Editorial Commentary

Genetic diversity in small cell lung carcinoma

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The development of next-generation sequencing (NGS) technology has led to many pivotal advancements in oncology. In particular, recurrent targetable alterations have been identified in non-small cell lung carcinoma (NSCLC), such as mutations in *EGFR*, *BRAF*, *ERBB2*, *KRAS*, rearranged *ALK*, *ROS1*, *RET*, *NTRK1*, and *MET* exon 14 skipping (1,2). The discovery of such actionable alterations in NSCLC has revolutionized the treatment of patients with NSCLCs. In contrast to NSCLC, small cell lung cancer (SCLC) was previously believed to be molecularly homogenous due to almost universal mutations in *TP53* and *RB1* (3,4). Over the last four decades, minimal changes have occurred in the therapy and survival outcomes of SCLC. In 2021, a transcriptome analysis reported that SCLC can be categorized into subgroups based on transcrriptome profiling (5). Three of the four subgroups are enriched in the predominant expression of specific transcription factors, namely *ASCL1* (SCLC-A), *NEUROD1* (SCLC-N), and *POU2F3* (SCLC-P), whereas the fourth is an inflamed subgroup (SCLC-I) associated with lack of expression of these transcription factors and higher levels of checkpoint proteins and interferon signaling (5). YAP1 was initially proposed to define a distinct subgroup; however, it was found to be absent or expressed only at low levels in tumors (5,6). Pathogenic mutations in *SMARCA4* were identified in six of eight YAP1-expressing SCLC (SCLC-Y) cell lines and correlated with reduced SMARCA4 RNA/protein expression, indicating that the characteristics of SCLC-Y are consistent with SMARCA4-deficient undifferentiated tumors rather than SCLC (7). The clinical implications of this transcriptional profiling are significant because each subgroup exhibits a unique susceptibility toward investigational therapies: SCLC-A, SCLC-N, and SCLC-P are sensitive to BCL-2, aurora kinase, and PARP inhibitors, respectively (5). Furthermore, patients with SCLC without the expression of these three transcription factors may benefit from immune checkpoint blockade (5). This new transcriptional profiling of SCLC has been validated at the protein level by immunohistochemistry in several studies (8-10). Nevertheless, the clinical significance of the molecular classification of SCLC based on mRNA profiling is not well defined, and the mechanisms underlying the effects of specific genetic alterations on the transcriptional landscape are yet to be elucidated.

Recently, Sivakumar *et al.* reported a genetic analysis of 3,600 patients with SCLC whose tissue samples were mainly obtained from community sites throughout the United States and submitted to Foundation Medicine, Inc. (11). This report presents several key observations related to SCLC pathobiology that may open novel therapeutic avenues for patients with SCLC. Firstly, approximately 5.5% of SCLC cases were identified as *TP53/RB1* wild-type tumors in the

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largest cohort study of patients with SCLC. While this finding is not surprising, alterations in genes that regulate RB and p53, such as *CDKN2A*, which codes for p16, a positive regulator of RB, *CCND1*, which codes for cyclin D1, a negative regulator of RB, and *MDM2*, a negative regulator of p53, were frequently identified in *TP53/RB1* wild-type tumors. Additionally, human papillomavirus (HPV) was also identified in 12.7% of *TP53/RB1* wild-type tumors. The p53 and RB proteins are well-characterized targets of the HPV E6 and E7 oncoproteins (12). HPV E7 proteins subvert G1–S arrest and induce hyperproliferation through the inhibition of RB family members and constitutive activation of E2F-responsive genes. Co-expression of HPV E6 with E7 abrogates p53-dependent apoptosis in response to the activities of E7, allowing replication in the presence of DNA damage and increased chromosomal instability (12). Thus, HPV^{+}*TP53/RB1* wild-type tumors are a distinct genetic subtype that may be uniquely responsive to strategies targeting HPV or reactivating p53 function to induce cell death in HPV-positive cancer cells. Basal cells in the bronchial epithelium can be infected with certain HPV strains (13); however, the lungs are not a common organ for HPV infection. Notably, some HPV-positive patients with NSCLCs (14) and SCLCs (15) may experience metastases due to the presence of HPV-positive tumors at other body sites. Thus, further analyses with detailed clinicopathological information are needed to elucidate the incidence of HPV infection in patients with SCLC and NSCLC. Second, driver mutations of receptor tyrosine kinase genes, including *EGFR* mutations (n=107) and *ALK* (n=5), *ROS1* (n=3), *RET* (n=5), and *NTRK1* (n=1) fusions, have been detected in SCLC (11). Subsequent genomic analysis of 41 patients with paired NSCLC and SCLC samples showed that NSCLC and SCLC tumor samples shared driver mutations in approximately 61% of cases. However, in approximately 17% of patients, NSCLC and SCLC tumor samples shared alterations, but no previously described driver mutations were detected in either biopsy. Therefore, these patients cannot be treated with tyrosine kinase inhibitors, suggesting that other treatment modalities, such as chemotherapy and immunotherapy, can also drive transformation (11). Additionally, a smoking-associated mutational signature is rare in *TP53/RB1* wild-type tumors (11). Frequently altered genes in NSCLC, such as *KRAS*, *BRAF*, *FGFR1*, and *KEAP1*, were enriched in *TP53/RB1* wild-type tumors in which a smoking-associated mutational signature is rare. Patients with *STK11* mutations were enriched for mutations in genes associated with NSCLC development, such as *KRAS* and *KEAP1*. These findings suggest that several of these tumors may have originated from NSCLC (11). Finally, the mutation spectrum of different sites of metastases showed that brain metastases were enriched for *PTEN* alterations compared with primary lung tumors, indicating that the PTEN pathway can play a unique role in SCLC brain metastasis (11).

Transformation of *EGFR*-mutated adenocarcinoma to SCLC in the case of acquired resistance to tyrosine kinase inhibitors has been observed in approximately 3–10% of patients (16). However, the results of a study by Sivakumar *et al.* suggested that SCLC transformation may occur across multiple distinct molecular cohorts of NSCLC (11). Populations of different ancestries are enriched for different genetic subtypes of SCLC such as *EGFR* mutations in patients with Asian ancestry and *STK11* mutations in patients with African ancestry (11). Molecular profiling, along with NGS, using formalin-fixed paraffin-embedded specimens has become essential for identifying predictive biomarkers for personalized therapy in advanced or metastatic NSCLC. The clinical application of NGS in patients with SCLC should be considered in future studies.

The major limitation of the study by Sivakumar *et al.* is the absence of RNA/protein expression profiles analyzed for SCLC. NSCLCs have emerged as successful examples of cancers that have been molecularly redefined through the discovery of actionable mutations. In addition to such genetic changes, differences in the pemetrexed response (17,18) and the efficacy of immune checkpoint inhibitors (19) have been reported in *EGFR/ALK*-negative lung adenocarcinoma with differential thyroid transcription factor 1 (TTF-1) expression, suggesting that more accurate and reliable treatments can be achieved by acquiring integrated genomic and transcriptomic information in NSCLC. Similarly, combined genomic analyses with transcriptome data of key transcription factors, including *ASCL1*, *NEUROD1*, and *POU2F3*, may be more efficient for patient stratification in SCLC. Another limitation of the study by Sivakumar *et al.* is the absence of a central pathological review because tissue samples with initial diagnosis were submitted by various practicing physicians throughout the United States. Notably, 1.5% of cases harbored inactivating mutations in *SMARCA4*, and these tumors should have been diagnosed as SMARCA4-deficient undifferentiated tumors. Nevertheless, before recognition of SMARCA4-deficient undifferentiated tumors as a distinct entity, these tumors were commonly categorized as neuroendocrine carcinomas in patient samples (20,21). Furthermore, the incidence

of combined SCLC in 3,600 patients with SCLC was unclear. Combined SCLC is defined as SCLC combined with elements of NSCLC within the primary tumor in which spontaneous transformation from NSCLC to SCLC may occur. Pathological analysis of surgically resected, treatment-naïve SCLC revealed that combined SCLC with adenocarcinoma accounts for approximately 9% of cases of SCLC (22). Another study showed that combined SCLC with adenocarcinoma, squamous cell carcinoma, and spindle cell carcinoma account for approximately 6.5%, 12%, and 1% of cases of SCLC, respectively (9). The driver mutations in the cohort studied by Sivakumar *et al.* were recurrently detected because some SCLCs with driver mutations may combine SCLCs with driver mutation-positive NSCLC components. The incidence of combined SCLC in real-world data remains largely unknown because combined SCLC is usually diagnosed in surgical specimens and not biopsy specimens. To address this limitation, a radiomic or radiogenomic approach can be useful to evaluate tumor heterogeneity in SCLC and differentiate pure SCLC and combined SCLC (23).

Subtyping of SCLC has been attempted many times since the first morphological classification in 1967 (24). In the era of precision medicine, novel subclassification of SCLC based on tissue-based biomarker assessment that can improve treatment strategies is required. Sivakumar *et al.* revealed the genetic diversity in SCLC. Although the number of patients with *TP53/RB1* wild tumors (5%) and transformed SCLC (4%) was limited, their tumors were genetically distinct from *TP53/RB1* mutated SCLC (11). Genetically, SCLC is categorized into *de novo* SCLC and transformed or combined SCLC. *De novo* SCLC is further categorized into *TP53/RB1* mutated type SCLC and *TP53/RB1* wild type SCLC. To validate the transcriptional profiling of SCLC and its therapeutic possibilities in BCL-2, aurora kinase, PARP inhibitors, and immunotherapy, future clinical trials should be planned for every genetically diverse group. Even when the ideal classification of SCLC is established, the limitation of access to tissue as diagnostic specimens may remain. Liquid biopsy can be used to overcome these limitations. Notably, a recent study showed that DNA methylation from tissues and liquid biopsy samples allows a subgroup of SCLC with ASCL1, NEUROD1, and POU2F3 (25). In addition to genomic and transcriptomic approaches, the ability of epigenomic approaches to predict pharmacological responses to drugs has the potential to revolutionize the subclassification of SCLC.

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