by the loss of *RB1* (20). In line with these findings, Meder *et al.* analyzed combined type SCLC from the point of view of the NOTCH-*ASCL1*-p53-RB axis (21) and suggested that non-SCLC tumor tissue harboring Notch abnormalities could transform into SCLC through the acquisition of *RB1* 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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## References

- Bunn PA Jr, Minna JD, Augustyn A, et al. Small Cell Lung Cancer: Can Recent Advances in Biology and Molecular Biology Be Translated into Improved Outcomes? *J Thorac Oncol* 2016;11:453-74.
- Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. *Lancet* 2019;394:1929-39.
- Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* 2018;15:81-94.
- Peifer M, Fernández-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nat Genet* 2012;44:1104-10.
- George J, Lim JS, Jang SJ, et al. Comprehensive genomic profiles of small cell lung cancer. *Nature* 2015;524:47-53.
- Liang X, Lin A, Wang Q, et al. Cell plasticity in patients with NSCLC: The controversial origins of transformed SCLC. *Biomed Pharmacother* 2022;149:112909.
- Rudin CM, Durinck S, Stawiski EW, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. *Nat Genet* 2012;44:1111-6.
- Tenjin Y, Matsuura K, Kudoh S, et al. Distinct transcriptional programs of SOX2 in different types of small cell lung cancers. *Lab Invest* 2020;100:1575-88.
- Travis WD, Brambilla E, Burke AP, et al. Introduction to The 2015 World Health Organization Classification of Tumors of the Lung, Pleura, Thymus, and Heart. *J Thorac Oncol* 2015;10:1240-2.
- Rudin CM, Poirier JT, Byers LA, et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nat Rev Cancer* 2019;19:289-97.
- Borromeo MD, Savage TK, Kollipara RK, et al. ASCL1 and NEUROD1 Reveal Heterogeneity in Pulmonary Neuroendocrine Tumors and Regulate Distinct Genetic Programs. *Cell Rep* 2016;16:1259-72.
- Lim JS, Ibaseta A, Fischer MM, et al. Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer. *Nature* 2017;545:360-4.
- Zhang W, Girard L, Zhang YA, et al. Small cell lung cancer tumors and preclinical models display heterogeneity of neuroendocrine phenotypes. *Transl Lung Cancer Res* 2018;7:32-49.
- Rizzo P, Osipo C, Foreman K, et al. Rational targeting of Notch signaling in cancer. *Oncogene* 2008;27:5124-31.
- Ito T, Kudoh S, Ichimura T, et al. 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* 2017;30:1-10.
- Wael H, Yoshida R, Kudoh S, et al. Notch1 signaling controls cell proliferation, apoptosis and differentiation in lung carcinoma. *Lung Cancer* 2014;85:131-40.
- Ito T, Udaka N, Yazawa T, et al. Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. *Development* 2000;127:3913-21.
- Fujino K, Motooka Y, Hassan WA, et al. Insulinoma-Associated Protein 1 Is a Crucial Regulator of Neuroendocrine Differentiation in Lung Cancer. *Am J Pathol* 2015;185:3164-77.
- Ito T. Intratumoral heterogeneity of Notch1 expression in small cell lung cancer. *J Thorac Dis* 2018;10:1272-5.
- Niederst MJ, Sequist LV, Poirier JT, et al. RB loss in resistant EGFR mutant lung adenocarcinomas that transform to small-cell lung cancer. *Nat Commun* 2015;6:6377.
- Meder L, König K, Ozretić L, et al. NOTCH, ASCL1, p53 and RB alterations define an alternative pathway driving neuroendocrine and small cell lung carcinomas. *Int J Cancer* 2016;138:927-38.
- Saito H, Tenjin Y, Yamada T, et al. The role of YAP1 in small cell lung cancer. *Hum Cell* 2022;35:628-38.
- Tenjin Y, Kudoh S, Kubota S, et al. 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* 2019;99:1622-35.
- Wang S, Tan Y, Li L, et al. A case report of pulmonary combined small cell carcinoma with enteric adenocarcinoma. *Transl Cancer Res* 2022. doi: 10.21037/tcr-22-1171
- Lee H, Fu Z, Koo BH, et al. The expression of TTF1, CDX2 and ISL1 in 74 poorly differentiated neuroendocrine carcinomas. *Ann Diagn Pathol* 2018;37:30-4.

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