Open chromatin region (OCR) based model predicts advanced adenoma in plasma cell-free DNA whole-genome bisulfite sequencing data

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Introduction

- Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality in the world[1].
- Majority of CRC are believed to originate from advanced adenomas (AA)[2].
- AA are under-diagnosed by current screening methods[2-3], leading to unmet demand for novel non-invasive biomarker-based tests for population-wide screening programs[4].
- Chromatin conformation of tumor derived cell free DNA (cfDNA) in plasma is tissue-specific and could enable the identification of AA[5].
- We report here a plasma cfDNA chromatin conformation marker panel performance for detection of patients with AA with good accuracy.

Methods

- Plasma cfDNA whole genome bisulfite sequencing (WGBS) data of 10 pooled samples (2 from AA, 3 from CRC stage I and 5 from control patients) were used as training set for open-chromatin region (OCR) filtering and model building.
- Small intestine OCRs previously described by Snyder et al. were used as a surrogate of colorectal tissue chromatin conformation[6].

- Sequencing-coverage-based features (OCR_{scores}) were computed to determine cfDNA chromatin conformation (Figure 1).
- OCRs showing maximum OCR_{score} difference between cases (AA + CRC stage I) and controls with low inter-sample variance were selected.
- A PLS-DA classification model was built on OCR_{scores}.
- Validation was performed using cfDNA WGBS data from 10 individual advanced adenoma patients and 10 age/gender matching colonoscopy verified control patients (Table 1).

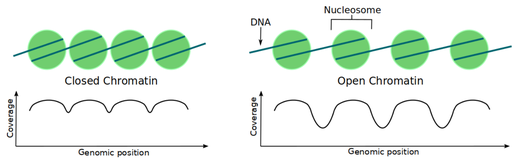


Figure 1. *OCR_{score} is based on sequencing coverage features. In cfDNA, sequencing coverage is ruled by chromatin conformation, where DNA that is not bound to nucleosomes is digested and coverage diminishes. Open chromatin conformation causes a higher drop in coverage between nucleosomes than closed chromatin conformation.*

Results

- A total of 1074 OCRs were chosen with the region filtering procedure. Further analysis was conducted using this set of OCRs.
- OCR_{scores}showed strong case-control separation power sample-wise in training data (Figure 2) and in validation data (Figure 3).

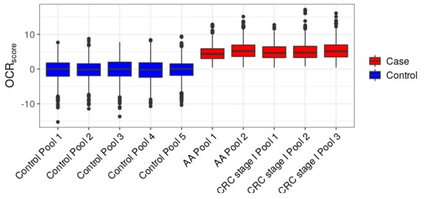
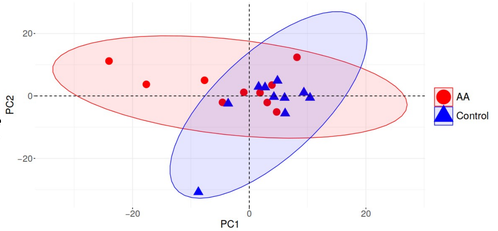


Figure 2. *OCR_{score} distribution of the 1074 selected regions in each case (red) and control (blue) sample in the train set.*

Figure 3. *PCA plot of the OCR_{score} distribution for the 1074 selected regions in each AA (red) and control (blue) sample in the validation set. They show differences with partial overlap.*



	Adenoma (N=10)	Control (N=10)
Age (average (min-max))	64.5 (52-71)	67.6 (53-76)
Gender Male (%)	50%	50%
Serrated with dysplasia	2	
Tubulovillous high grade	2	
Tubulovillous > 1cm	2	
Tubular high grade	2	
Tubular > 1cm	2	

Table 1. *Validation set patient cohort.*

	AA (real)	Control (real)
AA (predicted)	5	1
Control (Predicted)	5	9

Table 2. *Confusion matrix of the classification performance applying the PLS-DA model to validation data.*

- Contributions of cell-free DNA in a colon-tissue-specific chromatin conformation are found to be higher in cases than in controls (Figure 2).
- PLS-DA model validation resulted with 50% (5/10) of AA samples being correctly identified at 90% (9/10) specificity (Table 2).
- Model detected 100% (2/2) serrated adenoma, 50% (2/4) tubulovillous adenoma and 25% (1/4) tubular adenoma patients.
- Detection rate was comparable for patients with high grade dysplasia (50% [3/6]) and with low grade but > 1cm findings (50% [2/4]).

Conclusions

- We show that our chromatin-conformation-based model provides promising results to detect AA using plasma cfDNA.
- This method could serve as the basis for further development of a highly accurate and minimally invasive blood-based screening test and could potentially help to guide downstream clinical evaluation of the patients.

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Disclosure statement

- This study is sponsored by Universal Diagnostics S.L.
- P. Canal-Noguer, K. Kruusmaa, M. Bitenc are employees of Universal Diagnostics S.L.