Interrogating the Methylome of Small Cell Lung Cancer Patients Using Liquid Biopsies

Princess Margaret Cancer Centre UHN

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Abstract

Cell-free methylated DNA immunoprecipitation sequencing (**cfMeDIP-seq**) can be small cell lung cancer (SCLC) plasma samples to study the tumour methylome. cfMeDIP-seq bypasses the need for traditional tissue biopsies, is concordant with tumour tissue and can distinguish cancer from non-cancer. Using Peripheral Blood Leukocyte Methylation (PRIME) Subtraction, cfMeDIP-seq can identify subgroups in SCLC with prognostic associations.

Clinical Background

- 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 so methylome is understudied due to scarce tumour tissue

Methodology

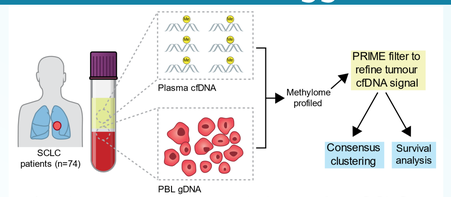


Figure 1. The pre-treatment methylome of 74 SCLC patients is examined through cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq) on plasma cell-free DNA (cfDNA) and on sheared genomic DNA (gDNA) from paired peripheral blood leukocytes (PBLs) (n = 74).

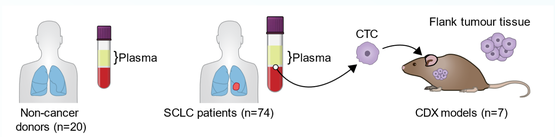


Figure 2. cfMeDIP-seq also performed on independent cohort of healthy non-cancer controls (n = 20) and on genomic DNA (gDNA) from flank tumour tissue from circulating tumour cell (CTC) derived xenograft (CDX) models (n = 7).

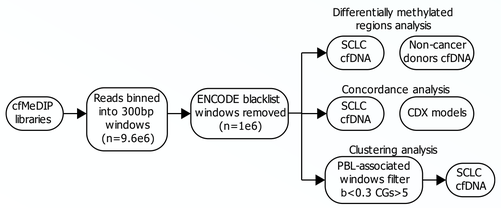
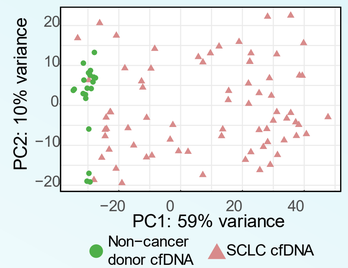


Figure 3. Overall workflow and analysis schematic.

Results



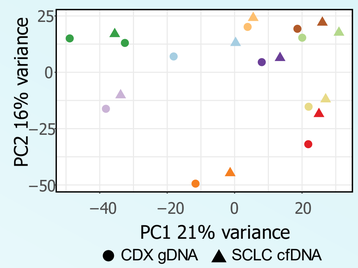


Figure 4. Whole-genome methylome profiles of SCLC cfDNA and non-cancer donor cfDNA (left). Concordance between genome-wide methylation profiles of SCLC cfDNA and CDX models is examined in the principal component analysis plot (right). Each unique color represents an SCLC patient/CDX model pair.

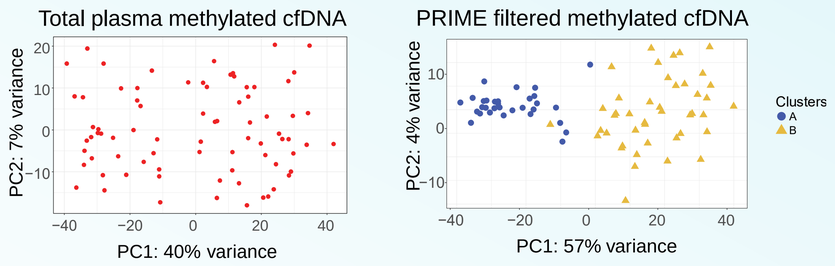


Figure 5. Principal component analysis (PCA) plot of total plasma cfDNA methylation profiles (left) of the 74 SCLC patients. Genome-wide methylation profiles are examined in this PCA plot (~8.5e6 300bp windows). Red filled circles correspond to cfDNA samples. PCA of PRIME- filtered plasma cfDNA methylation profiles of the 74 SCLC patients (right). In this PCA, methylation profiles for ~190,000 300bp PRIME windows (β<0.3 and CGs>5) are examined.

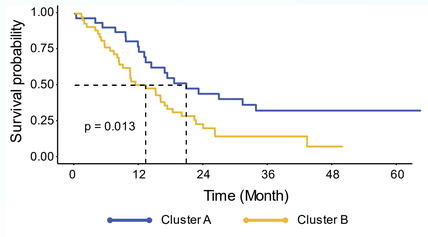


Figure 6. Kaplan-Meier survival analysis on cluster A and B identified by consensus clustering.

Results

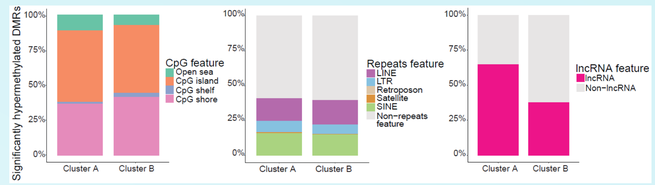


Figure 7. Bar plots of significantly hypermethylated DMRs observed in Cluster A (n=9037) and Cluster B (n=174) corresponding to CpG features (left), repeats features (centre), and long-noncoding RNA (right).

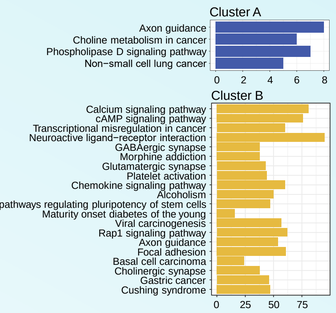


Figure 8. KEGG pathway analysis of hypermethylated DMRs in cluster A and cluster B. There are 174 hypermethylated DMRs in cluster A and 9037 in cluster B.

Conclusions

- We identified stage-specific methylation patterns in plasma of SCLC patients
- May reveal novel epigenetic and biologic mechanisms into SCLC disease progression
- CDX models recapitulate methylome of SCLC cell-free patient samples

Next Steps

- We have identified differentially hypermethylated lncRNA
- These lncRNA may mediate aggressiveness in SCLC
- We aim to explore these lncRNA in in vivo and in vitro model systems.

Acknowledgments

OMPRN Ontario Molecular Pathology Research Network

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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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Terry Fox New Frontiers Program Project Grant in Neuroendocrine Tumors (NETs) of the Lung, Prostate, and Pancreas: Triggers and Targets for NETs