Multimodal Analysis of cfDNA Methylation Sequencing Improves Early Colon Cancer Detection

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ABSTRACT

Introduction
In recent years, alterations in cell free DNA (cfDNA) methylation patterns have gained wide acceptance as powerful biomarkers for early cancer detection. Here, we evaluate a Multimodal Epigenetic Sequencing Assay (MESA) for colon cancer detection that combines features derived from both cfDNA fragmentomics and cfDNA methylation to improve test performance. Our study indicates that MESA's combined approach is superior to methods that use only methylation or only fragmentomics to distinguish between colon cancer patients and healthy individuals.

Methods
Blood specimens drawn from 64 subjects diagnosed with colon cancer and 67 control subjects were processed by using the ECLIPSE platform. This platform consists of molecular techniques for cfDNA extraction, conversion, library generation and targeted next-generation DNA sequencing to generate high quality sequencing reads from genomic regions of interest. The ECLIPSE platform also allows for the evaluation of both cfDNA methylation patterns and fragmentation features by using a non-disruptive, enzymatic conversion step, which minimizes degradation of cfDNA, unlike traditional bisulfite conversion methods. Custom bioinformatics pipelines and algorithms were used to process sequencing data, generate features and train models. Model performance was evaluated by using repeated 5-fold cross validation.

Results
The MESA combined feature models possessed a median AUC of 0.91. In contrast, models incorporating only cfDNA methylation features or only cfDNA fragmentation features possessed median AUCs of 0.89 and 0.83, respectively. We also observed >5% increase in sensitivity at 90% specificity for the MESA combined feature models. Therefore, our MESA approach of combining cfDNA fragmentomics and DNA methylation proved to be superior to using only a single class of features when distinguishing between colon cancer patients and healthy individuals.

Discussion
Our findings suggest that cfDNA fragmentation-derived features may carry useful information that is complementary and additive to the cfDNA methylation signal when distinguishing between patients with and without colon cancer. By utilizing improved molecular techniques and analysis methods, it is possible to evaluate both cfDNA methylation and cfDNA fragmentation features that reflect the underlying chromatin structure within a single assay. This multimodal approach is predicted to allow for the development of diagnostic tests with superior performance characteristics when compared to currently available testing methods.

INTRODUCTION

Cell free DNA (cfDNA), including circulating tumor DNA (ctDNA), carries molecular signatures that reflect the underlying epigenetic state of the cell(s) from which it originated. Most cfDNA methylation sequencing data analysis approaches explore the data in a unidimensional way, focusing on methylation patterns alone, and fail to capture the vast depth of epigenetic information carried by cfDNA.

Here, we demonstrate that a **multi-dimensional** analysis approach, which incorporates additional cfDNA features, is superior to cfDNA methylation alone in distinguishing between individuals with and without colon cancer.

METHODS AND MATERIALS

Table 1. Characteristics of study participants.

Colon Cancer	Stage I	Stage II	Stage III	Stage IV	Controls
Number of subjects	8	9	23	24	67
Age (median, IQR)	73 (54 - 82)	66 (58 - 73)	72 (58 – 80)	64 (54 - 69)	45 (35 – 59)
No. of males, % males	3 (37.5%)	5 (55.5%)	16 (69.5%)	15 (62.5%)	26 (38.8%)

ECLIPSE™ WET-LAB PLATFORM

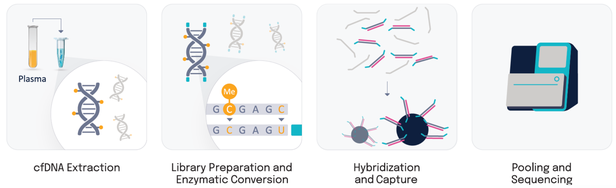

Figure 1. Helio's ECLIPSE™ platform for automated, high-throughput cfDNA methylation analysis.

ECLIPSE optimizes cfDNA methylation analysis by (1) using enzymatic conversation to minimize cfDNA degradation and preserve cfDNA integrity unlike traditional bisulfite conversion methods and (2) allowing for a single assay to simultaneously explore methylation and fragmentomics features.

MESA™ (MULTIMODAL EPIGENETIC SEQUENCING ASSAY)

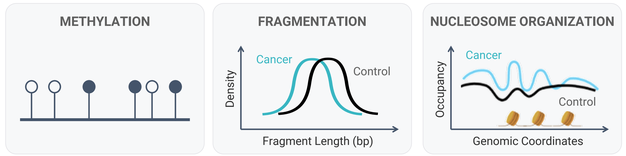

Figure 2. MESA™ allows for multi-modal feature analysis within a single assay.

MESA provides a multi-dimensional view of cancer, allowing for richer biological insights and different feature modalities that may capture complementary signals.

RESULTS

The model combining cfDNA methylation with nucleosome occupancy has a **higher AUC and sensitivity** at 90% specificity compared to either feature category alone, highlighting the utility of MESA's multimodal approach (Tables 2 and 3, Figure 3).

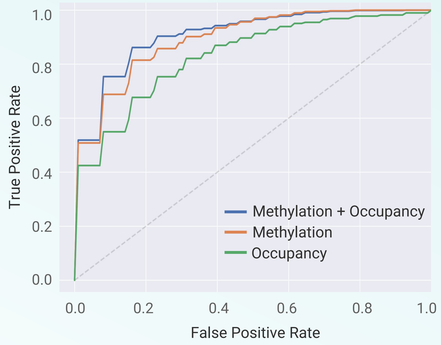

Figure 3. Mean ROC curve for individual modalities & MESA combined feature model.

Table 2. Model performance of individual modalities vs. MESA's combined features.

Features	AUC	Sens at 90% Spec
Methylation + Nucleosome Occupancy	0.91	75%
Methylation Only	0.89	68%
Nucleosome Occupancy Only	0.83	55%

Table 3. Model performance of individual modalities vs. MESA combined feature model. For every cancer stage, MESA outperforms methylation or nucleosome occupancy alone.

	Methylation + Occupancy		Methylation Only		Occupancy Only	
Stage	AUC	sens90	AUC	sens90	AUC	sens90
I	0.91	77%	0.90	69%	0.81	43%
II	0.92	70%	0.88	64%	0.84	58%
III	0.88	72%	0.87	65%	0.82	53%
IV	0.93	80%	0.92	73%	0.84	60%

WHAT'S NEXT? TAKE IT ONE STEP FURTHER.

Additional analytes such as protein tumor markers can provide complementary signals to detect cancer. In Figure 4. we demonstrate that adding protein information to our MESA combined feature model further improves model sensitivity at 90% specificity from 75% to 84% and improves AUC from 0.91 to 0.93, highlighting the **untapped power of Multimodal x Multiomics integration.**

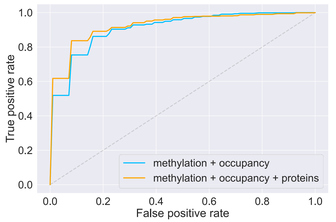

Figure 4. Mean ROC curve for Multimodal model (blue) vs. an integrated Multimodal x Multiomics model (orange).

DISCUSSION

- cfDNA methylation sequencing data can be featurized in multiple ways to provide insightful information beyond methylation patterns.
- Fragmentomics-based features provide additional information about the epigenetic & chromatin state of the tumor cell of origin and can be complementary to the methylation signal as demonstrated here.
- At Helio Genomics, several categories of features derived from cfDNA methylation sequencing data and multiple types of analytes are being explored to obtain the **most complete picture possible of the epigenetic landscape of cancer.**

Questions? Contactus@heliogenomics.com