New Insights into the Epigenomic Landscape of Small-Cell Lung Cancer: A Game Changer?

Lung cancer, the world's deadliest cancer, is categorized into two main histological groups: non-small-cell lung cancer (NSCLC) (85% of all lung cancers) and small-cell lung cancer (SCLC) (15% of all lung cancers). SCLC is marked by an exceptionally high rate of proliferation, a predilection for early metastasis, and a poor prognosis with only a 7% 5-year survival rate. The significant risk of metastasis is because of an unstable genome, rapid progression, and enhanced angiogenesis. Early detection by screening is challenging because of its indistinct preinvasive histological pattern (1). Although immunotherapy has made a significant contribution with the introduction of immune checkpoint inhibitors against PD-1/PD-L1 and CTLA-4 for the treatment of NSCLC, this is less of a success story for SCLC. In patients with SCLC who received atezolizumab combined with chemotherapy, the average survival improved by only 2 months (2, 3).

In contrast to NSCLC, the origin and evolution of SCLC are different, and its sequencing data is limited, which slows the development of personalized therapy (4). Tumor suppressor genes, such as RB1 (retinoblastoma 1) and TP53 (tumor protein p53), are inactivated in the majority of patients with SCLC (5). However, a growing body of evidence from molecular analyses (genome-wide methylation and transcriptomic analysis) of tumor tissue samples indicates considerable heterogeneity in the histology, cell morphology, degree of neuroendocrine differentiation, and role of neuronal lineage-specific transcription factors in this disease. Integrative analysis of these datasets led to four distinct subtypes driven by activation of transcription factors ASCL1 (SCLC-A), NEUROD1 (SCLC-N), YP1 (SCLC-Y), and POU2F3 (SCLC-P) (6). Other new subgroups have been described in human SCLC cell lines. One study proposed an inflammatory SCLC type (SCLC-I) (7), and another found a unique neuroendocrine variant (NEv2, or SCLS-A2) (8). In a nutshell, the recognition of tumor heterogeneity in SCLC suggests that new approaches are needed to treat these subtypes with their unique molecular features and therapeutic vulnerabilities.

The development of such new treatments will benefit from recent advances in high-throughput profiling that allows researchers to investigate changes in the genomic and epigenomic makeup of cancers, which aids the (epi)genotype-based classification into subtypes. Most somatic mutations in cancer impact protein-coding areas by turning oncogenes on or off. Other modifications that exert effects on the more significant noncoding regions of the genome by regulating gene expression have also been identified. These include epigenetic landmarks that reposition an oncogene adjacent to a highly active promoter or bring the promoters of oncogenes under the control of powerful transcriptional enhancers. Specific transcription factors and enhancers determine the cellular identity (9).

In this issue of the *Journal*, Kong and colleagues (pp. 1480–1494) add to the classification of SCLC molecular subtypes using histone modification-based epigenomic analysis. The study's achievements and highlights include *1*) genome-wide analysis of H3K27ac in 16 SCLC cell lines that identified two distinct super-enhancer landscapes, SCLC-Aα and SCLC-Aσ, which can be further distinguished by Nkx2–1 and TCF4 SEs; *2*) NKX2–1 cistrome profiling reveals that both SCLC-A and lung adenocarcinoma cell lines express NKX2–1, yet it promotes different transcriptional pathways; *3*) NKX2–1 regulates neural differentiation in SCLC-Aα, which is quite distinct from its role in lung epithelium in lung adenocarcinoma cell lines; and *4*) The specific cooccupancy of NKX2–1 and SOX1 in the SCLC-Aα genome regulates neural gene regulation, demonstrating a lineage-specific genomic-epigenomic interaction landscape in lung cancer. To explore the biological importance of the subtypes, the author used *in vitro* and *in vivo* models. These models suggest that a loss of NKX2–1 results in a stochastic predisposition to develop nonclassical SCLC-A morphology and thus a different lineage class of malignancies (10).

Challenges and Future Perspectives

The discoveries made by Kong and colleagues will undoubtedly inspire studies aimed at identifying epigenetic landmarks in molecular subtypes of additional tumor types. However, because of our incomplete understanding of the gene regulatory code, it is hard to precisely characterize such noncoding sequences. We believe that five critical challenges will hamper the adoption of epigenetic cancer subtyping in the future (Figure 1).

First, epigenetic profiling separates lineage classes more clearly than expression analysis. However, such processes in eukaryotes involve DNA methylation, histone modifications, chromatin compaction, and nuclear organization. TCGA (The Cancer Genome Atlas) research network allows unsupervised clustering of DNA methylation, DNA copy number, mRNA, microRNA, and protein array. Kong and colleagues argue that histone modifications and chromatin marks in promotor-enhancer–super-enhancer regions can be exploited for molecular subtyping. But which chromatin marks are the

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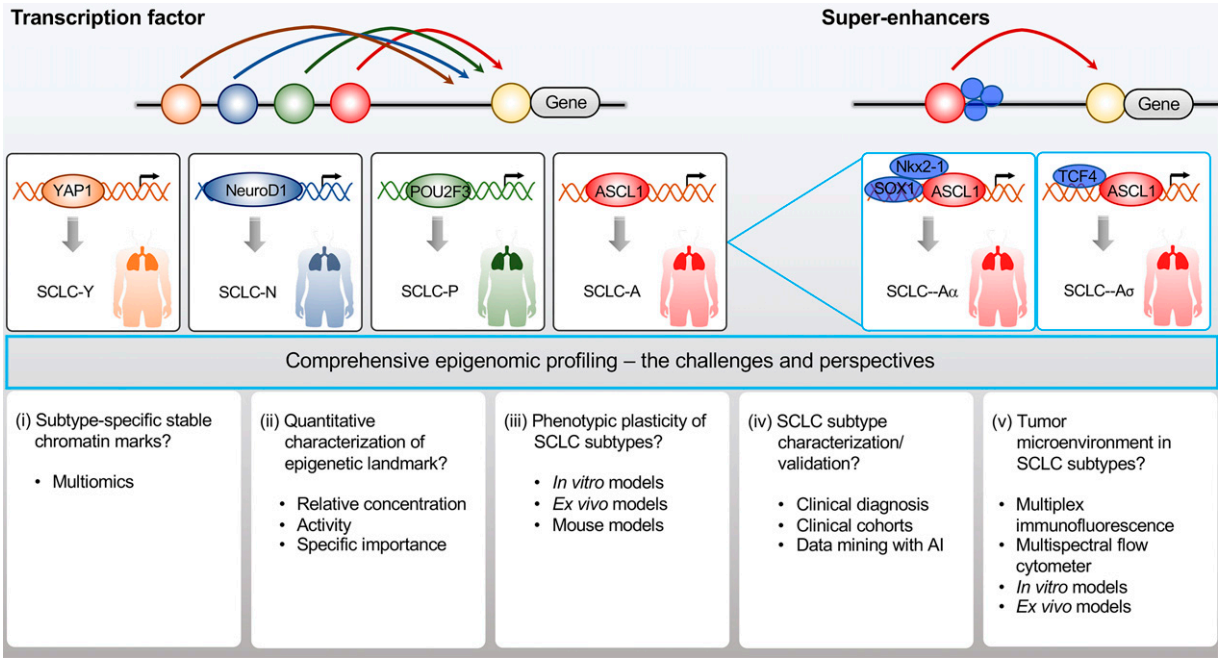

Figure 1. Small-cell lung cancer (SCLC) can be subclassified according to the relative activity of four key transcriptional regulators: SCLC-A (ASCL1 [achaete-scute homolog 1]), SCLC-P (POU2F3 [POU class 2 homeobox 3]), SCLC-N (NEUROD1 [neurogenic differentiation factor 1]), and SCLC-Y (YAP1 [yes-associated protein 1]) (left panel). Kong and colleagues applied epigenomic approaches to determine lineage heterogeneity of SCLC, which subdivides the major SCLC-A into two clusters (SCLC-Aα and SCLC-Aσ) with distinct super-enhancer landscapes. The study shows NKX2–1 (NK2 Homeobox 1) to be one of the most differentially enriched super-enhancers in SCLC-Aα and TCF4 (transcription factor 4) in SCLC-Aσ. NKX2–1 and SOX1 (SRY-Box transcription factor 1) cooccupy the SCLC-Aα genome and collaborate to control its neural lineage. This study provides functional significance of the SCLC lineage heterogeneity and mechanisms through which SCLC subtypes undergo cellular state reprogramming (right panel). Although a comprehensive epigenomic analysis will aid the development of subtype-specific therapeutic approaches to benefit patients with SCLC, future studies need to address specific challenges: *1*) Identification of subtype-specific stable chromatin marks using multiomics; *2*) Quantitative characterization of the identified epigenetic landmark; *3*) Determination of phenotypic plasticity within SCLC subtypes using *in vitro*, *ex vivo*, and mouse models; *4*) Characterization of the statistical and biological significance of the identified SCLC subtype in larger clinical cohorts aided by data mining with artificial intelligence in order to develop subtype-specific medications; *5*) Understanding SCLC subtype-specific tumor microenvironment on the basis of data generated by multiplex immunofluorescence, multispectral flow cytometry, and *in vitro* and *ex vivo* models.

ones that define disease-associated cell types? Although Trynka and colleagues observed that active gene regulation chromatin marks such as H3K4me3 were cell-type–specific, the author acknowledges that such techniques are vulnerable to cell-type variation and technological factors (11). Systematic studies are needed to find cell-type–specific stable chromatin marks for molecular subtyping.

Second, Kong and colleagues also show the functional significance of genomic characteristics that carry significant signals for enhancer-associated chromatin marks, which include regions like super-enhancers, DNase I super-hypersensitive sites, or locus control areas. These regions are not clearly defined yet. Unanswered questions include whether these elements are separate biological entities and whether the differences between them and "common" enhancers are quantitative or qualitative. Historically, qualitative research has been the focus of genomics, which has identified and mapped features including genes, transcripts, binding sites, and histone modifications. The relative concentration, activity, or specific importance of these features have received less attention. Numerous studies have consistently demonstrated that powerful enhancers make genes more vulnerable to changes that lower the activity of critical transcriptional regulators. During carcinogenesis, many driver genes come under the control of such potent enhancers. Considering how susceptible to perturbation enhancers are, researchers should carefully examine the characteristics of enhancer-associated genomic landmarks (12).

Third, despite genomic and epigenomic studies identifying various cancer types/subtypes, it is important to understand the biological significance of these molecular/epigenomic changes. *In vivo* genetically engineered mouse models need to be generated for further extensive cellular, molecular, and histological phenotyping. To accurately describe the subtypes, it needs molecular and clinical evidence in combination. When tumors are accurately classified into their subtypes, the next step will be to improve our understanding of their vulnerabilities for the generation of new drugs (13).

Fourth, several epigenetic regulators (DNA methyltransferases or histone deacetylases inhibitors) are effective targets in cancer

therapy. Without a doubt, chromatin-targeting medications could revolutionize how cancer is treated and result in more specialized treatments for complex molecular subgroups. But to minimize side effects, future epigenetic drugs will need to focus on particular chromosome regions (14).

Fifth, the tumor microenvironment (TME) and the tumor cell compartment are heterogeneous. The TME contains extracellular scaffolding, fibroblasts, *extracellular matrix*, connective tissue, adipose tissue, and immune cells. Despite significant interactions, TME-associated cell types have a different genetic architecture compared with tumor cells (15, 16). Therefore, the challenges that require future research are: How does epigenetic alteration in tumor cells regulate the microenvironment? Which cell type in the TME turns out to be the more efficient target? ■

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