Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/59/20241437

Utilizing gene expression profiling for the identification of small cell lung cancer subtypes

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Abstract. Small cell lung cancer is a highly aggressive form of lung cancer associated with poor prognosis and limited treatment options. Gene expression profiling has emerged as a valuable tool for classifying SCLC into distinct molecular subtypes, each with its own unique therapeutic vulnerabilities and biomarkers. Recent studies have identified four primary subtypes: SCLC-A, SCLC-N, SCLC-P, and SCLC-Y, with additional classification into neuroendocrine (NE) high, NE-low, and non-NE tumors. Advanced understanding of the molecular characteristics of these subtypes presents new opportunities for targeted therapeutic approaches. The paper underscores the potential for a shift toward personalized therapy for SCLC, taking into account the inter- and intratumoral heterogeneity and dynamic subtype interconversion in response to treatment. Incorporating subtype-specific biomarkers in clinical trial designs could lead to the first-ever molecular biomarker-driven approach in the management of SCLC, greatly enhancing treatment effectiveness and patient outcomes.

Keywords: SCLC, gene expression, molecular subtypes.

1. Introduction

Gene expression stands as a cornerstone process pivotal in orchestrating cellular functions, developmental pathways, and the broader spectrum of biological activities[1]. This process involves the intricate utilization of genetic blueprints to fabricate functional gene products, including proteins and RNA molecules, thereby serving as the molecular foundation for life's complexity and diversity[2, 3]. The meticulous modulation of gene expression is imperative for sustaining cellular equilibrium and adeptly navigating the plethora of internal and external environmental cues. Notably, aberrations in gene expression mechanisms have been linked to an extensive array of human pathologies, encompassing cancers, neurodegenerative diseases, and autoimmune disorders, among others. Techniques such as Western blotting, RT-qPCR, and RNA sequencing are employed to quantify and analyze gene expression, offering insights into the dynamic regulatory networks governing cellular function.

Small cell lung cancer (SCLC), distinguished by its rapid proliferation and propensity for metastasis, represents a formidable subtype of lung cancer, constituting roughly 15% of all lung cancer incidences. This malignancy is predominantly associated with tobacco smoke exposure and predominantly arises in individuals aged 65 and above. Despite progress in therapeutic strategies, SCLC prognosis remains dire,

DOI: 10.54254/2753-8818/59/20241437

with a five-year survival rate hovering around 6%, underscoring the urgent need for advanced diagnostic and treatment modalities[4-6].

The classification and precise identification of cancer subtypes hold paramount importance in the realm of oncology, serving as a linchpin for the customization of treatment regimens and the enhancement of therapeutic efficacy. This stratification enables clinicians and researchers to discern the unique molecular and genetic signatures that define different cancer subtypes, thereby facilitating the deployment of targeted therapies that are more likely to be effective against specific tumor profiles. In the context of small cell lung cancer, where heterogeneity in tumor characteristics can significantly influence disease progression and response to treatment, the ability to accurately classify and identify subtypes can dramatically impact clinical outcomes. Leveraging advanced gene expression analysis and other molecular diagnostic tools not only aids in the precise categorization of SCLC but also opens the door to novel therapeutic targets and strategies, ultimately aiming to improve survival rates and quality of life for patients afflicted with this aggressive cancer.

Within the ambit of this review, we aim to illuminate the contemporary understanding of SCLC's biological underpinnings, diagnostic criteria, and therapeutic avenues. Furthermore, we will explore how gene expression profiling can be harnessed to delineate SCLC subtypes, thereby paving the way for tailored treatment approaches that could potentially enhance patient outcomes in the battle against this aggressive cancer.

2. Molecular classification of small cell lung cancer subtypes

In stark contrast to non-small cell lung cancer (NSCLC), which inherently exhibits resistance to chemotherapy, SCLC initially demonstrates a high degree of susceptibility to cytotoxic agents[7]. Yet, despite this initial responsiveness, SCLC frequently experiences rapid recurrence, leading to a grim prognosis where complete cures are seldom achieved. Compounding this challenge is the absence of dependable biomarkers capable of forecasting responses to emerging immunotherapeutic strategies, such as immune checkpoint inhibitors. Surgical interventions remain infrequent for SCLC due to the tumor's brisk proliferation rate and its marked inclination towards early metastasis[8].

Historically, SCLC has been treated within the clinical framework as a monolithic entity, typified by a uniform morphological classification, which stands in sharp contrast to the increasingly individualized treatment paradigms applied to other lung cancer types. Consequently, conventional clinical trial designs for SCLC have predominantly focused on disease staging, neglecting the potential utility of unique molecular markers that could offer prognostic or predictive insights. Nevertheless, recent years have witnessed a reinvigorated global interest in SCLC research, marked by the advent of novel preclinical models, extensive genomic characterization, and the delineation of distinct molecular subtypes based on biological attributes[9-11].

Emerging evidence from recent investigations into primary human SCLC tumors has unveiled a model comprising discrete subtypes, each characterized by differential expression patterns of four pivotal transcriptional regulators: achaete-scute homolog 1 (ASCL1), neurogenic differentiation factor 1 (NEUROD1), yes-associated protein 1 (YAP1), and POU class 2 homeobox 3 (POU2F3)[12]. The interplay among these transcription factors and neuroendocrine (NE) expression profiles heralds the potential for identifying subtype-specific therapeutic targets[10].

Although traditionally viewed as a singular disease, extensive preclinical endeavors have unearthed biologically distinct SCLC subsets, enabling a contemporary classification into NE-high and NE-low subtypes. This distinction is predicated on the expression of various NE markers such as chromogranin A, synaptophysin , neural cell adhesion molecule 1, and gastrin-releasing peptide [13, 14]. A subset of SCLCs devoid of NE differentiation, termed non-NE tumors, further enriches this taxonomy. The dichotomy between NE-high and NE-low subtypes extends beyond mere expression profiles, encompassing significant variances in genetic mutations, morphological characteristics, growth dynamics, and immune cell infiltration, which collectively bear profound implications for therapeutic responsiveness[15, 16].

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Specifically, SCLC-A (ASCL1-dominant) tumors exhibit pronounced NE marker expression and traditional morphology, in contrast to the NE-low SCLC-N (NEUROD1-dominant) subtype, which presents variant morphological features[11, 17]. The transcriptional regulators ASCL1 and NEUROD1 are recognized as pivotal factors in the maturation of pulmonary NE cells, with both subtypes also preferentially expressing insulinoma-associated protein 1 (INSM1), a marker implicated in NE differentiation via modulation of the Notch signaling pathway. SCLCs with low or absent expression of NEUROD1, ASCL1, and INSM1 are categorized into either the SCLC-Y (YAP1-dominant) or SCLC-P (POU2F3-dominant) subtypes, predicated on their specific transcription factor expression profiles, with YAP1 activation being contingent upon the HIPPO signaling pathway's modulation[18, 19].

3. Targeted therapeutic approaches for neuroendocrine and molecular subtypes

The landscape of targeted therapies for SCLC has been fraught with challenges, starkly contrasting the notable successes achieved with immunotherapy in NSCLC. A pivotal factor underpinning the limited efficacy observed in SCLC treatments is the inherent heterogeneity of the disease[20]. This complexity poses significant obstacles, as therapeutic strategies effective in the more uniform molecular contexts of NSCLC may falter in the face of SCLC's diverse tumor biology.

In the clinical trial arena for SCLC, a critical disparity exists when compared to NSCLC studies: patients are often recruited without a thorough consideration of their unique molecular profiles. This approach, while expedient, overlooks the potential benefits of a more tailored therapeutic strategy that could be derived from an in-depth understanding of individual tumor characteristics[10].

The pursuit of subtype-specific molecular signatures and the identification of clinically relevant biomarkers stand as promising avenues toward refining SCLC treatment paradigms. Such endeavors aim not only to enhance the precision of therapeutic interventions but also to unveil novel targets that could underpin the development of more effective, individualized treatment strategies for SCLC. This nuanced understanding of the molecular underpinnings of SCLC subtypes could herald a new era of targeted therapy, potentially mirroring the advances seen in NSCLC treatment, and offering renewed hope for patients battling this formidable disease (table 1).

3.1. SCLC-A

The SCLC-A subtype is poised to exhibit responsiveness to therapies targeting Delta-like protein 3 (DLL3), leveraging the nuanced transcriptional interplay between DLL3 and ASCL1 within the context of Notch-inactive tumor cells. This interaction underpins the strategic application of the DLL3-targeted antibody-drug conjugate, Rova-T, heralding a precision medicine approach for SCLC-A management[21, 22]. Furthermore, the transcription factor ASCL1 also directly influences B-cell lymphoma-2 (BCL2) expression, implying that elevated BCL2 levels may herald susceptibility to the BCL2 inhibitor venetoclax, offering an additional targeted therapeutic avenue[23].

Moreover, the role of the histone demethylase Lysine specific demethylase 1 (LSD1) emerges as critical within the neuroendocrine subtypes SCLC-A and SCLC-N, with its activity intricately linked to the perturbation of INSM1. Recent advancements elucidate that inhibiting LSD1 not only precipitates NOTCH1 activation but also leads to the subsequent downregulation of ASCL1 in SCLC, mapping a novel therapeutic trajectory that intersects epigenetic modulation with tumor subtype-specific targeting. This convergence of molecular insights fosters the development of innovative, targeted interventions that promise to refine the therapeutic landscape for SCLC[24].

3.2. SCLC-N

The SCLC-N subtype is notably marked by MYC amplification, presenting a compelling molecular target for therapeutic intervention. This genetic aberration opens avenues for the deployment of agents specifically designed to counteract MYC's oncogenic effects. Moreover, this subtype is distinguished by elevated AURKA activity and enhanced arginine biosynthesis, further defining its unique molecular landscape. It is postulated that a synergistic therapeutic approach, combining AURKA inhibitors with

DOI: 10.54254/2753-8818/59/20241437

c-MYC targeting agents, could significantly amplify treatment outcomes, offering a strategic advantage in combating this subtype[25-27].

Intriguingly, the SCLC-N subtype demonstrates a peculiar susceptibility to the oncolytic Seneca Valley virus (SVV), which exhibits a predilection for infecting and lysing neuroendocrine cancer cells. This biological property of SVV underscores its potential utility as either a standalone therapeutic option or in concert with immunotherapeutic strategies, contingent upon meticulous, biomarker-driven patient stratification. In this vein, the ratio of NEUROD1 to ASCL1 expression emerges as a promising predictive biomarker, potentially guiding the selection of patients most likely to benefit from SVV-based therapies. This biomarker-driven approach not only exemplifies the principle of precision medicine but also heralds the potential of exploiting viral oncolytics as a novel modality in the targeted treatment arsenal against SCLC-N, thereby enriching the therapeutic landscape with innovative, subtype-specific interventions[28, 29].

3.3. SCLC-P

Emerging insights from CRISPR screening technologies have unveiled a distinct susceptibility within the SCLC-P subtype, characterized by a pronounced sensitivity to deficiencies in the insulin-like growth factor 1 receptor (IGF-1R). This vulnerability predicates the hypothesis that IGF-1R inhibitors could manifest as targeted therapeutic agents, offering a tailored approach to treating patients harboring this specific molecular signature[30].

Moreover, the potential efficacy of poly (ADP-ribose) polymerase (PARP) inhibitors within this subtype further diversifies the therapeutic portfolio, albeit with a caveat. The effectiveness of PARP inhibitors is not straightforwardly linked to the expression of Schlafen family member 11 (SLFN11), a factor not directly correlated with the transcriptional regulators defining the SCLC-P subtype. This observation suggests a more complex interplay of molecular mechanisms governing PARP inhibitor responsiveness, underscoring the need for a deeper understanding of the subtype-specific molecular landscape to optimize therapeutic strategies. The delineation of such targeted therapies, grounded in the molecular vulnerabilities of SCLC subtypes, heralds a promising frontier in the precision medicine approach to small cell lung cancer treatment[31].

3.4. SCLC-Y

The expression of programmed death-1 (PD-1) and its ligand (PD-L1) is not confined to specific subtypes of SCLC; however, there appears to be a predilection towards the SCLC-Y subtype. This inclination stems from the observation that YAP1, a hallmark of the SCLC-Y subtype, actively upregulates PD-L1 transcripts, thereby cultivating an immunosuppressive milieu within the tumor microenvironment. Furthermore, SCLC-Y cells exhibit heightened levels of CD38 and lymphocyte-activation gene 3, potentiating a more favorable response to immune checkpoint inhibitors (ICIs)[32, 33].

Expanding the therapeutic vista for SCLC-Y, gene expression analyses and computational models have pinpointed this subtype as particularly amenable to inhibitors targeting the mammalian target of rapamycin (mTOR), polo-like kinase (PLK), and possibly cyclin-dependent kinases 4 and 6 (CDK4/6). These findings underscore a potential for exploiting specific vulnerabilities within the SCLC-Y molecular architecture, offering avenues for targeted intervention[34].

The metabolic underpinnings of SCLC also present novel therapeutic opportunities, particularly through the lens of ferroptosis, an iron-dependent regulated necrosis. Non-neuroendocrine (non-NE) SCLCs exhibit a marked sensitivity to ferroptosis induction, in stark contrast to high-NE SCLCs, which show resistance to this form of cell death yet are distinctly vulnerable to inhibitors of the thioredoxin (TRX) pathway. This differential susceptibility highlights the potential for a dual therapeutic strategy combining ferroptosis induction with TRX pathway inhibition, aiming to target the intratumoral heterogeneity of NE/non-NE SCLC. Such a multifaceted approach could significantly enhance the treatment landscape for SCLC, offering a tailored regimen to circumvent the complex interplay of resistance mechanisms within the tumor microenvironment[35].

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Table 1. Molecular subtypes of SCLC associated with gene expression and targeted therapeutic approaches

SCLC subtypes	NE		Non-NE	
	ASCL1	NEUROD1	POU2F3	YAP1
Marker expression	DLL3 BCL2 LSD1 INSM1 NOTCH1	MYC AURKA	IGF-1R PARP SLFN11	PD-L1 mTOR PLK
Potential therapies approaches	DLL3 inhibitors BCL2 inhibitors LSD1 inhibitors	c-MYC inhibitors AURKA inhibitors	IGF-1R inhibitors PARP inhibitors	mTOR inhibitors PLK inhibitors CDK4/6 inhibitors

4. SCLC-I: Unveiling a new inflamed subtype of small cell lung cancer

Within the intricate landscape of SCLC subtypes, one particular group, distinguished not by a singular transcriptional hallmark but rather by a constellation of uniquely expressed genes including a spectrum of immune checkpoints and human leukocyte antigens (HLAs), has been aptly termed SCLC-inflamed, or SCLC-I. This designation reflects a heightened immune signature relative to other subtypes, an attribute of considerable interest given the generally muted responses of SCLC to immune checkpoint blockade (ICB), often attributed to sparse immune cell infiltration, particularly by cytotoxic T cells. The elevated expression of CD8A and CD8B in SCLC-I suggests a more robust cytotoxic T cell presence, offering a glimmer of therapeutic opportunity in a landscape often characterized by resistance[36].

Delving deeper into the immune landscape of SCLC-I, the utilization of CIBERSORTx deconvolution has facilitated a granular analysis of immune cell populations, revealing SCLC-I tumors as hotspots of immune activity, evidenced by superior total immune infiltrate and cytolytic activity scores. This immune-rich environment, juxtaposed with the broader context of SCLC's resistance mechanisms to ICB—including low HLA expression, dampened interferon signatures, and scant immune checkpoints—positions SCLC-I as a prime candidate for immunotherapeutic intervention[37].

Further exploration into SCLC-I's molecular terrain unveils an abundance of immune checkpoint molecules, such as PD-L1 and PD-1, alongside other actionable targets like CD38, IDO1, and LAG3, enriching the therapeutic blueprint for this subtype. The landmark IMpower133 trial, which underscored the potential of ICB in SCLC by demonstrating significant progression-free survival and overall survival benefits, hinted at the distinct responsiveness of the SCLC-I subgroup, although definitive subtype-specific conclusions remain elusive due to the trial's design limitations[38, 39].

Beyond conventional ICB targets, SCLC-I exhibits pronounced sensitivity to Bruton's tyrosine kinase (BTK) inhibition, as evidenced by the potent efficacy of ibrutinib in this context—a finding that not only broadens the therapeutic spectrum for SCLC-I but also underscores the potential of repurposing agents approved for hematologic malignancies. Additionally, the mesenchymal nature of SCLC-I, manifesting in epithelial-mesenchymal transition (EMT) traits, has been challenged by the histone deacetylase inhibitor mocetinostat, which demonstrated an ability to modulate EMT markers and exert tumor control in preclinical models[38, 39].

This evolving understanding of SCLC-I, marked by its unique immune and molecular characteristics, beckons a more nuanced therapeutic approach, leveraging the subtype's distinct vulnerabilities to innovate and refine treatment strategies for this formidable cancer.

5. Intratumoral diversity within small cell lung cancer subtypes

The exploration of TP53 and RB1 mutations in SCLC has unveiled a pronounced clonality in primary SCLC tumors compared to other lung cancer forms, such as adenocarcinoma[40]. This finding challenges the notion of a straightforward correlation between specific genetic alterations and the emergence of distinct SCLC subtypes within primary tumors. Nevertheless, evidence from murine

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models suggests that elevated MYC levels may predispose SCLC cells towards a NeuroD1-high phenotype. Similarly, the overexpression of the Notch intracellular domain in certain genetically engineered mouse models has been shown to foster the development of tumors with low ASCL1 and NeuroD1 expression levels, hinting at the potential influence of Notch pathway mutations in human SCLC on subtype differentiation[41].

The intricate interplay between Notch signaling activation and ASCL1 expression highlights a broader, yet underexplored, landscape of functional interactions between Notch pathway activity and the transcriptional regulators that delineate SCLC's four principal subtypes. Delving deeper into these molecular relationships is imperative to fully comprehend the genetic underpinnings that govern SCLC subtype differentiation and proliferation.

Moreover, the concept of intratumoral heterogeneity within SCLC, characterized by functionally diverse cell subpopulations, adds another layer of complexity to the disease's progression and chemotherapy resistance. Patient-derived xenograft models, established from biopsy specimens or circulating tumor cells both before and after treatment, are invaluable tools for elucidating the dynamic nature of intratumoral heterogeneity and its implications for therapeutic resistance[42, 43].

In vitro SCLC cultures often exhibit morphologically distinct cell types, with suspended cells displaying typical epithelial neuroendocrine traits, whereas adherent cells manifest a more mesenchymal, non-neuroendocrine phenotype[44]. This dichotomy is mirrored in mouse SCLC tumors, where the coexistence of neuroendocrine and non-neuroendocrine cells significantly augments metastatic potential, particularly to the liver. A paracrine signaling mechanism, mediated by the ETS family transcription factor PEA3 in non-neuroendocrine cells, has been implicated in the enhanced metastatic prowess of neuroendocrine cells[45].

Recent findings also indicate that the activation of Notch signaling, typically repressed in the predominant ASCL1-high SCLC subtype, can trigger a neuroendocrine to non-neuroendocrine cellular fate transition. This transition involves the induction of the transcriptional repressor REST and subsequent HES1 expression in non-neuroendocrine phenotype cells, leading to a population characterized by slower proliferation but heightened chemoresistance, ostensibly supporting the survival and expansion of neuroendocrine cells within mixed tumors[46].

The potential connections between this heterogeneity—including mesenchymal-like, Notch-high/REST-high, and vascular-like SCLC cells—and the established SCLC subtypes remain an open field for investigation, promising to unravel further the complexities of SCLC biology and therapeutic responsiveness.

6. Discussion

SCLC has posed significant challenges in the context of personalized therapy, largely due to its complex inter- and intratumoral heterogeneity that has remained underexplored. Traditional classification schemes primarily focused on the high-NE SCLC tumors, which are predominantly driven by ASCL1 and NEUROD1, along with ancillary factors like cMYC and TTF1. However, the identification of a non-NE, tuft-cell variant driven by POU2F3 has necessitated a reevaluation of SCLC classification, revealing the existence of at least two distinct non-NE molecular subtypes, namely SCLC-I and SCLC-P[10, 30].

SCLC-I is characterized by EMT features and an inflamed phenotype, marked by heightened expression of genes associated with HLAs, interferon-gamma activation, and immune checkpoints[47]. This subtype's definition extends beyond mere inflammatory characteristics, as it has been identified in contexts devoid of a tumor immune microenvironment, indicating that both tumor-intrinsic and -extrinsic factors contribute to its unique biology and therapeutic vulnerabilities.

While ICB has become a standard treatment for SCLC, the search for predictive biomarkers for this class of therapy has been challenging, with tumor mutational burden and PD-L1 expression being considered potential markers[48, 49]. Intriguingly, single-cell analyses suggest that intratumoral heterogeneity within SCLC subtypes may be a dynamic phenomenon, particularly with the observation of an increase in chemoresistant, triple-negative SCLC-I cells following platinum-based chemotherapy.

DOI: 10.54254/2753-8818/59/20241437

This suggests a potential for subtype switching as an underlying resistance mechanism, further supported by evidence of transcriptional shifts from SCLC-A towards SCLC-I subtypes[39].

The ability to classify most SCLC tumors into one of the four defined subtypes offers a promising avenue for implementing prospective subtyping in clinical trials, potentially enabling patient assignment to specific treatment arms based on their SCLC subtype. This approach, which could employ transcriptional, proteomic, or epigenetic profiling for subtype determination, represents a pivotal step toward the realization of personalized therapy for SCLC. Should any of these subtype-specific treatment strategies demonstrate significant efficacy, it could herald the advent of the first standard-of-care molecular biomarker-driven selection in SCLC treatment, marking a significant milestone in the pursuit of personalized therapeutic interventions for this aggressive disease.

7. Summary

Significant strides in understanding SCLC biology have been made, revealing distinct subtypes characterized by unique gene expression profiles, a discovery stemming from both human and mouse studies. This finer subclassification has unveiled variability in therapeutic targets across subtypes, informing ongoing and future clinical trials. Delving deeper into the specifics of each subtype is imperative to delineate their biological behaviors, interconversion potentials, and therapeutic vulnerabilities. Advancing this knowledge and applying it to subtype-specific clinical research could be pivotal in devising more effective treatments for SCLC, marking a promising direction in the ongoing quest to combat this aggressive disease.

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