Development of a single-cell methylation workflow to understand the biology of circulating cells in early-stage non-small cell lung cancer

Abstract Submitter: Hannah Sheedy, United Kingdom*

Co-Authors: Janhavi Rastogi, Steven Hill, Seva Makeev, Florent Mouliere, Caroline Dive, Alexandra Clipson, Dominic Rothwell

*Cancer Research UK National Biomarker Centre, Manchester, UK.

Abstract

Lung cancer remains the leading cause of cancer-related deaths worldwide, with non-small cell lung cancer (NSCLC) accounting for 85% of cases. While early-stage NSCLC patients may undergo surgery with curative intent, approximately 50% experience recurrence, highlighting the need for biomarkers to predict relapse and enhance our understanding of metastasis. Liquid biopsies such as circulating tumour cells (CTCs) and cell-free DNA offer a minimally invasive alternative to tissue sampling, enabling real-time insights into tumour biology and disease progression.

In the TRACERx study, pulmonary vein (PV) blood sampling during NSCLC resection surgery demonstrated prognostic significance of PV-CTCs in stratifying relapse risk. PV-CTCs more closely resembled metastatic disease than primary tumours, revealing their role in early dissemination. Interestingly, PV-derived circulating epithelial cells (CECs) were prognostic but lacked aberrant copy number profiles or tumour-specific mutations, suggesting a distinct biological role in the development of recurrent disease.

Methylation profiling offers a promising approach to understand the biology of CECs and elucidate their role in NSCLC progression. However, single-cell methylation analysis is technically challenging due to the small amounts of DNA in a single cell. Current bisulfite-based methods for CTC methylation profiling lead to DNA degradation and limited CpG coverage (<4%). Here we describe a novel workflow for single cell methylation sequencing, utilising an enzyme-based conversion approach, which overcomes low input limitations by implementing barcodes to enable the pooling of genetic material from isolated single cells. Initial experiments using NSCLC cell lines and white blood cells from healthy donors confirm feasibility, achieving robust library preparation. Preliminary sequencing data indicates successful enzymatic conversion and reproducible copy number profiling down to a single cell.

Future work will validate this protocol in diverse cell types and apply it to clinical samples. This method could uncover novel epigenetic mechanisms of metastasis, informing predictive biomarkers that could improve post-surgical surveillance in NSCLC.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.