Circulating cell-free DNA methylation assay: towards early cancer detection and minimal residual disease

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INTRODUCTION

DNA methylation is one of the earliest frequent signatures during cancer development, and its distinct patterns among different cancer types enable the potential of early detection of specific cancers. Several methods are available for sequencing DNA methylation throughout the genome, including whole genome bisulfite sequencing (WGBS) and antibody-dependent DNA immunoprecipitation (MeDIP). As a non-invasive approach, plasma cell-free DNA (cfDNA) has been widely used for clinical applications. In this study, we report the preliminary data of PredicineMETH, a proprietary cfDNA-based methylation assay for early cancer detection, minimal residual disease assessment, and further identification of tissue-of-origin in cancer.

MATERIALS AND METHODS

Cell line gDNA with known methylation profiles, plasma cfDNA, and urinary cfDNA (ucfDNA) from patients and healthy donors were used in our methylation study. Methylation results were verified by comparing with public methylation data. Methylation profiles of healthy donors and patients were also compared to assess the assay’s ability to distinguish cancer patients from healthy donors.

Figure 1. PredicineMETH - DNA methylation NGS workflow.

A. Sample preparation and NGS workflow

- Sample collection
- cDNA extraction
- Methylation assay and library construction
- Next-generation sequencing

B. DNA methylation analysis workflow

- Sequencing alignment
- Quality control
- Differential analysis
- Feature selection
- Classification

C. IGV graphic view of in-house methylation data and public data

RESULTS

Figure 2. PredicineMETH NGS assay can reliably detect methylation using as low as 5ng DNA. High concordance was observed between GM12878 methylation data from our assay and that from public database.

A. GM12878 gDNA was fragmented by sonication to mimic cfDNA and subjected to our methylation assay

B. GM12878 CpG island signals from Predicine assay were compared with public MeDIP database

Figure 3. PredicineMETH can distinguish samples from different sources.

A. DNA methylation profile correlation coefficient between different sources

B. Multidimensional scaling plot to separate different cell types based on differential DNA methylation profiles.

Figure 4. PredicineMETH can classify healthy donors and bladder cancer patients.

A. DNA methylation profile correlation coefficient between different samples from healthy donors or cancer patients, where each patient has multiple DNA methylation profiles before and after treatment.

B. Multidimensional scaling plot indicated the effectiveness of the treatment, where all the patients’ DNA methylation profiles after treatment are more similar to healthy donors.

CONCLUSIONS

- Predicine has developed PredicineMETH, a proprietary methylation NGS assay to detect methylation profiling in gDNA, plasma cfDNA, and ucfDNA.
- Preliminary data suggest the potential of PredicineMETH in early cancer detection, therapeutic monitoring, and minimal residual disease assessment.

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