Identifying Circulating DNA Methylation Patterns in Small Cell Lung Cancer Patients

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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

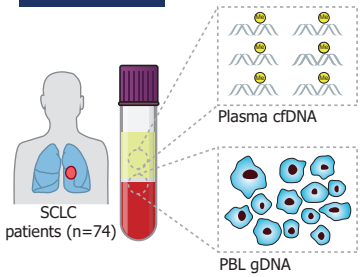

Figure 1. The pre-treatment methylome of 74 SCLC patients is examined through cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq) on plasma cell-free DNA (cfDNA) and on sheared genomic DNA (gDNA) from paired peripheral blood leukocytes (PBLs) (n = 74).

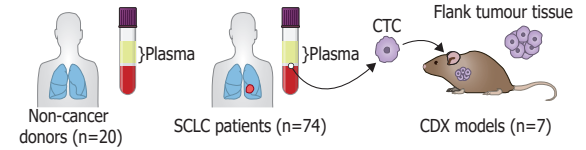

Figure 2. cfMeDIP-seq also performed on independent cohort of healthy non-cancer controls (n = 20) and on genomic DNA (gDNA) from flank tumour tissue from circulating tumour cell (CTC) derived xenograft (CDX) models (n = 12).

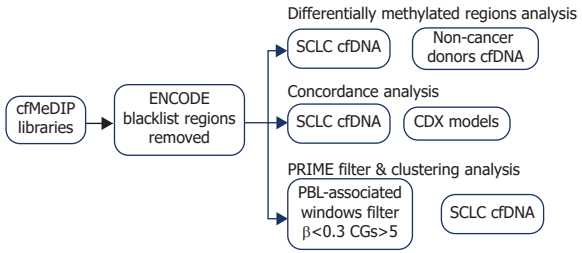

Figure 3. Overall workflow and analysis schematic using our Peripheral Blood Leukocyte Methylation (PRIME) cfDNA filter method.

RESULTS

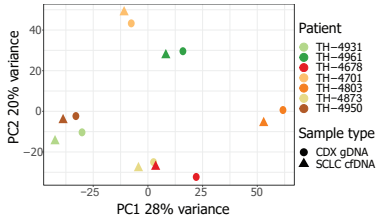

Figure 4. Concordance between genome-wide methylation profiles of SCLC cfDNA and CDX models is examined in the principal component analysis plot. Each unique color represents an SCLC patient/CDX model pair.

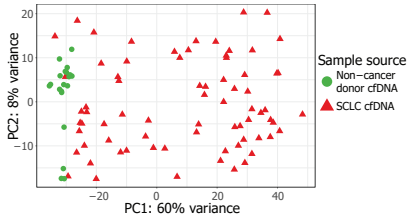

Figure 5. Whole-genome methylome profiles of SCLC cfDNA and non-cancer donor cfDNA.

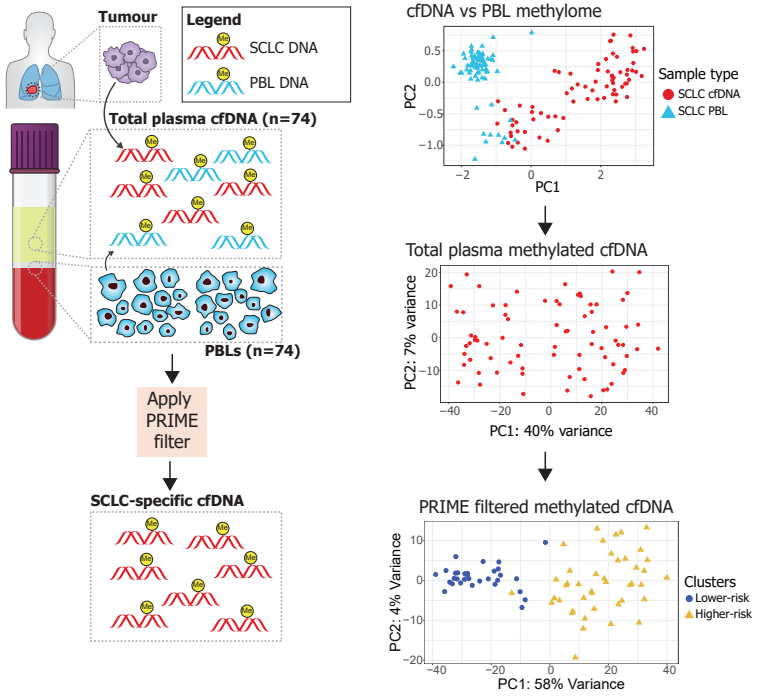

Figure 6. Overall schematic outlining our approach to first compare SCLC total cfDNA and SCLC PBL gDNA methylome (top right), SCLC total cfDNA methylome (middle right), and PRIME-filtered cfDNA methylome (bottom right). For each patient, plasma cfDNA and PBL gDNA are extracted using peripheral blood samples collected prior to starting first-line chemotherapy. Genome-wide methylation profiles are ~8.5e6 300bp windows and PRIME-filtered profiles are ~190k 300bp windows. Red filled circles correspond to cfDNA samplesand blue triangles are PBL samples.

RESULTS

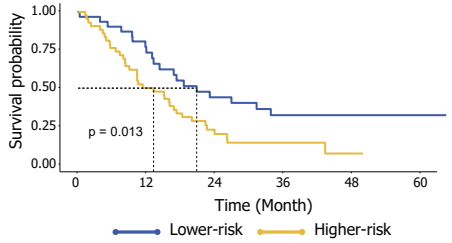

Figure 7. Kaplan-Meier survival analysis on Lower-risk and Higher-risk cluster identified by consensus clustering.

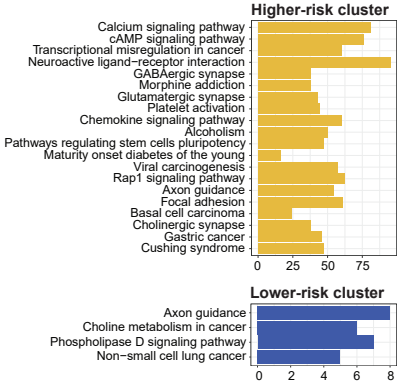

Figure 8. KEGG pathway analysis of hypermethylated DMRs in Lower-risk (left) and Higher-risk (right) clusters.

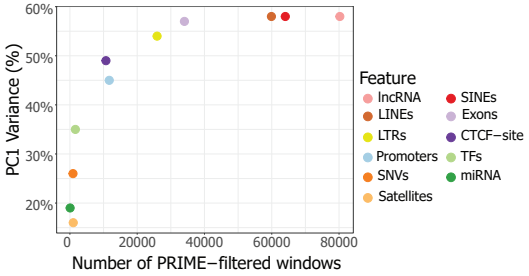

Figure 9. Correlating the number of PRIME-filtered hypermethylated 300bp windows corresponding to specific methylomic features with principal component 1 (PC1) variance.

CONCLUSIONS

We identified methylation patterns in the plasma of SCLC patients using the cfMeDIP-seq assay and **identified two distinct clusters** with stage and prognostic associations. These clusters are differential methylated at **regulatory and non-coding features** suggesting biologic mechanisms of SCLC disease progression. Lastly, we developed an **SCLC-specific cfDNA filter, PRIME**, that will help inform future liquid biopsy analyses.

ACKNOWLEDGMENTS

OMPRN Ontario Molecular Pathology Research Network
CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
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