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Brief Communication

Cell-free DNA fragmentomics: a universal framework for early cancer detection and monitoring

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Abstract: Cell-free DNA (cfDNA) fragmentomics has emerged as a powerful and noninvasive approach for cancer detection, characterization, and monitoring. By analyzing genome-wide fragmentation patterns - including fragment length distributions, end motifs, nucleosome footprints, and copy number variations - cfDNA fragmentomics provides high-resolution insights into tumor-specific biological signals even at low tumor burden. This technology offers advantages over conventional mutation-based assays by capturing aggregate structural and epigenomic alterations without requiring prior knowledge of driver mutations. In non-small cell lung cancer (NSCLC), cfDNA fragmentomics enables early detection, discrimination of malignant pulmonary nodules, and post-surgical monitoring of minimal residual disease. Recent studies have demonstrated that fragmentomic risk scores can accurately stratify recurrence risk and improve prognostic sensitivity beyond traditional genomic assays. In hepatocellular carcinoma (HCC), integration of fragment size selection, CNV profiling, and end-motif analysis has led to high-performing models for early diagnosis, particularly in high-risk populations. Moreover, cfDNA fragmentomics has proven effective in detecting malignant transformation in patients with neurofibromatosis-associated peripheral nerve sheath tumors, distinguishing benign from premalignant or malignant lesions with high precision. Expanding beyond these major cancers, fragmentomic approaches have demonstrated diagnostic potential in gastric, urological, hematologic, and pediatric malignancies. Notably, the DELFI-TF (DNA Evaluation of Fragments for early Interception-Tumor Fraction) framework has shown prognostic relevance by correlating pre-treatment cfDNA features with survival outcomes in colorectal and lung cancer patients, outperforming conventional imaging. All of these results highlight the translational importance of cfDNA fragmentomics as a cutting-edge precision oncology tool. Its continued integration into clinical workflows may redefine early cancer detection, facilitate subtype-specific interventions, and enable real-time, individualized treatment monitoring.

Keywords: Cell-free DNA, fragmentomics, early cancer detection, liquid biopsy

Introduction

Cell-free DNA (cfDNA) fragmentomics analyzes genome-wide fragmentation patterns - such as fragment size distributions, end motifs, nucleosome footprints, and copy-number changes - to detect cancer [1, 2]. Unlike mutation-based assays, fragmentomic biomarkers do not require prior knowledge of tumor mutations and instead exploit aggregate cfDNA signals. For example, cancer patients often exhibit a left-shifted cfDNA size profile, characterized by an increased proportion of short fragments, and distinctive end-motif frequencies [2]. These features correlate with tumor chromatin structure and nuclease activity, allowing inference of tissue-of-origin and tumor burden [3]. Recent studies confirm that cfDNA fragmentomic profiles differ markedly between healthy individuals and cancer patients [4]. Accordingly, fragmentomics has emerged as a powerful noninvasive approach for cancer detection, screening, and monitoring. Here, we briefly review its applications across various tumor types.

Non-small cell lung cancer (NSCLC)

Non-small cell lung cancer (NSCLC), comprising the two major histological subtypes - lung ade-

nocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) - accounts for the majority of lung cancer cases and presents significant genomic and therapeutic heterogeneity [5]. Despite recent progress in targeted therapies and biomarker-driven treatment strategies, early diagnosis remains challenging due to the overlapping clinical manifestations and the complex molecular landscapes of LUAD and LUSC [5]. Conventional diagnostic approaches, including imaging and protein-based markers, are often insufficient for detecting early-stage disease or capturing tumor-specific genomic alterations, particularly in heterogeneous or mixed histological contexts.

To address these limitations, cell-free DNA (cfDNA) fragmentomics has emerged as a promising noninvasive diagnostic strategy. By analyzing genome-wide fragmentation signatures - such as fragment size distributions, end motifs, coverage profiles, and copy-number variations (CNVs) - cfDNA fragmentomics enables the detection of tumor-derived signals from plasma with high sensitivity, even at low tumor burden. This approach holds particular promise for capturing the molecular complexity of NSCLC and overcoming the diagnostic blind spots of traditional methods.

Recent mechanistic studies have further underscored the biological diversity of NSCLC. Distinct regulatory pathways and molecular drivers have been identified, including CD147 post-translational methylation that promotes apoptosis [6], circDONSON-mediated activation of the MAPK pathway [7], and TRIM29-dependent upregulation of SLC7A5 in response to nicotine exposure [8]. These findings highlight the intricate molecular architecture of NSCLC and support the need for advanced diagnostic modalities that can integrate such complexity at the epigenetic and transcriptomic levels - capabilities that cfDNA fragmentomics may offer.

Importantly, NSCLC has been one of the earliest malignancies investigated using cfDNA fragmentomic techniques. Xu et al built a machine-learning model using multiple fragmentomic features (coverage, size, end motifs, etc.) to classify suspicious nodules. The ensemble model maintained robust performance in external validation (AUC=0.860), achieving 89.7% sensitivity [9]. Thus, fragmentomics could reduce unnecessary interventions in CT-screened patients.

Fragmentomics also excels in minimal residual disease (MRD) detection after NSCLC surgery. Wang et al performed whole-genome cfDNA sequencing on postsurgical NSCLC patients and derived fragmentomic risk scores [10]. High-risk fragmentomic profiles conferred 4.6-8.3-fold higher relapse risk, and combining fragmentomics with mutation results yielded 78.3% sensitivity for recurrence (versus 43.5% for mutation-only) [10]. In short, cfDNA fragmentomics showed “great sensitivity” in predicting early-stage NSCLC recurrence and can guide adjuvant therapy [10]. These studies demonstrate that fragmentome-based assays offer accurate early detection and prognosis in lung cancer [9, 10].

Hepatocellular carcinoma (HCC)

Liver cancer is a global killer for which sensitive screening is needed. Recent work has leveraged fragmentomics to detect HCC in high-risk populations. Foda et al applied genome-wide cfDNA fragmentome profiling in cohorts of HCC patients and controls [11]. A machine-learning model integrating multiple fragmentation features achieved 88% sensitivity at 98% specificity in average-risk individuals, and 85% sensitivity at 80% specificity in high-risk (cirrhotic/hepatitis) cohorts [11]. Notably, cfDNA fragmentation patterns closely reflected underlying tumor chromatin structure in HCC, providing biological rationale for detection [11].

In HBV-related HCC, Jin et al found that selecting short cfDNA fragments (<150 bp) greatly enriched tumor-derived DNA and CNV signals [12]. They identified recurrent CNV regions (e.g., chr1p, 4q losses; 1q, 8q gains) and specific 4-mer end motifs (e.g., CCCA, CCTG, CCAG) enriched in HCC cfDNA [12]. Overall, combining CNV markers, fragment-size selection, and end-motif profiling has potential for effective detection of HCC [12]. These innovations have yielded a high-performing, cost-effective cfDNA test for liver cancer [11, 12].

Neurofibromatosis-associated nerve sheath tumors

In cancer predisposition syndromes, fragmentomics can enable early malignant transformation detection. Sundby et al applied cfDNA fragmentomic analysis in NF1 patients with peri-

pheral nerve sheath tumors (PNST) [13]. Using genome-wide fragment length ratios, end-motif patterns, and deconvolved fragment contributions, they could distinguish benign plexiform neurofibromas, atypical neurofibromas, and malignant peripheral nerve sheath tumors [13]. In particular, fragmentomics correctly classified cases that were ambiguous by clinical criteria, pioneering early detection of malignant and premalignant PNST in NF1 [13]. This method distinguishes atypical neurofibromas from benign plexiform neurofibromas and MPNST, enabling more precise clinical diagnosis [13]. These results echo earlier findings that combining fragment-size and CNV analysis can reliably separate benign from malignant lesions [14]. In summary, cfDNA fragmentomics shows great promise for surveillance in tumor-prone patients.

Other cancer types (gastric, urological, lymphoma, pediatric)

Fragmentomics is also being explored across diverse malignancies. In gastroesophageal cancers, multi-dimensional cfDNA assays including fragmentomic features have achieved remarkable accuracy. For example, Yu et al used whole-genome cfDNA sequencing (fragment length, CNVs, nucleosome footprint, SNVs) in early gastric cancer [15]. Their classifier distinguished stage I-II disease with AUROC ~0.96 in both discovery and validation sets [15].

In urological cancers, Chauhan et al analyzed urine cfDNA from bladder, prostate, and renal cancer patients, deriving genome-wide tumor fractions and fragmentomic metrics [16]. A cfDNA model combining fragmentation and CNV features predicted pathologic complete response (pCR) with AUC≈0.89 [16]. In validation, fragmentomic end-motif signatures achieved AUCs ~0.84-0.85 for bladder or prostate cancer classification [16].

In lymphomas, Meriranta et al reported that B-cell lymphomas exhibit nonrandom cfDNA fragmentation patterns that augment ctDNA detection [17]. In pediatric oncology, fragment size analysis combined with CNV profiling enables detection of neuroblastoma and sarcoma, even with low ctDNA burden [13]. Moreover, cfDNA fragmentomics helped distinguish tumor grades in pediatric NF1 patients [13].

Conclusions

Cell-free DNA (cfDNA) fragmentomics is rapidly emerging as a transformative tool in cancer management. By analyzing genome-wide fragmentation patterns - such as fragment length distributions, end motifs, coverage patterns, and copy-number variations (CNVs) - fragmentomics can detect tumor-specific signals even at low tumor fractions. This approach has demonstrated impressive accuracy in early detection, diagnosis, and stratification across various cancers, including lung, liver, gastrointestinal, urological, lymphomas, hereditary tumors, and pediatric malignancies. Beyond detection, cfDNA fragmentomics shows strong potential for monitoring therapy response and disease progression. For instance, the DELFI-TF (DNA Evaluation of Fragments for early Interception-Tumor Fraction) approach utilizes fragmentomic tumor fraction to estimate disease burden. In studies involving colorectal and lung cancer patients, pre-treatment DELFI-TF scores correlated with survival outcomes and outperformed imaging in predicting treatment response. Patients with lower DELFI-TF scores during treatment exhibited significantly longer overall survival compared to those with higher scores. Moreover, DELFI-TF provided prognostic information independent of traditional mutation-based assays, highlighting its utility in real-time disease monitoring [18]. In summary, cfDNA fragmentomics - through innovations in analyzing fragment size, end motifs, and CNVs - is reshaping noninvasive cancer diagnostics. Its applications in sensitive screening and real-time monitoring hold promise for enhancing personalized cancer care and improving patient outcomes.

Disclosure of conflict of interest

None.

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