Unlocking the Potential of Non-Mutational Characteristics of Cell-Free DNA as a Biomarker for Oral Cancer

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Abstract

Plasma cell-free DNA (cfDNA) is a developing source of biomarkers for oral cancer and potentially other diseases. Previous studies have used Broad Range whole genome sequencing to identify various patterns in cfDNA such as methylation patterns, acetylation patterns, and g-quadruplex abundance. In our study on plasma cfDNA for oral cancer detection, we analyzed short to long fragment ratios, G-quadruplex abundance, and end-motif profiles throughout the genome. For each feature, we examined three cfDNA populations: mononucleosomal (mncfDNA), ultrashort (uscfDNA), and short cfDNA (scfDNA). The results demonstrated distinct fragmentomic ratios across the different cfDNA populations and microenvironments. Additionally, G-quadruplex abundance was notably higher in certain cfDNA populations, indicating potential as biomarkers for oral cancer detection. Our comprehensive approach highlights the diagnostic potential of cfDNA characteristics, providing insights into their role in the complex microenvironment of oral cancer.

Introduction

Oral cancers are diagnostic challenges due to their complex biology, heterogeneous nature, and lack of effective biomarkers. CfDNA provides information of non-somatic mutational characteristics including fragment size ratios, g-quadruplex abundance, and end-motif profiles to act as biomarkers for early cancer detection. In previous studies, scientists have used library preparation and construction abilities of single-stranded DNA of the Broad-Range pipeline. Using the Broad-Range cfDNA pipeline, they conducted a low-coverage non targeted whole genome sequencing study where non-mutational characteristics of cfDNA such as end-motif profiles, fragmentomics, and alignment to genes associated with cancer tumor microenvironments were quantified in a search for oral cancer-specific signatures. Moreover, plasma-based diagnostics are minimally invasive, making it an attractive option for large population-based screening of oral cancer. Unraveling the secrets of cfDNA in plasma and the unique populations uncovered by BRcfDNA-Seq opens new opportunities for oral cancer diagnosis, treatment, and prevention.

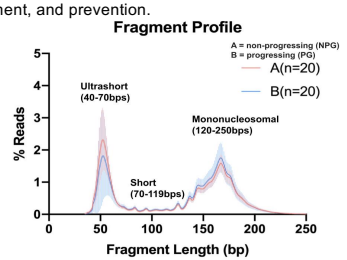
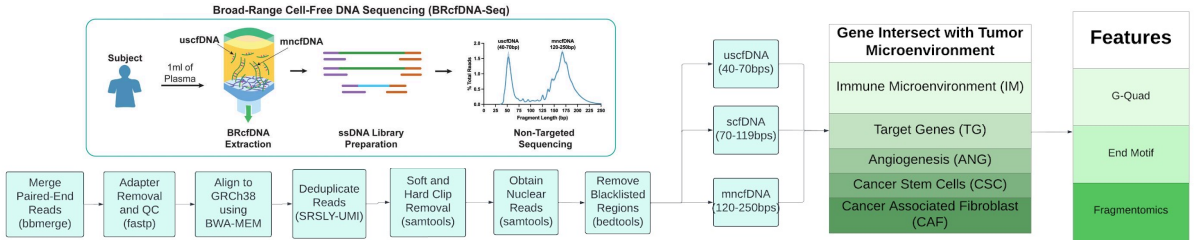


Figure 1. Used library preparation/construction techniques of single-stranded DNA of Broad-Range pipeline to discover presence of three cfDNA populations for non-progressing and progressing groups: ultrashort (uscfDNA), mononucleosomal (mncfDNA), and short (scfDNA).

Methods



Results

Comparing cfDNA Populations

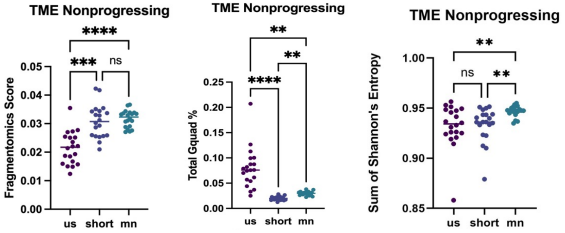


Figure 3. *ANOVA was performed for the TME nonprogressing group for all three cfDNA populations analyzing fragmentomics score (short to long ratio of fragments), Gquad abundance (% of all reads containing g-quadruplex motifs), and end-motifs (sum of Shanon's entropy scores). Significant difference observed when comparing ultrashort to mononucleosomal cfDNA population.*

Potential Biomarker Development

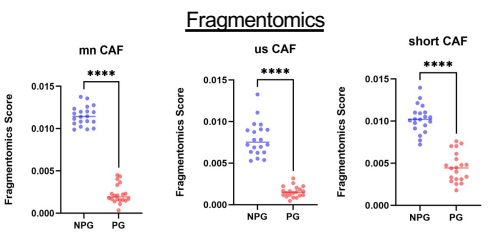


Figure 3. *CAF fragmentomics scores for all three cfDNA populations, comparing nPG and PG groups (same results observed for ANG and CSC populations)*

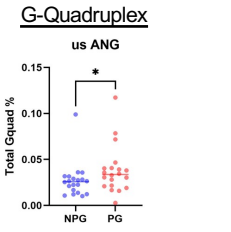
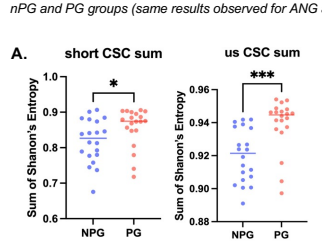


Figure 4. *G-quadruplex abundance for nPG and PG groups for uscfDNA ANG sample.*



End-Motif

B.

Most Common End-Motifs for ultrashort tumor microenvironment	Most Common End-Motifs for short tumor microenvironment	Most Common End-Motifs for mononucleosomal tumor microenvironment
CCCC	CCCA	CCCA
CCCA	CCTG	CCTG
CCAG	CCAG	CCAG
CCCT	CCCC	CCCT
CCTC	CCCT	CCCC

Figure 5. A) *The uscfDNA CSC population demonstrates a significant difference ($p<0.001$) between sum of Shannon's entropy scores between the nPG and PG groups. There are five other groups that demonstrate a significant difference ($p<0.05$) between sum of Shannon's entropy scores between the nPG and PG groups.* ***B)*** *Five most common end-motifs for all three cfDNA populations ranked from most to least common: ultrashort, mononucleosomal, and short.*

Discussion

This study demonstrates the potential that non mutational characteristics of cfDNA hold as promising biomarkers for early cancer detection of oral and other cancers. As non mutational characteristics analysis improves, doctors will be able to use compact devices to conduct liquid biopsies with just one drop of blood or saliva, enabling early cancer diagnosis or detection. Further research as well as validating non-mutational characteristics as clinical biomarkers within plasma cfDNA can allow us to draw parallels to saliva methods, which will facilitate the development of non-invasive solutions for early cancer detection.

Fragmentomics – (short to long fragment ratios)

- ANG, CSC, and CAF for uscfDNA, scfDNA, mncfDNA populations had significant differences, while no significant differences were found between progressing (PG) and non-progressing (nPG) groups for other microenvironments.

G-Quadruplex – (string of 4 or more guanine nucleotides)

- Gquad structures have been demonstrated to be another metric for genomic instability and associated with oncogenic process[7]. Interestingly, only the us ANG group showed significant differences between PG and nPG groups.

End-Motif – (4 nucleotide motifs at the end of fragments)

- Cleavage of cfDNA suggests activity of nucleases in circulation and intracellular nucleases, producing unique 4-mer end-motif profiles of cfDNA at C termini ends[5].
- Most results were insignificant between nPG and PG groups except for the us CSC metric. Five other metrics showed a significant difference as well: scfDNA ANG, mncfDNA CSC, scfDNA CSC, scfDNA IM, mncfDNA TG.

References

PMID: [1]37817261; [2]37817261; [3]31142840; [4]37075072; [5]32111602; [6]38797520;

Additional Resources

Wong Lab GitHub

Wong Lab Website

Gastric Cancer Paper