Copy number alteration signal from plasma DNA LINE-1 targeted bisulfite sequencing: a new non-invasive multi-cancer detection marker

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Abstract

Detection of circulating tumor DNA (ctDNA) allows non-invasive retrieval of tumor molecular profiles and disease monitoring. However, detecting small fractions of ctDNA shed when the tumor burden is reduced remains a challenge. It is therefore necessary to develop more sensitive biomarkers.

Our team has recently developed machine learning classifiers, based on cancer-associated LINE-1 hypomethylation, to discriminate with great accuracy cancer patients and healthy individuals from cell-free DNA (DIAMOND assay, Michel et al., medRxiv 2023). We observed that hypomethylation levels were similar in primary and metastatic tumor tissues, confirming that alteration of LINE-1 methylation is an early event in carcinogenesis. However, we detected higher hypomethylation levels in metastatic compared to localized stages in plasma, reflecting the fraction of circulating tumor DNA, which correlates with the tumor burden. It has also been shown that copy number alteration (CNA) detected from cell-free DNA strongly correlates with tumor burden and can be inferred from PCR-based targeted LINE-1 sequencing.

I investigated whether CNA signal could be extracted from the DIAMOND data and used as a multicancer biomarker.I developed a pipeline quantifying CNA on a genome-wide scale (Z score). This approach was validated on cell lines that were also characterized with CGH arrays, a classical method for CNA analysis. In plasma samples, high Z scores were observed specifically in cancer samples. Z score and hypomethylation levels were only moderately statistically correlated, demonstrating that these are partially independent markers providing

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distinct signals. Therefore, I created a 2-step classifier using the DNA methylation model and the CNA-scores, which improved cancer detection, particularly for localized breast cancer.

Altogether, our results support the idea that LINE-1-based CNA, along with hypomethylation, can be used as a non-invasive multi-cancer biomarker.

Keywords: L1 elements, Copy number alteration, ctDNA, Multi, cancer, biomarker, Liquid biopsy, Machine learning