Methylation patterns in cell-free dna for pan-cancer detection

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Abstract

Background

Epigenetic modifications, such as DNA methylation, are established early in cancer development and may offer higher sensitivity for cancer detection than mutations in circulating cell-free DNA (cfDNA).

Objective

This study aimed to investigate cfDNA methylation differences between cancer patients and individuals with severe, nonspecific symptoms.

Methods

cfDNA was extracted from plasma samples of 229 patients undergoing cancer evaluation due to severe, nonspecific symptoms. Among them, 34 were diagnosed with cancer, while 195 had non-malignant diseases and served as controls. Samples underwent enzymatic conversion, library preparation, and target enrichment using the NEBNext workflow with the Twist pan-cancer alliance panel, followed by sequencing. Methylation analysis was performed using nf-core/methylseq. Differentially methylated regions (DMRs) were identified using DMRichR, and classification between groups was optimized with support vector machine (SVM) algorithms and cross-validation.

Results

The method showed high feasibility, as all samples with measurable cfDNA generated high-quality libraries with >200X sequencing depth. Cancer samples exhibited significantly higher overall methylation than controls (1.88% vs. 1.31%, $p<0.001$). A total of 1,585 DMRs were identified, with 61.6% located in promoter regions. Most (96.9%) were hypermethylated in cancer. Ontology analyses revealed associations with nuclear origin and transcription regulation. The 10 highest-ranked DMRs were used to construct a classifier, achieving a sensitivity of 94.1% at 100% specificity, corresponding to an area under the curve (AUC) of 99.6%.

Conclusion

Enzymatic conversion library preparation is clinically feasible even with limited cfDNA. Significant methylation differences were observed between cancer cases and controls with severe nonspecific symptoms, and machine learning effectively distinguished malignant from non-malignant conditions. However, validation in a larger dataset is needed to mitigate the risk of overfitting. Our findings support methylation as a promising biomarker for cancer detection despite background noise from confounding diseases.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.