Identification of Small Cell Lung Cancer Stage-specific DNA Methylation in Patients Using Liquid Biopsies

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Abstract Control #4183

INTRODUCTION

Small cell lung cancer (SCLC) is a deadly disease and patients often suffer from recurrent disease. Biologic mechanisms of recurrence are unclear. Epigenetic mechanisms, like DNA methylation, may be operant. SCLC is rarely resected; therefore, the SCLC methylome is understudied due to scarce tumour tissue.

METHODS

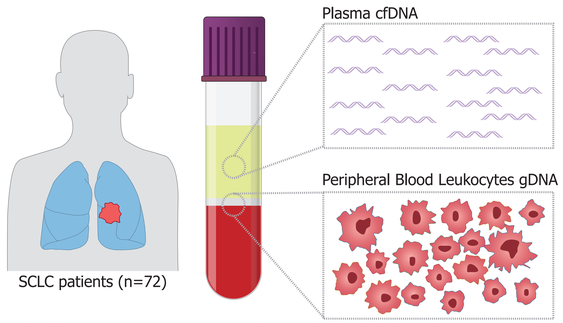

Figure 1. The pre-treatment methylome of 72 SCLC patients is examined through cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq) on plasma cell-free DNA (cfDNA) and on sheared genomic DNA (gDNA) from paired peripheral blood leukocytes (PBLs) (n = 72).

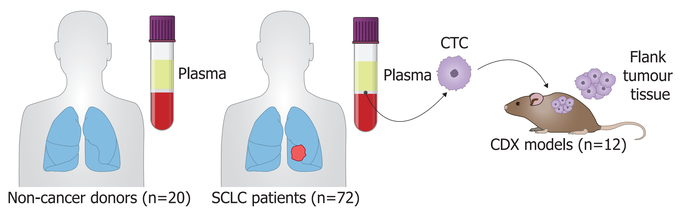

Figure 2. cfMeDIP-seq also performed on independent cohort of healthy non-cancer controls (n = 20) and on genomic DNA (gDNA) from flank tumour tissue from circulating tumour cell (CTC) derived xenograft (CDX) models (n = 12).

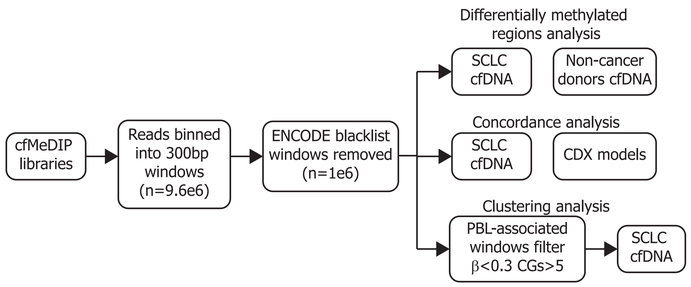

Figure 3. Overall workflow and analysis schematic.

RESULTS

	Extensive-stage	Limited-stage	Total	p-value
Total N (%)	41 (56.9)	31 (43.1)	72	
Age (years)				
Median (IQR)	66.0 (61.1 to 75.7)	68.0 (62.0 to 74.6)	67.4 (61.7 to 75.5)	0.69
Sex				
Female	12 (29.3)	16 (51.6)	28 (38.9)	0.09
Male	29 (70.7)	15 (48.4)	44 (61.1)	
Smoking Status				
Current smoker	20 (48.8)	13 (41.9)	33 (45.8)	
Former smoker	19 (46.3)	13 (41.9)	32 (44.4)	0.35
Never smoker	2 (4.9)	5 (16.1)	7 (9.7)	

Table 1. Select demographics and staging information for the 72 SCLC patients. To test for statistical significance, Mann-Whitney (continuous) and Fishers exact (categorical) tests were done.

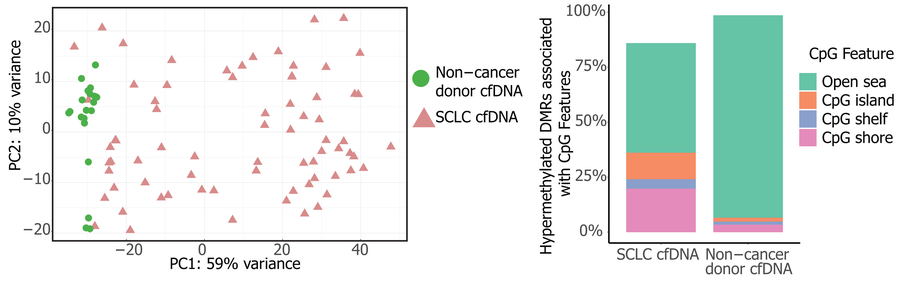

Figure 4. Whole-genome methylome profiles of SCLC cfDNA and non-cancer donor cfDNA (left). The proportion of differentially methylated regions (DMRs) associated with CpG features that were significantly hypermethylated in either SCLC (44,359/51,666) or non-cancer (1,004/1,019) are examined in the stacked barplot (right). A small proportion of these significant hypermethylated regions in either SCLC (7,307/51,666) or non-cancer (15/1,1019) did not map to any CpG features.

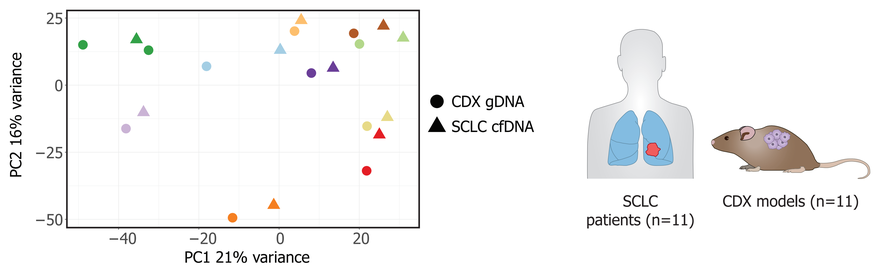

Figure 5. Concordance between genome-wide methylation profiles of SCLC cfDNA and CDX models is examined in the principal component analysis plot. Each unique color represents an SCLC patient/CDX model pair.

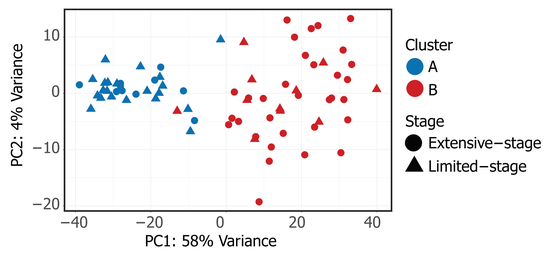

Figure 6. Identification of methylome-defined clusters within SCLC cfDNA. PBL-associated windows with β<0.3 and CGs>5 per window were kept (n=190,769). Consensus clustering was done on these windows to identify clusters. Cluster A (9 ES, 22 LS) and Cluster B (33 ES, 11 LS) were significantly different by stage ($X^2(1) = 13.79$, $p < 0.001$) but not by sex (p = 0.81) or concentration of cfDNA (p = 0.33).

RESULTS

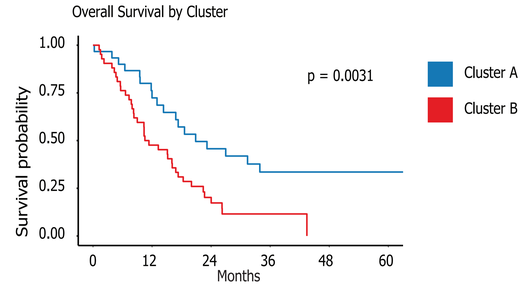

Figure 7. Kaplan-Meier analysis of overall survival in cluster A and B. Patients in Cluster A (median OS = 21 months) had a significantly better survival outcome than patients in Cluster B (median OS = 11 months) by univarate analysis (HR: 2.3, p = 0.0031). However, this pattern was not significant after multivariate analysis accounting for stage and cluster (HR: 1.5, p = 0.2)

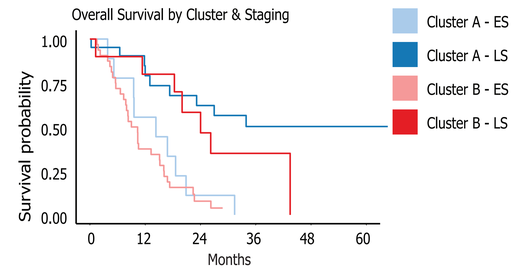

Figure 8. Kaplan-Meier analysis of overall survival in cluster A and B stratified by limited-stage (LS) and extensive-stage (ES) SCLC.

CONCLUSION

We identified stage-specific methylation patterns in the plasma of SCLC patients using the cfMeDIP-seq assay that may reveal novel epigenetic and biologic mechanisms of SCLC disease progression. Moreover, CDXs recapitulate the methylome of SCLC cell-free patient samples highlighting their utility in future work as representative models for methylome analysis.

Acknowledgments

OMPRN Ontario Molecular Pathology Research Network
CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
STARS 21
University of Toronto
UHN Toronto General, Toronto Western, Princess Margaret, Toronto Rehab, Michener Institute