Novel Liquid Biopsy-based Determination of Lung Cancer Histology Using A Comprehensive Epigenomic Platform Reveals Enhancer Activity as A Key Determinant for Accurate Classification

Jamey Guess, Aparna Gorthi, Anthony D'Ippolito, Jonathan Beagan, Travis Clark, Michael Coyne, Tyrone Tamakloe, Baovy Tran, Hyun-Hwan Jeong, Kristian Cibulskis, Hathairat Sawaengsri, Juliann Chmielecki, Corrie A. Painter, J Carl Barrett, Matthew Eaton
Precede Biosciences, Inc., Boston, MA

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INTRODUCTION

- Accurate molecular characterization of lung cancer including histology classification and therapeutic target profiling is critical for treatment planning and resistance monitoring.
- Detecting lineage transformation from lung adenocarcinoma (LUAD) to small cell lung carcinoma (SCLC) following treatment, an established resistance mechanism, is challenging due to deteriorating patient health and complicated invasive procedures.
- We describe an accurate lung cancer histological classification using an innovative, multi-analyte epigenomic liquid biopsy method that comprehensively profiles tumor-specific transcription activation from 1 mL of patient plasma.

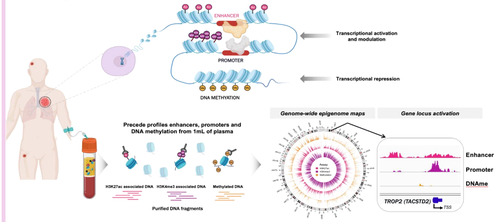
METHODS

- We developed a novel multi-analyte assay that reveals genome-wide transcriptional activity of promoters, enhancers from chromatin and the DNA methylome using 1mL of patient plasma.[1]
- Genome-wide epigenomic marks corresponding to activated promoters, activated enhancers, and methylated DNA was recovered from circulating chromatin and quantified via high throughput sequencing to produce a read out for transcriptionally activated and repressed regions of the genome.
- 37 LUAD and 34 SCLC patient plasma samples collected from commercial biobanks, along with reference cell lines were profiled using this technology to identify epigenomic loci characteristic of LUAD and SCLC (Table 1).
- Circulating tumor DNA (ctDNA) estimation was performed using ichorCNA.[2]
- A multimodal classifier was developed using genome-wide features to classify SCLC from LUAD.

Table 1: Lung cancer cohort

	N	Stage III	Stage IV	Female	ctDNA >3%
LUAD	37	0	37	14	21
SCLC	34	4	30	14	30
Healthy	24	-	-	12	-

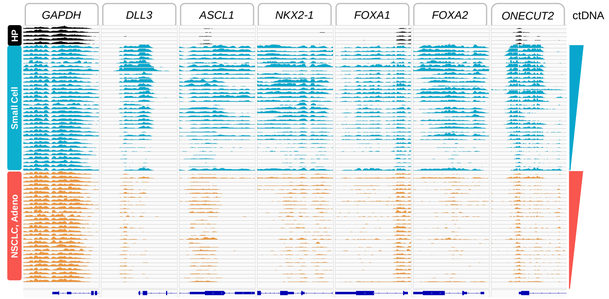
Figure 1. Comprehensive Epigenomic Platform Offers Dynamic Resolution into Target and Pathway Biology from 1 mL of Plasma



Cell free DNA derived from tumors exist in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against H3K27ac marking active enhancers, H3K4me3 marking active promoters and DNA methylation are used to enrich for associated DNA fragments from 1 mL plasma and sequenced to define genome-wide epigenomic maps that capture the underlying transcriptional state of the tumor cells.

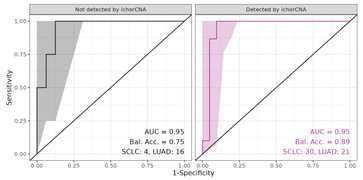
RESULTS

Figure 2. Dynamic Range of Signal at Promoters Seen Across LUAD and SCLC Regulators



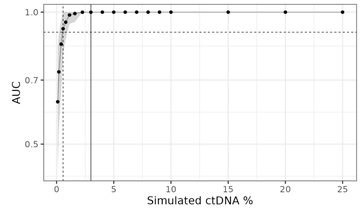
Patient-specific promoter signals at genes representing differential transcriptional biology across SCLC and LUAD show clear differences across histology. Samples are grouped by histology and sorted by ichorCNA ctDNA fraction estimate within each group.

Figure 3. Multi-analyte Classifier Accurately Discriminates Between SCLC and LUAD



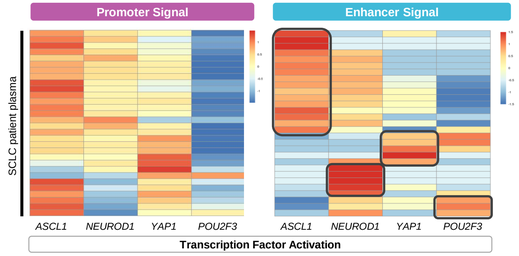
We developed a classifier using enhancer, promoter and methylation signal quantified at loci determined to be differential between SCLC and NSCLC in cell lines. 5-fold cross validation was performed using regularized logistic regression to estimate the predictive performance of classifying SCLC and LUAD. Selected features comprise 65% promoters, 23% enhancers and 13% methylation. Predictive performance remained high even for samples with ctDNA levels below the ichorCNA limit of detection suggesting that the assay is sensitive below 3% ctDNA.

Figure 4. In Silico Simulations Suggest Classification Performance Maintained Down to 0.5% ctDNA



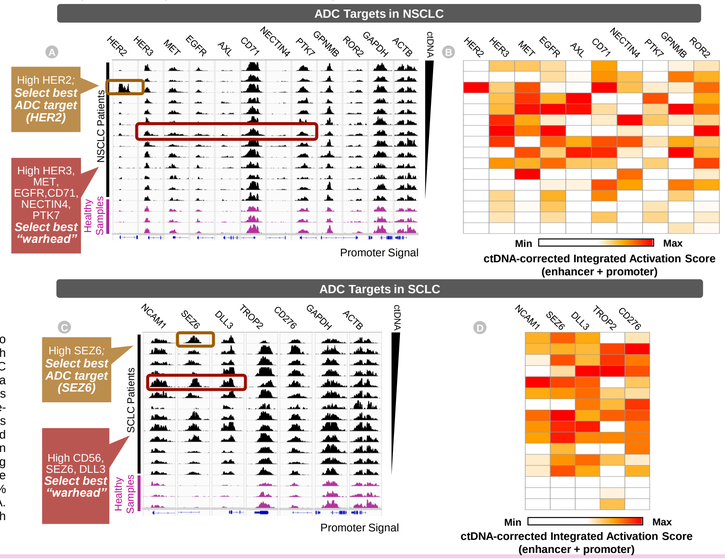
In silico plasma samples were created to simulate lower ctDNA levels by diluting the high ctDNA profiles of 17 LUAD and 12 SCLC plasma with each of the 24 healthy plasma samples. Regularized logistic regression was used to classify in silico mixtures using a leave-one-out scheme in which all mixtures generated from a given pair of cancer and healthy samples were held out and then predicted based on a model fit to the remaining mixture samples. AUC estimates from these simulations remain above 0.90 even at 0.5% ctDNA and above 0.80 at 0.4% ctDNA. Performance is expected to improve with further feature engineering.

Figure 5. Enhancer Activity Shows Potential in Identifying SCLC Subtypes



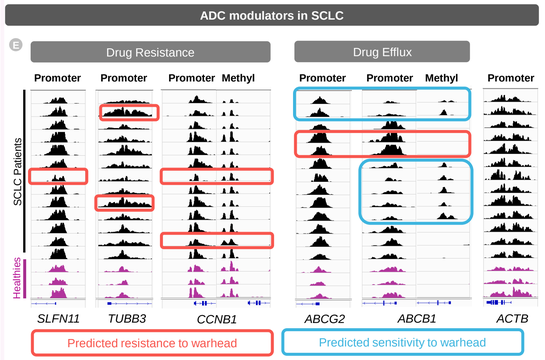
Heatmaps of quantified and scaled enhancer and promoter activity at established SCLC subtype driver genes (columns) in SCLC patient plasma samples (rows). Unsupervised clustering of enhancer activity shows distinct groups of SCLC samples suggesting the ability to infer SCLC subtypes using the epigenomic-based liquid biopsy platform. The observed distribution of SCLC subtypes is within the expected distribution of 70% SCLC-A, 11% SCLC-N, 17% SCLC-P and 2% SCLC-Y[3] (p=0.2).

Figure 6. Detecting Epigenomic Activation Status of Multiple Antibody-drug Conjugate (ADC) Targets and Response Modulators in Lung Cancer



RESULTS

Figure 6. Detecting Epigenomic Activation Status of Multiple Antibody-drug Conjugate (ADC) Targets and Response Modulators in Lung Cancer *(continued)*



Epigenomic activation panel to guide patient/therapy selection for ADC targets. (**A**) Qualitative track view displaying promoter signals across NSCLC cancer-relevant ADC target antigens and housekeeping genes GAPDH/ACTB. Rows represent select NSCLC cancer patient plasma samples sorted by ichor ctDNA fraction estimate or healthy volunteer plasma samples. (**B**) Quantitative heatmap of normalized and ctDNA-corrected epigenomic activation score (combining enhancer and promoter signals) for each ADC target antigen. Patient-specific epigenomic activation scores for each gene are represented on a white (low) to red (high) gradient. (**C,D**) Same as (**A,B**) in SCLC. (**E**) Promoter epigenomic activation signals at select genes known to modulate ADC response/resistance in SCLC patient plasma samples. Altered expression of these genes could impart payload resistance (*SLFN11*: topoisomerase inhibitor resistance, *TUBB3*: microtubule inhibitor resistance, *CCNB1*) or induce payload efflux (increase in drug efflux pumps *ABCG2*, *ABCB1*).

CONCLUSIONS

- We demonstrate the feasibility of epigenomic mapping from 1 mL of plasma in lung cancer patients to distinguish LUAD vs SCLC lung cancer histology.
- The resulting data is biologically interpretable through the lens of transcriptional activation of key histology specific genes.
- The transcriptional regulation of ADC targets is apparent through comprehensive epigenomic profiling of plasma in both NSCLC and SCLC, with the expression of *HER2, DLL3* and others showing a strong dynamic range independent of ctDNA levels.
- This novel liquid biopsy-based platform captures the transcriptional complexity of underlying disease biology and may provide a non-invasive approach to challenging tissue biopsies for biomarker investigation and therapeutic decision making.

Contact: Matthew Eaton, PhD
Precede Biosciences, Inc
Email: matthew.eaton@precede.bio

References

1. Baca SC, Seo JH, Davidsohn MP et al. Liquid biopsy epigenomic profiling for cancer subtyping. *Nat Med* 29, 2737–2741 (2023).
2. Adalsteinsson VA, Ha G, Freeman SS et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat Commun* 8, 1324 (2017).
3. Rudin CM, Poirier JT, Byers LA, et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. Nat Rev Cancer (2019).

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