Detection of met exon 14 skipping mutation by maldi-tof based liquid biopsy in non-small cell lung cancer

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Abstract

Background & Objectives

Skipping of exon 14 in the MET-gene due to differential splicing causes MET over-accumulation and oncogenic signaling, correlated with poor prognosis in non-small cell lung cancers (NSCLC). In this study we assessed the possibility of detecting circulating MET mRNA in blood of NSCLC patients as a novel liquid biopsy assay.

Methods

Since MET exon 14 skipping (METΔex14) can be caused by >500 different mutations, which are challenging to screen on DNA level due to their low mutation allele frequency, we decided to use circulating METΔex14 mRNA. A PCR-based custom assay on the Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF) based MassARRAY® System was used to detect differentially spliced MET RNA from METΔex14 mutations in plasma samples. As naked mRNA is rapidly degraded in blood by extracellular RNases, a model system based on cell-free medium of H596 cell line was developed to analytically verify the assay performance.

Results

Cell-free medium after 2 days of cultivation of a NSC H596 cell line containing secreted exosome-protected and protein-bound mRNA was spiked into blood from healthy donors in different concentrations (containing 100, 50, 10, 5, and 2.5 RNA input copies on average). The effects of storage time at room temperature and repeated freezing/thawing cycles on cell-free RNA (cfRNA) stability were investigated. METΔex14 fusion RNA could successfully be detected in all dilutions and all replicates.

Conclusion

We have developed and analytically validated a novel liquid biopsy assay that is capable of detecting METΔex14 fusion cfRNA in our model system down to 2.5 input copies. To further assess whether our assay might provide an alternative diagnostic method for MET-tyrosine kinase inhibitor treatment in absence of tumor tissue, we invite future collaborations to provide blood samples from patients with known METΔex14 genotype to us.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.