Comprehensive Epigenomic Profiling from Plasma to Inform Therapy Selection: A Proof-of-Concept Study in Cancer

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Abstract #963

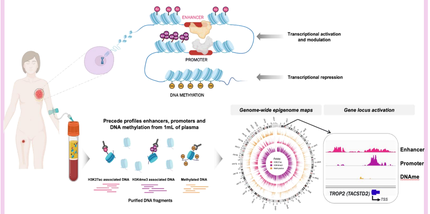
INTRODUCTION

- Analysis of genomic alterations in circulating tumor DNA (ctDNA) is gaining traction in clinical oncology and can provide insights into tumor biology without the need for invasive tissue sampling.
- Current ctDNA next-generation sequencing (NGS) based tests focus on genomic alterations (e.g. mutations, copy number alterations, and fusions) but fail to effectively capture changes associated with epigenomics and transcriptional biology.
- Epigenomic reprogramming can lead to transcriptional dysregulation resulting in distinct molecular and histologic subtypes within a single tumor type as well as drive non-genetic mechanisms of therapeutic resistance.
- Here, we describe a novel liquid biopsy (LBx) assay that interrogates genome-wide transcriptional biology from 1 mL of plasma through analysis of DNA associated with histone marks of active transcription.
- This comprehensive multi-analyte epigenomic profiling approach has the potential to investigate the transcriptional status of individual genes as well as genome-wide biology that is correlated or anti-correlated with targets of interest.
- Examples of applications include expression of relevant drug targets [e.g. antibody drug conjugates (ADCs) and radio-immune conjugates (RICs)], classification of molecular subtypes using a genome wide transcriptional activation signal, analysis of into non-genetic mechanisms of therapeutic resistance, and insight into immune dysregulation in disease.

METHODS

- We developed a novel multi-analyte assay that reveals genome-wide transcriptional activity of promoters, enhancers from chromatin and the DNA methylome using 1 mL of patient plasma.
- 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 readout for transcriptionally activated and repressed regions of the genome.
- Circulating tumor DNA (ctDNA) estimation was performed using ichorCNA[2].
- We have applied this assay to over 2,000 patient plasma samples from 15 cancer and 5 non-cancer indications.

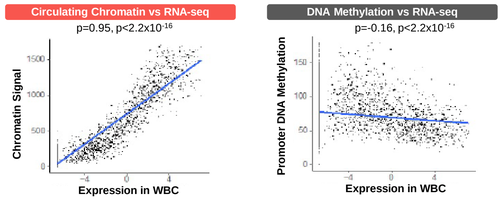
Figure 1. Comprehensive Epigenomic Platform Offers Dynamic Resolution into Target and Pathway Biology from 1 mL Of Plasma



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.

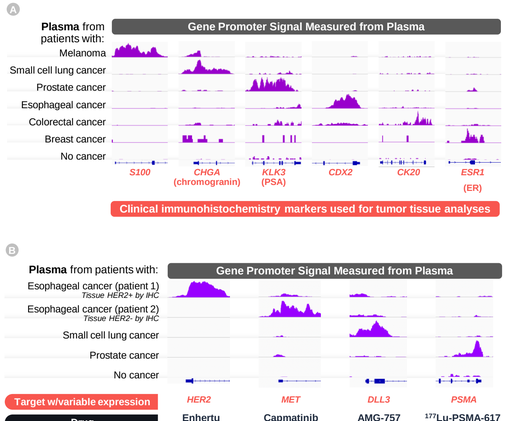
RESULTS

Figure 2. Precede Plasma Signal Robustly Correlates with Gene Expression as Measured by RNA-seq



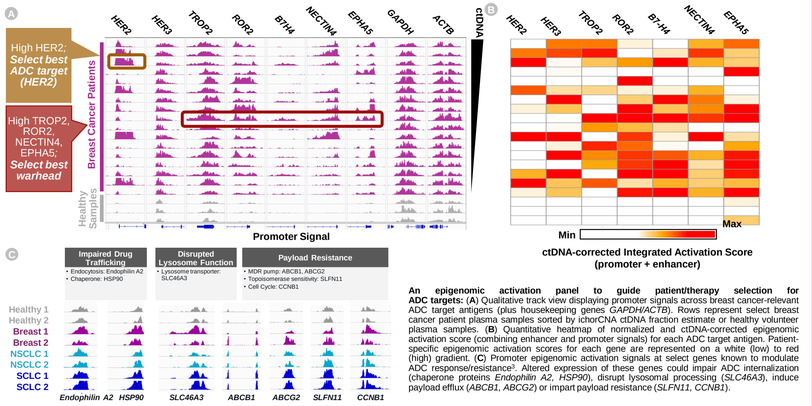
Correlation between RNA-seq and Precede output. Compared to DNA methylation, analysis of promoter marks (H3K4me3) from WBCs was superior in identifying transcriptionally active regions as evidenced by high concordance with RNA-seq (transcriptome) readouts (left panel). In contrast, DNA methylation alone did not reproduce gene expression patterns from RNA-seq (right panel).

Figure 3. Precede Detects Clinically Biologically Relevant Signal and Identifies Potential Therapeutic Vulnerabilities from A Single Sample



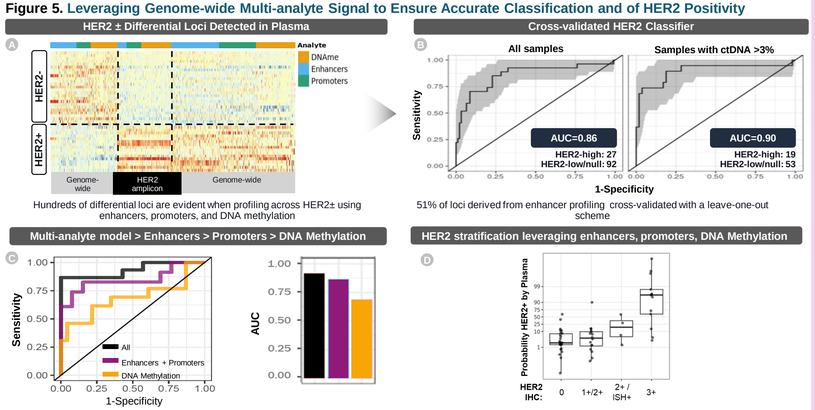
Promoter signal at genes differentially expressed across cancers reveal relevant biology and potential therapeutic vulnerabilities: Patient-specific (by row) promoter signals at genes analyzed routinely by immunohistochemistry (IHC) across different cancer types (**A**). Disease-specific transcriptional activity, as indicated by signal peak at the promoter region, is observed for IHC targets of interest. Additionally, transcriptional activity can also be investigated in potential drug targets of interest (**B**) from the same sample. Here, we show representative signals from disease-specific drug targets. For the esophageal cancer patients, HER2 promoter activity from the plasma analysis correlated with the HER2 IHC analysis performed on the tissue samples. A healthy sample is included as a negative control for transcriptional activity in oncogenic targets.

Figure 4. Detecting Activation Status Of Multiple Antibody-drug Conjugate (ADC) Targets and Response Modulators from 1mL of Plasma from Breast Cancer Patients



An epigenomic activation panel to guide patient/therapy selection for ADC targets: (**A**) Qualitative track view displaying promoter signals across breast cancer-relevant ADC target antigens (plus housekeeping genes *GAPDH*/*ACTB*). Rows represent select breast cancer patient plasma samples sorted by ichorCNA 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**) Promoter epigenomic activation signals at select genes known to modulate ADC response/resistance[3]. Altered expression of these genes could impair ADC internalization (chaperone proteins *Endophilin A2*, *HSP90*), disrupt lysosomal processing (*SLC46A3*), induce payload efflux (*ABCB1*, *ABCG2*) or impart payload resistance (*SLFN11*, *CCNB1*).

Figure 5. Leveraging Genome-wide Multi-analyte Signal to Ensure Accurate Classification and of HER2 Positivity



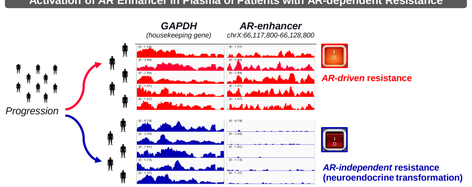
Hundreds of differential loci are evident when profiling across HER2± using enhancers, promoters, and DNA methylation

51% of loci derived from enhancer profiling cross-validated with a leave-one-out scheme

Accurate classification of HER2 positivity from plasma using a multi-analyte classifier. Genomic loci with differential signal (FDR ≤ 0.05, log2 fold-change ≥ 1) between HER2 3+ and HER2 0 patients at enhancers, promoters, and sites of DNA methylation. Signal has been normalized (ctDNA %) and the z-score was calculated for each locus (**A**). Genome-wide enhancer, promoter, and DNA methylation signal was used to train a regularized logistic regression model to classify HER2 IHC status (0 vs 3+). Performance was assessed via AUC in a leave-one-out cross-validation scheme (**B**). Performance was stratified by whether ctDNA was detectable using ichorCNA (>3%) on patient-matched low-pass whole genome sequencing data[2]. The most robust classifier incorporate signal from promoters, enhancers, and methylated regions (**C**). Using the HER2 3+ vs 0 classifier described previously, the probability of HER2 positivity was determined for samples classified as HER2-low by IHC, demonstrating a positive association of the probability of HER2 positivity and IHC score. (**D**) HER2 classifier development has been described previously[4].

RESULTS

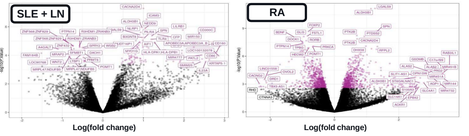
Figure 6. Detection of Enhancer-driven Resistance in Prostate Cancer Plasma



Activation of the AR gene enhancer drives treatment resistant castration-resistant prostate cancer (CRPC)[5].

Gene enhancer analysis of plasma samples from prostate cancer patients at the time of treatment progression revealed reactivation of the androgen receptor (AR) in a subset of patients (top), suggesting an AR-driven resistance mechanism for which AR-directed therapies may be appropriate. In contrast, no reactivation was observed in a cohort of patients (bottom), suggesting AR-independent mechanisms of resistance such as neuroendocrine transformation.

Figure 7. Applications in Auto-immune Diseases Can Reveal Insights into Immune Biology



Pathway	Adj. p-value
HALLMARK_ALLOGRAFT_REJECTION	4.32E-09
HALLMARK_INTERFERON_GAMMA_RESPONSE	2.18E-07
HALLMARK_INFLAMMATORY_RESPONSE	6.1E-06
HALLMARK_IL6_JAK_STAT3_SIGNALING	0.00963
HALLMARK_INTERFERON_ALPHA_RESPONSE	0.00963
HALLMARK_IL2_STAT5_SIGNALING	0.02083
HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.03408

Pathway	Adj. p-value
HALLMARK_HEME_METABOLISM	<0.0001
HALLMARK_INTERFERON_ALPHA_RESPONSE	<0.0001
HALLMARK_ALLOGRAFT_REJECTION	<0.0001
HALLMARK_INTERFERON_GAMMA_RESPONSE	<0.0001
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATH	0.047
HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.0049
HALLMARK_HYPOXIA	0.0139
HALLMARK_INFLAMMATORY_RESPONSE	0.0139

*Lupus patient samples sourced from a biobank FDR <0.05

Applied to systemic lupus erythematosus (SLE) plus lupus nephritis (LN) and rheumatoid arthritis (RA), Precede identifies canonically up-regulated genes and pathways vs healthy controls.

Volcano plots showing differences in gene activation from transcriptional signatures between different auto-immune disease states. Pathway analysis identified signaling mechanisms that aligned with known biology and/or drug targeting approaches in these indications. Insights from transcriptional analysis of these auto-immune states can potentially be applied to oncology indications for which an enhanced understanding of immune activation may help inform therapeutic response and resistance.

CONCLUSIONS

Applications to Inform and De-risk Drug Development

Oncology | *Autoimmunity* | *Other Conditions w/Detectable cfDNA*

1. Patient selection
2. Tumor stratification
3. Dynamic signaling changes & PD
4. Mechanisms of resistance
5. Toxicity

- Here, we present a highly differentiated comprehensive epigenomic assay that provides unique insights into the biology of cancer and non-cancer conditions.
- Using 1 mL of plasma, we are able to profile the transcriptional state of the genome through analysis of histone-associated marks of active promoters and enhancers plus transcriptional repression derived from the DNA methylome.
- This multi-analyte assessment enables serial assessment of drug targets, pathways, and cancer phenotypes including:
  - Proxy for expression of key genes routinely assessed by IHC-based assays
  - ADC target antigen expression as well as expression of genes involved in ADC-warhead sensitivity
  - Genome-wide pathway analysis associated with oncogenic activation
  - Novel mechanisms of therapeutic resistance beyond mutational profiles
  - Relevant immune biology
- Additionally, the platform is poised for delivering information about drug toxicity [e.g., interstitial lung disease (ILD) and Idiopathic pulmonary fibrosis (IPF)].
- Additional work to identify and validate new epigenomic biology as well as novel biomarkers is ongoing.

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