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Review

Review

Understanding SCLC heterogeneity and plasticity in cancer metastasis and chemotherapy resistance

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Abstract
Small cell lung cancer (SCLC) accounts for approximately 15% of all lung cancer cases and features a strong predilection for early metastasis and extremely poor prognosis. Despite being highly sensitive to chemotherapy and/or radiotherapy initially, most SCLC patients develop therapeutic resistance within one year and die of distant metastases. Multiple studies have revealed the high heterogeneity and strong plasticity of SCLC associated with frequent metastases and early development of therapeutic resistance as well as poor clinical outcome. Importantly, different SCLC subtypes are associated with different therapeutic vulnerabilities, and the inflamed subtype tends to have the best response to immunotherapy, which highlights the importance of precision medicine in the clinic. Here, we review recent advances in SCLC heterogeneity and plasticity and their link to distant metastases and chemotherapy resistance. We hope that a better understanding of the molecular mechanisms underlying SCLC malignant progression will help to develop better intervention strategies for this deadly disease.

Key words small cell lung cancer, heterogeneity, plasticity, metastases, chemotherapy resistance

Introduction

Small cell lung cancer (SCLC) accounts for approximately 15% of all lung cancer cases [1]. SCLC frequently occurs in lifetime heavy smokers (5 or more cigarettes a day), with only 2% of cases arising in never-smokers [2–4], which is possibly linked to exposure to air pollution [5] and radon [6] and histological transformation from non-small cell lung cancer (NSCLC) [7]. SCLC is rapidly growing, highly metastatic, and relatively immune-cold, with an extremely poor prognosis compared to other solid tumors [8]. SCLC can be classified into limited stage and extensive stage. Most patients are initially diagnosed at an extensive stage, characterized by nearby lung and/or distant organ metastases [9]. The most common organs for SCLC metastases include the contralateral lung, brain, bone and liver [10,11] (Figure 1). A high frequency of early metastasis and therapeutic resistance contributes to poor clinical outcomes of SCLC, with a 5-year survival rate of less than 7% [12]. Current first-line SCLC therapy remains chemotherapy and radiotherapy, which were established decades ago [13–16]. Although SCLC patients initially exhibit a strong response to chemotherapy, most relapse with acquired drug resistance within one year [17–20].

Based on studies of human specimens and mouse models, emerging recognition of the high heterogeneity and plasticity of SCLC has implicated the complexity of this disease [21–33]. In this review, we will summarize the progression of current findings in SCLC heterogeneity and plasticity, as well as their link to distant metastases and chemotherapy resistance. The improved understanding of the molecular mechanisms underlying SCLC heterogeneity and plasticity might help the development of novel therapeutic strategies for clinical management.

SCLC Subtyping and Therapeutic Vulnerabilities

Although neuroendocrine (NE) cells serve as the predominant cell of origin of SCLC, alveolar type II (AT2) cells and club cells are also endowed with this ability [34–36]. Growing evidence has supported the multiple cells of origin of SCLC, indicating the potential heterogeneity of this disease. Since the 1980s, both “classic” and “variant” phenotypes have been detected in established human SCLC cell lines [37,38]. Nearly 70% of these cell lines feature a

"classic" phenotype, grow as tight aggregates and highly express NE-associated proteins [39]. The rest belong to "variant" cell lines, which can be further classified into morphological and chemical variant subtypes, with the former adherent to the dishes in cell culture and the latter growing as tightly aggregates with reduced NE markers [39].

The differential expression of four lineage-related transcription factors distinguishes SCLC into the achaete-scute homolog 1 (ASCL1) (SCLC-A), neuronal differentiation 1 (NEUROD1) (SCLC-N), POU class 2 homeobox 3 (POU2F3) (SCLC-P) and Yes1 associated transcriptional regulator (YAP1) (SCLC-Y) subtypes, which are associated with distinct therapeutic vulnerabilities [30]. Delta-like ligand 3 (DLL3), a direct transcriptional target of ASCL1, tends to be highly expressed in the SCLC-A subtype and minimally expressed in normal tissues [40], which enables the development of therapeutics to specifically target SCLC cells (Table 1). The DLL3-targeted antibody drug conjugate (ADC) rovalpituzumab tesirine (Rova-T) has been evaluated in clinical trials [41] (Table 1). Unfortunately, the following phase II study demonstrated associated toxicities [42], and additional DLL3 targeting approaches are currently under development [43] (Table 1). Other important aberrations in the SCLC-A subtype include amplifications of BCL2 apoptosis regulator (BCL2) [44] and enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) [45,46] and a decrease in CREB binding protein (CREBBP) [47] (Table 1).

Upregulation of C-MYC is related to the SCLC-N subtype [30] and serves as a potential target for therapeutic agents [61,62]. SCLC with high C-MYC expression is selectively vulnerable to aurora A/B kinase, checkpoint kinase 1 (CHK1), inosine monophosphate dehydrogenase 1/2 (IMPDH1/2) inhibitor treatment, and arginine deprivation [48–56] (Table 1). Although C-MYC shares major features with its paralogues N-MYC and L-MYC, the sensitivity to aurora kinase inhibitors seems unique for C-MYC-driven SCLC based on the results of a clustered regularly interspaced short palindromic repeats (CRISPR) activation model [54]. A recent double-blind clinical study confirmed that C-MYC may be a potential predictive biomarker of response to the aurora A inhibitor alisertib [63]. The SCLC-P subtype has been shown to preferentially depend on insulin-like growth factor 1 receptor (IGF-1R) and poly(ADP-ribose) polymerase 1 (PARP) signaling [44,57] and is

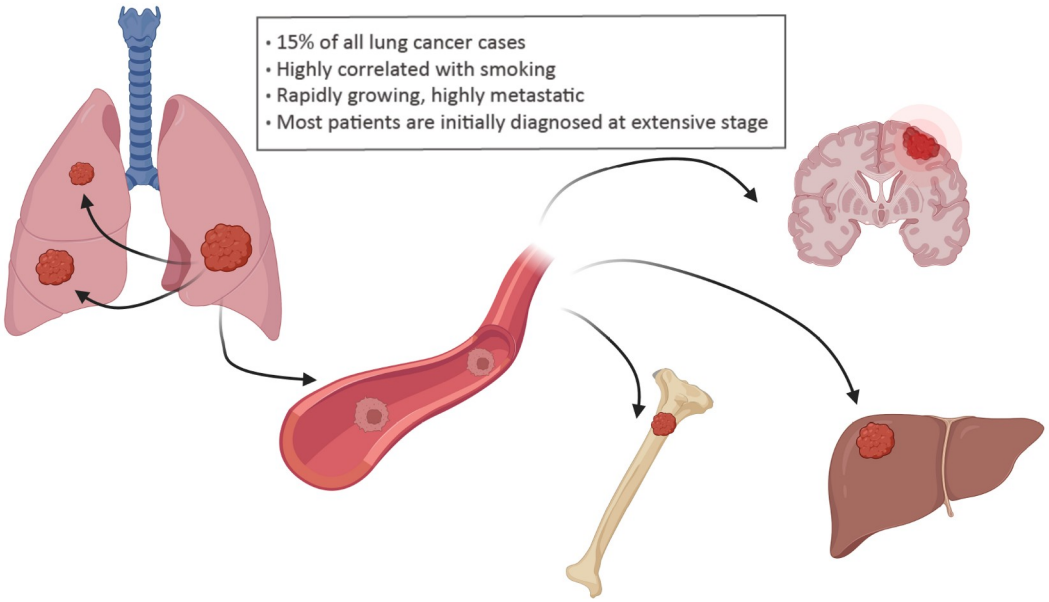

Figure 1. **Various organs for SCLC metastasis**

Table 1. **SCLC subtyping and therapeutic strategies**

Subtype	Characteristic	Therapeutic strategies
SCLC-A	High DLL3 level [40] Amplifications of BCL2 [44] or EZH2 [45,46] Decrease of CREBBP [47]	DLL3 inhibitor (Rova-T) [40–43]
SCLC-N	Upregulation of C-MYC [30]	Aurora A/B kinase, CHK1, IMPDH1/2 inhibitors, arginine deprivation [48–56]
SCLC-P	Activation of IGF-1R or PARP signaling [44,57] High MCL1 level [58]	MCL1 inhibitor (S63845) [58]
SCLC-Y	Inflamed tumor microenvironment [59]	Immunotherapy [59]
SCLC-I[a]	Low expression of ASCL1, NEUROD1 and POU2F3 [44]	Combined chemotherapy and immunotherapy [44]
SCLC-I[b]	Immunosuppressive feature and high genomic instability, high POU2F3 level [60]	Combined chemotherapy and immunotherapy [60]

enriched with myeloid cell leukemia 1 (MCL1) expression, indicating a potential response to targeted therapy with the MCL1 inhibitor S63845 [58] (Table 1). The SCLC-Y subtype is associated with an inflamed tumor microenvironment, suggesting a potential benefit from immune checkpoint blockade treatment [59] (Table 1).

Studies show the close association between increased tumor mutational burden (TMB) and response to immunotherapy in multiple cancer types [64,65]. Considering the high TMB of SCLC [66] and better prognosis of patients with increased tumor-infiltrating lymphocytes (TILs) [67,68], immunotherapy is approved for patients with extensive stage or relapsed SCLC [69–71]. However, response to anti-PD-1/PD-L1 therapy only occurs in a small group of patients with SCLC [71,72]. A phase III clinical trial of anti-PD-1 (nivolumab) in combination with anti-CTLA-4 (ipilimumab) in patients with extensive stage SCLC failed to meet its primary endpoint of overall survival [70]. Moreover, the correlation between PD-L1 expression and the effect of immunotherapy is ambiguous [73,74]. These studies imply that the efficacy of immunotherapy for unselected patients with SCLC is modest; thus, it is urgently needed to identify the patients who may benefit most from immunotherapy. Using tumor expression data and nonnegative matrix factorization, a previous study identified a SCLC subtype (SCLC-I^{a}) with low expression of ASCL1, NEUROD1 and POU2F3 and featured an inflamed gene signature [44] (Table 1). SCLC-I^{a} is sensitive to the addition of immunotherapy to chemotherapy [44] (Table 1). Through integrative analysis of multi-omics data, we also uncovered the immune features of SCLC (SCLC-I^{b}) with immunosuppressive features and high genomic instability [60]. Importantly, we found that POU2F3 is effective in predicting the SCLC-I^{b} subtype, and patients with high POU2F3 expression exhibit better responses to immunotherapy [60] (Table 1). SCLC-A (70%), SCLC-N (10-15%) and SCLC-P (12%) are the dominant subtypes of SCLC [57,75,76]. However, only 2% of SCLC shows YAP1 expression at quite low levels relative to ASCL1, NEUROD1 and POU2F3 [76,77]. YAP1 and its transcriptional targets are higher in both POU2F3 and SCLC-I^{a} subtypes than in the other two subtypes [44]. Therefore, it is proposed that YAP1 alone may not define a single group [78–80]. In contrast to the SCLC-I^{a} subtype with low POU2F3 expression, the SCLC-I^{b} subtype shows high POU2F3 level [60]. However, the relationship between the SCLC-P and SCLC-I^{b} subtypes is unclear and needs further study.

Notch signaling is positively correlated with the non-NE phenotype and significantly predicts the clinical benefit of immunotherapy [67]. Cyclin-dependent kinase 7 (CDK7) is a central regulator of the cell cycle and gene transcription [81]. Combining CDK inhibitors with anti-PD-1 offers a significant survival benefit in SCLC, providing a rationale for new combination regimens and immunotherapies [82]. More recently, certain combination chemotherapy plus immunotherapy regimens (including the anti-PD-L1 drugs atezolizumab or durvalumab) have been recommended in the National Comprehensive Cancer Network (NCCN) Guidelines for SCLC as preferred options for patients with extensive stage SCLC [69,83–88].

Molecular and Cellular Mechanisms Involved in SCLC Metastasis

Concurrent loss of p53 and RB1 occurs frequently in SCLC [89,90]. Homozygous deletion of these two alleles in mouse lung epithelia promotes SCLC development and dramatic metastasis, which closely recapitulates the clinical disease [91]. SCLC derived from the *Rb1*$^{L/L}$/*Trp53*$^{L/L}$ (*RP*) mouse model typically expresses NE markers, including neural cell adhesion molecule 1 (NCAM) and ASCL1, and frequently metastasizes into distant organs. Concurrent deletion of RB family members p107 and p130 or *Pten* in the *RP* model significantly accelerates malignant progression and SCLC metastasis [22,92–94].

Based on the study of these autochthonous SCLC mouse models, we recently identified NCAM^{hi}CD44^{lo} cells as the major subpopulation responsible for liver metastasis [32] (Figure 2). During SCLC malignant progression, the phenotypic transition of NCAM^{hi}CD44^{lo} cells (SCLC metastasizing cells, SMCs) from NCAM^{lo}CD44^{hi} cells (non-SCLC metastasizing cells, non-SMCs) is driven by the down-regulation of the Hippo pathway co-activator *Taz*/*Wwtr1*. Moreover, the SWI/SNF chromatin remodelling complex plays an important role in silencing *Taz* during this process. Liver metastasis from SCLC patients showed decreased *Taz* expression and an increased NCAM^{hi}CD44^{lo} phenotype. To study the heterogeneity and tumor microenvironment of clinical SCLC specimens, Chan *et al.* [95] used single-cell transcriptome sequencing and imaging techniques and identified a phospholipase C gamma 2 (PLCG2)-high-expressing subpopulation linked to increased brain metastasis and poor prognosis, as well as an enrichment of a monocyte/macrophage population with a profibrotic, immunosuppressive phenotype.

A previous study showed that NE and nonneuroendocrine (non-NE) share common cell origins and play different roles during SCLC metastasis [21]. Further study revealed that non-NE cells secrete fibroblast growth factor 2 (FGF2) and enhance the expression of polyomavirus enhancer activator 3 (Pea3) in NE cells, resulting in metastatic dissemination of the NE subclone to the liver [26] (Figure 2). Interestingly, we recently found that the non-NE subtype of SCLC is composed of mesenchymal and epithelial-like subsets, and the activation of TGF-β signaling in the mesenchymal subset promotes cancer metastasis to the liver [96] (Figure 2). Moreover, depletion of the TGF-β signaling pathway in mice depresses the liver metastasis capability of SCLC [96]. In addition, *Nfib* is oncogenic in SCLC, promotes pro-metastatic neuronal gene expression programs and drives SCLC liver metastasis [23,28,29]. Mechanistically, NFIB promotes SCLC metastasis by increasing the accessibility of global chromatin during cancer progression [23] (Figure 2).

Mechanistic Insights into SCLC Chemotherapy Resistance and Overcoming Strategy

The current first-line treatment for SCLC, combining etoposide and cisplatin (E/P), has been the clinical standard of care since the 1970s [97]. Despite the initial sensitivity to chemotherapy, resistance emerges rapidly. The paucity of tumor specimens from chemotherapy-resistant SCLC patients has greatly hindered the current mechanistic understanding of chemotherapy resistance in the clinic. Therefore, the clinical outcome has not significantly improved over the past few decades, and chemotherapy resistance remains the central problem for SCLC treatment [8].

Through comprehensive bioinformatics analyses, we found that adherent or semi-adherent SCLC cells are enriched with increased PI3K/Akt/mTOR pathway activity and high chemotherapy resistance [98] (Figure 3). Activation of this pathway promotes the transition from the suspension to adhesion growth pattern of SCLC cells and confers chemotherapy resistance [98]. Such chemotherapy

resistance could be largely overcome by combining chemotherapy with PI3K/Akt/mTOR pathway inhibitors [98]. A phase I/II clinical trial (NCT03366103) targeting mammalian target of rapamycin kinase (mTOR) and BCL-2 is currently ongoing [1].

Activation of the Kras or Notch pathway results in an NE to non-NE fate switch, which enhances SCLC chemotherapy resistance [21,23,24,51] (Figure 3). MYC drives the temporal evolution of SCLC subtypes by reprogramming neuroendocrine fate through activation of the Notch pathway and promotes SCLC chemotherapy resistance [31,51,99,100] (Figure 3). YAP can signal through Notch-dependent or Notch-independent pathways to promote the fate conversion from NE to non-NE tumor cells [101] (Figure 3). Notch blockade in combination with chemotherapy suppresses tumor growth and delays relapse in pre-clinical models [24]. Moreover, EZH2 is upregulated in chemoresistant SCLC and promotes drug resistance through epigenetic silencing of schlafen family member 11 (*SLFN11*) [102]. Combined EZH2 inhibition and chemotherapy treatment is currently being explored in a phase I/II clinical trial of

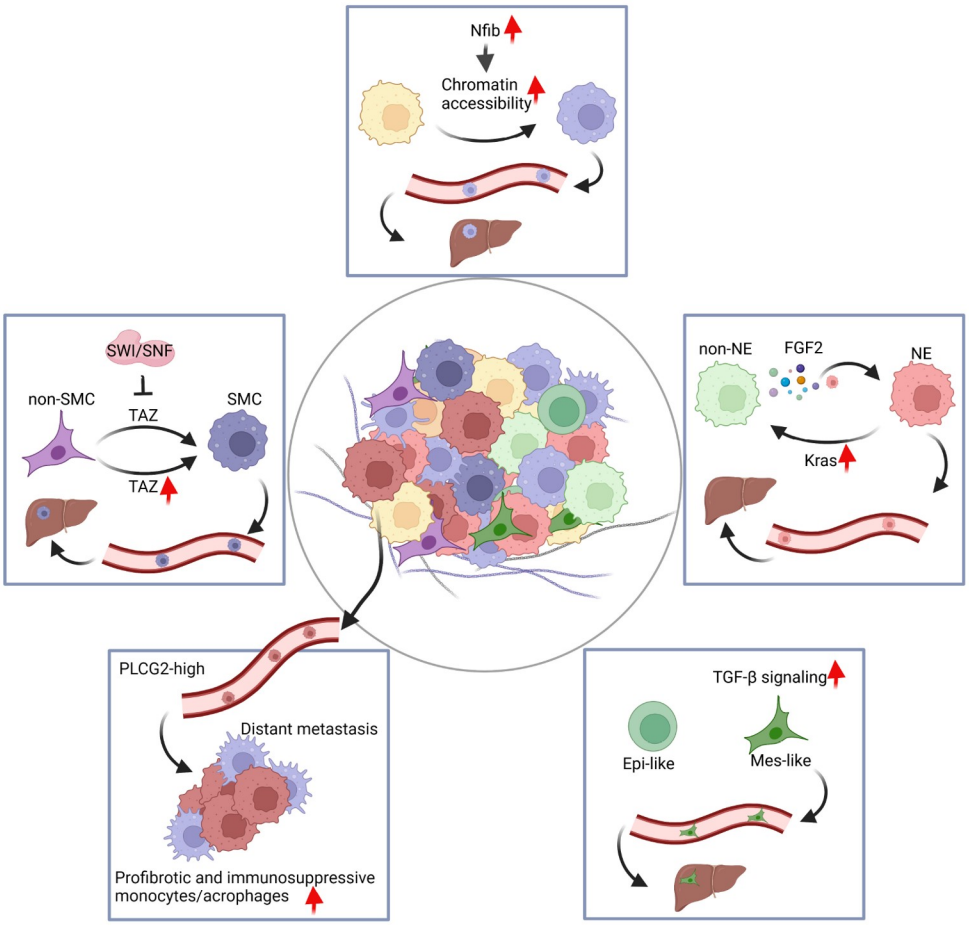

Figure 2. The link of SCLC heterogeneity and plasticity to distant metastases SMC, SCLC metastasizing cell. non-SMC, non-SCLC metastasizing cell. NE, neuroendocrine. non-NE, nonneuroendocrine. FGF2, fibroblast growth factor 2. Epi, epithelial. Mes, mesenchymal.

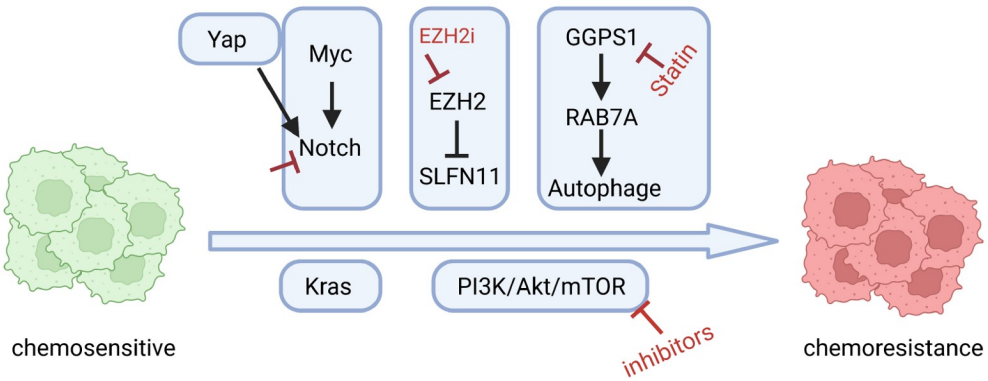

Figure 3. Mechanisms in regulating SCLC chemoresistance

recurrent SCLC (NCT038979798) [103].

Recently, we found that chemoresistant SCLC undergoes metabolic reprogramming relying on the mevalonate (MVA)–geranylgeranyl diphosphate (GGPP) pathway, which can be targeted by clinically approved statins [104]. Mechanistically, statins induce oxidative stress accumulation and apoptosis through the GGPP synthase 1 (GGPS1)–RAB7A–autophagy axis [104]. Statin treatment overcomes both intrinsic and acquired SCLC chemotherapy resistance *in vivo* across multiple SCLC patient-derived xenograft (PDX) models bearing high GGPS1 levels. Importantly, GGPS1 expression is negatively associated with survival in SCLC patients, and combined statin and chemotherapy treatment resulted in durable responses in three SCLC patients who relapsed from first-line chemotherapy [104].

Perspective

SCLC is the most malignant type of lung cancer with an extremely poor prognosis, and most patients are diagnosed at an extensive stage. Patients with SCLC exhibit a remarkable initial response to chemotherapy and/or radiotherapy followed by the quick development of drug resistance. Although an increasing number of targeted therapies have emerged in many other cancer types [105,106], treatments for recurrent or refractory SCLC are limited and unsatisfactory [12,107–109]. Most SCLC patients eventually die of distant metastases and chemotherapy resistance. Recent advances in SCLC have revealed the heterogeneity and plasticity of SCLC and have uncovered pivotal roles during cancer metastasis and chemotherapy resistance. The phenotypic evolution during malignant cancer progression emphasizes the importance of timely and precise diagnosis and related therapeutic interventions in the clinic.

Different molecular subtypes of SCLC have been defined by gene expression profiling and exhibit distinct vulnerabilities to targeted therapies. Precise analysis of the patients at initial diagnosis may help to improve the therapeutic outcomes. Immunotherapy has been recommended for extensive-stage and recurrent SCLC clinical treatment, and recent studies have identified the inflamed or immune subtype that may benefit from immunotherapy. The combination of molecularly targeted therapy or immunotherapy with traditional chemotherapy may improve the clinical outcomes in the future. A better understanding of SCLC biology will hopefully uncover novel vulnerabilities that might be amenable to clinical therapeutic approaches.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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