Cell-free dna methylation detection in small plasma volumes using bisulfite-free multiplex digital pcr demonstrated in an early-onset colorectal cancer pilot study

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Abstract

Background: Colorectal cancer (CRC) is a major global health burden, with rising incidence in young adults, called early-onset CRC (EO-CRC). Targeted methylation detection of CRC-specific biomarkers in blood plasma offers a minimally invasive diagnostic approach. Digital PCR (dPCR) enhances clinical feasibility, especially for longitudinal monitoring, due to its absolute quantification and rapid processing. However, conventional bisulfite-based dPCR assays require large plasma volumes up to 16 mL, limiting practical implementation.

Objective: This study aimed to develop a bisulfite-free, low-plasma-volume dPCR methylation assay coupling cell-free methylated DNA immunoprecipitation (cfMeDIP) and highly sensitive multiplexed mediator probe dPCR (cfMeDIP-dPCR). To demonstrate feasibility for detection of EO-CRC, we conducted a pilot study using small plasma volumes.

Methods: Promising CRC-specific methylation markers, identified in bisulfite-based plasma studies, were selected for a multiplex target panel. The multiplex assay was technically validated using dilutions of cfDNA surrogates from HCT116 cells and then applied in a pilot study involving 32 EO-CRC patients and 29 non-CRC controls, using only 2 mL of plasma per sample.

Results: Three out of four methylation markers from literature passed the technical assay validation with LoD of 0.01 ng (SEPT9), 0.01 ng (KCNQ5) and 0.06 ng (C9orf50). In the pilot study, each marker exhibited significantly higher median methylation ratios ($p \leq 0.001$) in EO-CRC patients compared to healthy controls. The marker KCNQ5 demonstrated the best performance, achieving 85% sensitivity at 90% specificity. Moreover, its methylation ratios correlated significantly with tumor stage.

Conclusions: This study introduces cfMeDIP-dPCR as a highly sensitive and easy-to-implement liquid biopsy assay for targeted methylation analysis. Combining degradation-free cfMeDIP with sensitive multiplex dPCR achieved clinically relevant sensitivities in 2 mL of plasma only, up to 8 times less than existing assays. Crucially, this study confirms that three well-established CRC markers can be applied to EO-CRC detection. The correlation between methylation ratios and tumor stages highlights its potential for both cancer detection and monitoring. These findings support the development of cfMeDIP-dPCR assays for accessible and patient-friendly diagnostics of CRC. Future research should aim to enhance multiplexing, conduct larger studies, and validate assay performance across a broader range of diseases with epigenetic signatures.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.