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Brief Communication

Ultrashort plasma cell-free DNA: a novel non-invasive maker for cancer diagnostics

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Abstract: Recent studies have shown that ultrashort plasma cell-free DNA (uscfDNA), a novel type of cfDNA fragment approximately 50 nucleotides long, differs from conventional monocyte-derived cfDNA in several aspects, including specific extraction requirements and a higher incidence of tumor-specific genetic alterations. uscfDNA shows promise in enhancing liquid biopsy sensitivity for cancer detection, with distinct methylation profiles observed in cancer patients. These findings suggest uscfDNA analysis could significantly improve non-invasive cancer diagnostics, offering new avenues for early detection and personalized medicine strategies.

Keywords: uscfDNA, mncfDNA, plasma

Plasma cell-free DNA refers to DNA fragments, typically around 167 base pairs (bp) in length, known as mononucleosomal cell-free DNA (mncfDNA), released into the bloodstream from cells undergoing apoptosis or necrosis. These fragments have proven valuable as markers for various medical conditions, including cancer detection [1] and non-invasive prenatal testing (NIPT) [2]. Recently, a novel class of cfDNA, termed ultrashort plasma cfDNA, with lengths of approximately 50 nucleotides, has been identified, offering unique biological markers that could enhance the sensitivity and specificity of liquid biopsies. These ultrashort fragments exhibit distinct characteristics from mncfDNA, existing at sizes below nucleosomal DNA and likely being single-stranded or partially single-stranded. Consequently, conventional DNA extraction or next-generation sequencing (NGS) methods are not compatible with ultrashort single-stranded cfDNA (uscfDNA).

Hudecova et al. (2022) shed new light on the landscape of plasma cfDNA, particularly focusing on the characteristics and diagnostic potential of ultrashort cfDNA [3]. This study introduces a novel approach using high-affinity magnetic bead-based DNA extraction and single-stranded DNA sequencing library preparation (MB-ssDNA), uncovering a population of cfDNA fragments centered around ~50 bp. The findings suggest that these ultrashort cfDNA fragments are more abundant in healthy individuals than in cancer patients and are potentially linked to accessible chromatin regions with the capacity to form G-quadruplex structures. Additionally, the study demonstrated that ultrashort cfDNA fragments exhibit a higher proportion of tumor-specific genetic alterations, making them a promising target for non-invasive cancer detection. The stability of these fragments in circulation further enhances their viability as diagnostic markers.

Cheng et al. (2023) developed a novel method called broad-range cfDNA sequencing (BRcfDNA-Seq) to detect uscfDNA in lung cancer plasma, and identified unique functional elements and structural motifs in that are significantly associated with lung cancer [4]. The study reported a 45.2-fold increase in promoter, intronic, and exonic peaks for uscfDNA compared to mncfDNA in noncancer subjects. Furthermore, uscfDNA exhibited distinct end-motif frequency profiles and a notable increase in G-Quadruplex structures, which are indicative of cancerous alterations. These findings suggest that uscfDNA can serve as a highly

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specific marker for lung cancer, enhancing early detection capabilities.

Cheng et al. (2024) introduced a novel 5mCAdpBS-Seq workflow, which effectively captures the methylation profile of uscfDNA, a task previously hindered by the limitations of conventional bisulfite sequencing (BS-Seq). By employing pre-methylated adapters, the researchers elegantly circumvent the biases introduced by bisulfite-induced degradation, offering a more accurate profile of the uscfDNA methylome [5]. This study demonstrated lower methylation levels for uscfDNA compared to mncfDNA, only 41.4 ± 5% of uscfDNA CpG sites were found within mncfDNA, with most sites unique to mncfDNA. Methylation tissue-of-origin analysis indicated that uscfDNA is mainly derived from peripheral blood cells. Additionally, non-small cell lung cancer patients exhibited unique methylation signatures in their ultrashort cfDNA, which were absent in healthy controls. This innovative approach holds promise for advancing our understanding of uscfDNA methylation characteristics, paving the way for improved diagnostic tools and personalized medicine strategies.

Disclosure of conflict of interest

None.

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