Developing a methylation-sensitive restriction enzyme-droplet digital pcr assay for the identification and monitoring of non-small cell lung cancer

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Abstract

Background

DNA methylation is an epigenetic modification crucial for regulating gene expression in various cellular processes. Aberrant DNA methylation is linked to numerous pathologies, including non-small cell lung cancer (NSCLC). Emerging evidence suggests that DNA methylation-based biomarkers can be used for diagnosis, prognosis, and predicting therapy response using circulating tumor DNA (ctDNA). However, studies on DNA methylation analysis in body fluids are limited, posing challenges in developing lung cancer-specific and quantitative assays with sufficient sensitivity.

Objective

This study aims to develop a methylation-sensitive restriction enzyme (MSRE)-based multiplex digital droplet PCR (ddPCR) assay to detect hypermethylated regions in >80% of NSCLC patients and evaluate its use in monitoring ctDNA levels during treatment.

Methods

In silico analysis of The Cancer Genome Atlas (TCGA) methylation data was conducted to identify differentially hypermethylated regions specific for NSCLC in comparison to healthy tissue. The main criteria for marker selection were: mean methylation percentage in healthy blood <5% and at least 80% of lung tumor samples should be hypermethylated. A multiplex ddPCR assay was designed to detect NSCLC-specific hypermethylated CpG dinucleotides in five selected genes. This explorative study was performed using lung cancer cell lines, in vitro methylated DNA and tissue samples from lung cancer patients (n=18) and non-cancer individuals (n=10).

Results

The developed six-color multiplex ddPCR assay successfully detected hypermethylated regions in MSRE-digested methylated cell lines and in vitro methylated DNA, while no methylated DNA was detected in leukocyte DNA. In tumor-tissue of 18 patients with NSCLC at least 4 of the 5 selected genes were hypermethylated, while none of the lung tissues from the 10 non-cancer individuals revealed hypermethylation in these 4 genes. Results on a larger cohort of tissues as well as plasma-derived cfDNA are pending and will be presented.

Conclusion

We designed a MRSE-ddPCR assay that specifically discriminates between tissues from patients with NSCLC and healthy individuals. The validation of the MRSE-ddPCR assay on plasma-derived ctDNA from patients with lung cancer versus non-cancer plasmas and its use as a quantitative tool for monitoring therapy response in follow-up plasma samples, is ongoing.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.