Chen *et al.*
European Journal of Medical Research (2025) 30:136
https://doi.org/10.1186/s40001-025-02313-z

European Journal of Medical Research

REVIEW

Open Access

Application of plasma cell-free DNA in screening of advanced colorectal adenoma

Bing-Hong Chen[1,2], Hoi-Ioi Ng[1*], Yong Liu[1], Wei Zhang[2] and Gui-Qi Wang[1]

Abstract

Background Currently, due to the invasive nature of colonoscopy and the associated pain, people avoid undergoing the procedure, making it difficult to detect the majority of potential early stage colorectal carcinoma/precancerous lesions or advanced adenoma. Advanced colorectal adenoma is the main precursor to the development of colorectal carcinoma. Therefore, improving advanced colorectal adenoma detection rate can significantly decrease the development and morbidity of colorectal carcinoma. Accordingly, a non-invasive method to screen high-risk people for colonoscopy in clinical practice is urgently needed.

Main text With the development of medical technology, screening methods for colorectal carcinoma are emerging rapidly, and diverse non-invasive methods are being developed. Cell-free DNA (cfDNA), commonly referred to as liquid biopsy, has promising application prospects as a minimally invasive strategy for early screening of colorectal cancer. CfDNA has already been applied in the field of prenatal diagnosis, advanced carcinoma, and organ transplantation, and the application cfDNA in advanced colorectal adenoma is at the cutting-edge of current research. Thus, this review summarizes the progress in research on different biological characteristics of cfDNA and its utility in the screening of advanced colorectal adenoma, including sizes of cfDNA molecules, end signature of cfDNA (preferred ends, end motifs, jagged ends), nucleosomal footprints, cfDNA topology, cfDNA methylation, and cfDNA integrity.

Conclusions We hope that this review will advance this promising research field.

Keywords Advanced colorectal carcinoma, Cell-free DNA, Non-invasive screening, Liquid biopsy, Biological characteristics

Background

Colorectal cancer (CRC) is a common malignant cancer. According to the latest statistics from the American Cancer Society for 2023, the incidence and mortality rates of CRC among men and women remain the third highest among those of all cancers [1]. Owing to the growing awareness about a healthy lifestyle, advancements in early diagnosis and treatment of CRC, and improvements in treatment methods, the mortality rate of CRC has been decreasing annually [2]. The early diagnosis and treatment of CRC have been the focus of extensive research. Colorectal cancer develops relatively slowly, and the process of developing from polyp to cancer usually takes 5–10 years or even longer (Fig. 1), in which the timely detection and treatment of advanced adenomas, which are now known as precancerous lesions, can effectively reduce the incidence rate of and mortality associated with CRC [3]. Up to now,

*Correspondence:
Hoi-Ioi Ng
15811329134@139.com
[1] Department of Endoscopy, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China
[2] Department of Endoscopy, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen 518116, China

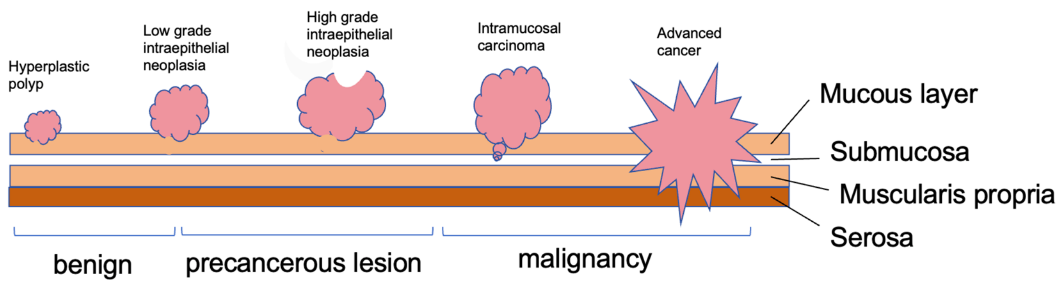

Fig. 1 Colorectal cancer (CRC) development

there are various detection methods for CRC, which are summarized in Table 1. Among them, colonoscopy is the gold standard for screening and diagnosis of CRC [4]. However, because some patients do not want to accept or cannot tolerate invasive procedures, such as colonoscopy [5] and due to certain standards and quality requirements of colonoscopy for endoscopists [6], it is difficult to promote colonoscopy for early cancer screening. Therefore, there is an urgent need to develop more accurate and convenient non-invasive screening methods.

With advancements in medical technology, screening methods for CRC are emerging rapidly, and non-invasive methods are diversifying. Cell-free DNA (cfDNA), also known as DNA that circulates freely in the bloodstream, is derived from a variety of sources, including circulating tumor cells that escape from primary tumors, degraded tumor cell DNA released from primary tumor cells (secondary to tumor cell death), and secreted tumor cell exosomes [6, 7]. cfDNA, which is also used in liquid biopsy, has shown promise as a minimally invasive strategy for early CRC screening [8]. In this review, different detection methods of cfDNA are discussed. The application progress of different biological characteristics of cfDNA in advanced colorectal adenoma is summarized and elaborated, with a focus on the latest research (Table 2).

Different analytical methods for cfDNA

There are several methods for detecting cfDNA. Real-time polymerase chain reaction (PCR)-based assay methods are widely used for cfDNA measurement but are less sensitive for early disease detection. Many PCR-based improved tests have since been developed, Including digital PCR, droplet digital PCR, allele-specific amplification refractory mutation system PCR, allele-specific PCR, beads, emulsion, amplification and magnetics [9–12]. PCR can better detect genetic changes in cfDNA, but only a small number of loci can be analyzed at a time. Next generation sequencing (NGS), however, can simultaneously measure the genetic changes of the whole genome in a single sequencing, and has unique advantages in detecting unknown sequences, unknown mutations, and high-throughput multilocus [13]. The main technologies include tag-amplicon deep sequencing, safe-sequencing system, and personalized profiling by deep sequencing [14, 15]. There are also enzyme-based cfDNA mutation detection methods that can detect mutations in target genes in cfDNA [16]. Different detection methods complement each other in the study of cfDNA.

Fragmentation patterns of cfDNA

The study of patterns of cfDNA fragments, also known as fragmentomics, is currently an area of intense interest in biomarker research, which focuses on sizes, preferred ends, end motifs, jagged ends, and nucleosomal

Table 1 Different detection methods for colorectal cancer

Detection methods	Advantages	Disadvantages
FOBT, FIT	Inexpensive and easy to check	Less sensitive and susceptible to interference
Stool DNA testing	Non-invasive, safe, and easy	Relatively expensive, with uncertain inter-screening periods
Colonoscopy	Intuitive, efficient, "Gold standard"	requires bowel preparation, poor compliance
Computed tomographic colonography	Alternative to colonoscopy	requires bowel preparation, expensive and radiation hazards
cfDNA	Non-invasive, allows real-time detection of comprehensive genomic information about the tumor	Unstable sensitivity, difficult for early disease screening

FOBT fecal occult blood test, *FIT* fecal immunochemical test, *cfDNA* cell-free DNA

Table 2 Different biological characteristics of cfDNA

				Features	Application
Biological characteristics of cfDNA	Fragmentation patterns of cfDNA	Sizes of cfDNA Molecules		Usually small in tumor-derived cfDNA	Identify tumor DNA fragments and improve cancer detection
		End signature of cfDNA	Preferred Ends	A subset of genomic coordinates that are cut preferentially	Ratio of tumor-associated and non-tumor-associated preferred endpoints distinguishes tumor patients from healthy individuals
			End motifs	Several bases of the 5′-end of the sequence of a cfDNA molecular fragment	Excellent predictive performance in early stage cancer
			Jagged ends	Single-stranded DNA at the ends of double-stranded cfDNA	Tumor cfDNA molecules have more jagged ends
		Nucleosomal Footprints		Epigenetic characteristics	Non-invasively identify the tissue origin of cancer cells
		Topology of cfDNA		Different forms of DNA molecules, including circular and linear forms	Distinguish between physical and neoplastic populations
	Detection of cfDNA methylation			A form of chemical modification of cfDNA	High sensitivity and specificity for CRC screening and early detection
	Integrity of cfDNA			The degree of cfDNA fragmentation	High in CRC, a prognostic marker

footprints characterized by the distribution patterns of cfDNA fragments in the genome and topology [17].

1. Sizes of cfDNA molecules

The size distribution of cfDNA is an important biological characteristic for its clinical application, and fragment size can be obtained through single-base resolution sequencing. Existing studies have confirmed that tumor-derived cfDNA molecules in the plasma of cancer patients are usually small [18, 19], and short genes typically prioritize tumor-related copy number aberrations. The size characteristics of tumor-derived cfDNA in the plasma of cancer patients can be used to identify tumor DNA fragments and improve cancer detection [20]. Based on research on the size characteristics of cfDNA, Cristiano et al. [21] developed a method to evaluate the size pattern of cfDNA throughout the entire genome and found that the size spectrum of cancer patients was distorted. Analysis of fragmentation characteristics in 236 patients with cancer and 245 healthy individuals showed that the size spectrum was helpful in identifying a limited number of tissues of cancerous origin in 75% of the cases, which further confirmed that the size of cfDNA provided information related to the origin tissues. In a study on the prediction of advanced adenomas using cfDNA, Peng et al. [22] obtained additional information on a cancer group (advanced adenomas and early CRC) and a healthy control group based on an improved cfDNA fragment size and distribution model, revealing more differences between the two, thus improving the predictive performance of the model. Studying the mechanism underlying differential changes in the cfDNA size spectrum in precancerous lesions, such as advanced colorectal adenomas, is a promising future research direction with great significance.

2. End signature of cfDNA

Preferred ends

A subset of genome coordinates is preferentially cut to form the so-called "preferred end" [23], which was first discovered during the generation of cfDNA in the plasma from pregnant women. The results indicated that 25% of cfDNA fragments were present in at least one peer sharing the same terminal site. Clusters of preferred ends are consistent with the nucleosome pattern in the genome [24], which also depends on tissue specificity. Using the ratio of tumor-related and non-tumor-related preferred terminals, the AUC for distinguishing patients with hepatocellular carcinoma from healthy subjects was 0.88 [24], indicating that the preferred terminal could serve as a biomarker for cancer and facilitate its early detection.

End motifs

End motifs refer to several bases (such as 4-nucleotide motifs) of the 5′-end of the sequence of a cfDNA molecular fragment. The most common end motif, CCCA, in the cfDNA of healthy human subjects was found to be reduced in patients with liver cancer and was found to be prevalent in multiple cancer types, including CRC, further proving its potential clinical application in oncology [25]. Adapted from the end motifs originally reported by Jiang et al. [25], Peng et al. [22] extended the end motifs to 6 bp and detected their frequency. This motif carries more information (patterns of fragment origin sites in the genome), thereby increasing the predictive ability of the end motif for advanced adenomas and early CRC.

Jagged ends

Fragment omics features are largely based on sequencing results generated by double-stranded cfDNA molecules after the completion of DNA terminal repair, where in the 5′ single-stranded protruding end of cfDNA molecules is repaired and filled, while the 3′ single-stranded protruding end is trimmed. Consequently, the presence of single-stranded DNA (also known as jagged ends) at the ends of double-stranded cfDNA has been overlooked. Jiang et al. [26] developed a method to detect the jagged ends of cfDNA molecules by introducing differential methylation signals into the complementary strands of single-stranded cfDNA existing at the 5’ end and found that tumor cfDNA molecules have more jagged ends than the background DNA molecules dominated by hematopoietic DNA in cancer patients.

3. Nucleosomal footprints

Studies have shown that the fragmentation pattern of cfDNA is non-randomly distributed across the genome [27] and that the fragment spectrum of cancer patients is more diverse than that of healthy individuals [21]. Based on these findings, epigenetic characteristics, such as nucleosomal footprint and gene expression, can be inferred from the fragment pattern of cfDNA. CfDNA fragments are enriched in nucleosome sequences, whereas nucleosome footprints are different in cells [28], making it possible to non-invasively identify the tissue origin of cancer cells. Various methods have been developed to characterize the epigenome using cfDNA fragment patterns to determine tissue origin [29, 30]. Tissue origin and footprint analysis of cfDNA is a current research hotspot, which is expected to expand our current understanding of fragment pattern of cfDNA. However, this is still in the exploratory stage and has not been studied in advanced adenomas.

4. Topology of cfDNA

DNA topology refers to different forms of DNA molecules, including circular and linear forms. Previous studies focused on double-stranded linear plasma DNA. With advancements in detection methods, an increasing number of ultralong double-stranded DNA, ultra-short single-stranded cfDNA, and circular cfDNA have been discovered and studied. Mead et al. [31] distinguished between physical and neoplastic populations using an optimal model containing DNA markers, including circular cfDNA. The final detection rate had a positive predictive value of 81.1% for polyps and a negative predictive value of 73.5% for early cancer diagnosis. In-depth research on cfDNA with different fragments and topological structures will help gain a more systematic and comprehensive understanding of cfDNA, providing a foundation for further applications.

In a prospective study, Peng et al. [22] constructed a multi-omics early screening model, based on five machine learning models and five cfDNA fragment omics features for CRC, to distinguish healthy individuals from patients with advanced adenomas/early CRC. The specificity of this model was 94.8%, and the AUC for distinguishing between healthy individuals and those with advanced adenomas/early CRC was 0.988. At a 94.8% specificity, the sensitivities for advanced adenomas and early CRC were 95.7% (95% CI 85.2–99.5%) and 98.0% (95% CI 94.2–99.6%), respectively. Further subgroup analysis showed that the early screening model exhibited high sensitivity for different levels of advanced adenomas and pedunculated/non-pedunculated adenomas. This indicates that the cfDNA fragment omics model is a promising detection and screening method for advanced adenomas and early CRC, and non-invasive clinical screening of CRC is a new and efficient detection method.

Detection of cfDNA methylation

The *SEPT9* gene methylation detection method for plasma cfDNA was the first FDA-approved blood testing method for CRC screening [32], with high sensitivity and specificity for CRC screening and early detection. In 2014, Church et al. [33] prospectively evaluated the accuracy of circulating methylated SEPT9 DNA (mSEPT9) in detecting CRC in the screened populations. This study found that in asymptomatic high-risk populations undergoing screening, blood-based mSEPT9 detection can detect CRC signals in the blood; however, the detection sensitivity for advanced adenomas and early cancers is relatively low. With the advancement of cfDNA research and the development of detection technology, combining multiple biomarkers is an effective strategy to improve the sensitivity of advanced adenoma diagnosis and screening. Various research groups have established and verified non-invasive cfDNA methylation models of multiple cfDNA methylation biomarkers to detect advanced adenoma and early CRC. Zhao et al. [34] reported a new qPCR-based detection method that combined methylated SEPT9 and SDC2 (named the ColorDefense test). Compared to individual detection methods, combined detection has high sensitivity and specificity for detecting advanced adenomas. Gómez et al. [35] found that 11 methylation biomarker models based on the plasma cfDNA could reliably identify patients with advanced adenomas. Mo et al. [36] achieved cfDNA multigene methylation haplotype detection using NGS technology; the detection sensitivity of this method was 79.0% in patients with advanced adenomas, 86.6% in patients with CRC, and 88.1% in the control population. The performance of this method in detecting advanced adenomas was superior to that of fecal immunochemical testing and carcinoembryonic antigen. Multiple studies have reported satisfactory results [37–39]. Methylated cfDNA is a promising biomarker for estimating the prognosis of patients with advanced adenomas and CRC and will possibly be widely used in clinical practice in the future.

Integrity of cfDNA

The integrity of plasma cfDNA has been extensively studied as a diagnostic and prognostic marker in several cancers [40]. CfDNA integrity was calculated as the ratio of the concentration of longer DNA fragments at a specific genetic locus to the concentration of shorter DNA fragments, indicating the degree of cfDNA fragmentation [41]. Compared to healthy controls, many cancer patients have high plasma-free DNA concentrations, which can serve as potential predictive markers [42]. Naoyuki et al. [43] found that the integrity of cfDNA in the plasma of patients with CRC was higher than that in healthy individuals; they used the integrity of cfDNA as a prognostic marker independent of cfDNA. In a study of advanced adenomas, Bedin et al. [44] assessed the presence and integrity of plasma cfDNA in subjects using an ALU sequence-based qPCR method. The amount and pattern of cfDNA differed significantly among the control group, patients with advanced adenomas, and patients with CRC, and increased with tumor occurrence and histopathological grade. The distribution of the cfDNA integrity index was similar between the control group and patients with CRC; however, there was a significant difference between the cfDNA integrity index of patients with adenoma and patients with CRC ($p<0.01$), which confirmed that a strategy based on cfDNA integrity can improve the diagnosis of advanced adenoma and early CRC.

Discussion

Since cfDNA was first found in healthy and diseased individuals, its presence has gradually been discovered in patients with cancer, patients with systemic lupus erythematosus, and organ transplant recipients [45]. Owing to the advancement of cfDNA research and technological progress, cfDNA has great potential for application in non-invasive prenatal diagnosis, single-gene diseases, and cancer screening [46]. Liquid biopsy has been widely used in cancer screening, but it still has limitations in the early screening and diagnosis of CRC. Compared with the cost of fecal immunochemical test or multi-target stool DNA testing, cfDNA screening is more expensive and the sensitivity of screening is not significantly improved [47]. Because the levels of related molecules in the blood of patients with early CRC are very low, the sensitivity requirements for liquid biopsy are very high. Owing to the limitations of the current technology, a shift towards studying other molecular features of cfDNA in the blood (cfDNA fragmentation patterns or cfDNA methylation) to explore the correlation of cfDNA with tumors and improve detection accuracy is currently another approach. Research has shown that detection technology for cfDNA fragment omics features plays an important role in the early diagnosis of CRC. An early screening model for CRC based on the characteristics of plasma-free DNA multi-omics fragments can effectively distinguish among patients with advanced adenomas, patients with CRC, and healthy populations and has potential application value [22]. This provides a good hint to determine the relationship between advanced colorectal adenoma and cfDNA, because many previous studies on early screening or diagnosis of CRC using cfDNA included only healthy subjects and patients with CRC. In fact, CRC development takes a long time. As an important intermediate stage, advanced adenoma has a window of several years to develop into cancer. Therefore, a good early screening model for CRC should include advanced adenomas or "precancerous lesions" as an important part. Although cfDNA fragment omics characteristics provide a very good method for the early screening of CRC using liquid biopsy, research on cfDNA related to CRC screening is particularly challenging for the diagnosis of advanced adenomas. With the deepening of research, an in-depth study of plasma cfDNA fragment genomics will help reveal the origin and fragmentation mechanism of cfDNA in the physiological and pathological processes of diseases and lay the foundation for further exploration and development of potential diagnostic tools based on cfDNA fragment genomics [17, 48]. In addition, an increasing number of studies have focused on exploring detection methods for cfDNA methylation in the blood and early screening of CRC based on the integrity of plasma cfDNA and have achieved satisfactory results. Conversely, Chung et al. integrated cfDNA genomic alterations, aberrant methylation status, and fragmentomic patterns to screen for CRC and showed that this cfDNA blood-based test had 83% sensitivity for CRC, 90% specificity for advanced neoplasia, and only 13% sensitivity for advanced precancerous lesions [49]. The non-invasive detection of precancerous lesions is still a challenge, and further improvement in the sensitivity and specificity of detection techniques and increasing the number of actual tested samples for practical clinical applications remain challenges for technical exploration.

In summary, the application of cfDNA analysis for the clinical diagnosis of advanced colorectal adenomas presents enormous challenges. cfDNA fragments are prone to chemical damage at low concentrations, which results in low clinical sensitivity of the liquid biopsies based on cfDNA for cancer detection [50]. How to utilize multi-omics as a complementary method to identify cancer patients as early as possible and sensitively monitor the dynamic changes of the disease are an urgent problem in liquid biopsy. Combining cfDNA multi-omics, such as fragment omics and epigenetics, will help in improving the accuracy of cfDNA-based detection. It is necessary to develop additional bioinformatic methods and tools to improve the sensitivity of detection and explore the biological characteristics of cfDNA. Overall, plasma cfDNA is a promising strategy for non-invasive detection of advanced colorectal adenomas.

Conclusions

The development of CRC is a comprehensive process involving genetic, epigenetic, and structural modifications, from benign adenoma to invasive cancer. Early detection and complete endoscopic resection at the adenoma stage are the key factors for survival. The non-invasive detection of advanced adenomas based on cfDNA is still evolving, and larger and long-term prospective studies are needed to confirm its clinical application in the prediction of advanced adenomas. As a potential minimally invasive tool in the field of liquid biopsy for advanced colorectal adenomas, plasma cfDNA has extensive and far-reaching research prospects.

Abbreviations

CRC	Colorectal cancer
cfDNA	Cell-free DNA
PCR	Polymerase chain reaction
NGS	Next generation sequencing
mSEPT9	Methylated SEPT9 DNA

Author contributions

Bing-Hong Chen wrote the manuscript; Hoi-Ioi Ng design the study, wrote and edit the manuscript; Yong Liu and Wei Zhang performed the literature research, Gui-Qi Wang provided financial and clinical support.

Funding

This work was supported by CAMS Innovation Fund for Medical Sciences [Grant numbers 2021-1-I2M-061, 2021-1-I2M-010] and Beijing Hope Run Special Fund of Cancer Foundation of China [LC2022B35].

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 16 June 2024 Accepted: 20 January 2025
Published online: 25 February 2025

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics 2023. CA Cancer J Clin. 2023;73(1):17–48.
2. Global, regional, and national burden of colorectal cancer and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The lancet Gastroenterology & hepatology. 7(7): 627–647.
3. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology. 2007;50(1):113–30.
4. Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, Shi W, Bond JH, Schapiro M, Panish JF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. N Engl J Med. 2012;366(8):687–96.
5. Sly JR, Edwards T, Shelton RC, Jandorf L. Identifying barriers to colonoscopy screening for nonadherent African American participants in a patient navigation intervention. Health Educ Behav Off Publ Soc Publ Health Educ. 2013;40(4):449–57.
6. Lhewa DY, Strate LL. Pros and cons of colonoscopy in management of acute lower gastrointestinal bleeding. World J Gastroenterol. 2012;18(11):1185–90.
7. Overman MJ, Modak J, Kopetz S, Murthy R, Yao JC, Hicks ME, Abbruzzese JL, Tam AL. Use of research biopsies in clinical trials: are risks and benefits adequately discussed? J Clin Oncol Off J Am Soc Clin Oncol. 2013;31(1):17–22.
8. Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: current technology and clinical applications. J Hematol Oncol. 2022;15(1):131.
9. Zhang BO, Xu CW, Shao Y, Wang HT, Wu YF, Song YY, Li XB, Zhang Z, Wang WJ, Li LQ, et al. Comparison of droplet digital PCR and conventional quantitative PCR for measuring EGFR gene mutation. Exp Ther Med. 2015;9(4):1383–8.
10. Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci USA. 2011;108(23):9530–5.
11. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, Thornton K, Agrawal N, Sokoll L, Szabo SA, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008;14(9):985–90.
12. Hindson CM, Chevillet JR, Briggs HA, Gallichotte EN, Ruf IK, Hindson BJ, Vessella RL, Tewari M. Absolute quantification by droplet digital PCR versus analog real-time PCR. Nat Methods. 2013;10(10):1003–5.
13. Deveson IW, Gong B, Lai K, LoCoco JS, Richmond TA, Schageman J, Zhang Z, Novoradovskaya N, Willey JC, Jones W, et al. Evaluating the analytical

validity of circulating tumor DNA sequencing assays for precision oncology. Nat Biotechnol. 2021;39(9):1115–28.
14. Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DW, Kaper F, Dawson SJ, Piskorz AM, Jimenez-Linan M, Bentley D, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Trans Med. 2012;4(136):136ra168.
15. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, Liu CL, Neal JW, Wakelee HA, Merritt RE, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014;20(5):548–54.
16. Zhang W, Zhao S, Xie Z, Chen S, Huang Y, Zhao Z, Yi G. The fluorescence amplification strategy based on 3D DNA walker and CRISPR/Cas12a for the rapid detection of BRAF V600E. Anal Sci Int J Japan Soc Anal Chem. 2022;38(8):1057–66.
17. Ding SC, Lo YMD. Cell-Free DNA Fragmentomics in Liquid Biopsy. Diagnostics. 2022. https://doi.org/10.3390/diagnostics12040978.
18. Mouliere F, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, Mair R, Goranova T, Marass F, Heider K, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Trans Med. 2018. https://doi.org/10.1126/scitranslmed.aat4921.
19. Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, Diaz LA Jr, Goodman SN, David KA, Juhl H, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci USA. 2005;102(45):16368–73.
20. Jiang P, Chan CW, Chan KC, Cheng SH, Wong J, Wong VW, Wong GL, Chan SL, Mok TS, Chan HL, et al. Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. Proc Natl Acad Sci USA. 2015;112(11):E1317-1325.
21. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, Jensen S, Medina JE, Hruban C, White JR, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature. 2019;570(7761):385–9.
22. Ma X, Chen Y, Tang W, Bao H, Mo S, Liu R, Wu S, Bao H, Li Y, Zhang L, et al. Multi-dimensional fragmentomic assay for ultrasensitive early detection of colorectal advanced adenoma and adenocarcinoma. J Hematol Oncol. 2021;14(1):175.
23. Chan KC, Jiang P, Sun K, Cheng YK, Tong YK, Cheng SH, Wong AI, Hudecova I, Leung TY, Chiu RW, et al. Second generation noninvasive fetal genome analysis reveals de novo mutations, single-base parental inheritance, and preferred DNA ends. Proc Natl Acad Sci USA. 2016;113(50):E8159-e8168.
24. Jiang P, Sun K, Tong YK, Cheng SH, Cheng THT, Heung MMS, Wong J, Wong VWS, Chan HLY, Chan KCA, et al. Preferred end coordinates and somatic variants as signatures of circulating tumor DNA associated with hepatocellular carcinoma. Proc Natl Acad Sci USA. 2018;115(46):E10925-e10933.
25. Jiang P, Sun K, Peng W, Cheng SH, Ni M, Yeung PC, Heung MMS, Xie T, Shang H, Zhou Z, et al. Plasma DNA end-motif profiling as a fragmentomic marker in cancer, pregnancy, and transplantation. Cancer Discov. 2020;10(5):664–73.
26. Jiang P, Xie T, Ding SC, Zhou Z, Cheng SH, Chan RWY, Lee WS, Peng W, Wong J, Wong VWS, et al. Detection and characterization of jagged ends of double-stranded DNA in plasma. Genome Res. 2020;30(8):1144–53.
27. Ivanov M, Baranova A, Butler T, Spellman P, Mileyko V. Non-random fragmentation patterns in circulating cell-free DNA reflect epigenetic regulation. BMC genomics. 2015. https://doi.org/10.1186/1471-2164-16-S13-S1.
28. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci USA. 2008;105(42):16266–71.
29. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA Comprises an In Vivo Nucleosome Footprint that Informs Its Tissues-Of-Origin. Cell. 2016;164(1–2):57–68.
30. Sun K, Jiang P, Cheng SH, Cheng THT, Wong J, Wong VWS, Ng SSM, Ma BBY, Leung TY, Chan SL, et al. Orientation-aware plasma cell-free DNA fragmentation analysis in open chromatin regions informs tissue of origin. Genome Res. 2019;29(3):418–27.
31. Mead R, Duku M, Bhandari P, Cree IA. Circulating tumour markers can define patients with normal colons, benign polyps, and cancers. Br J Cancer. 2011;105(2):239–45.
32. Song L, Jia J, Peng X, Xiao W, Li Y. The performance of the SEPT9 gene methylation assay and a comparison with other CRC screening tests: A meta-analysis. Sci Rep. 2017;7(1):3032.
33. Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, Castaños-Vélez E, Blumenstein BA, Rösch T, Osborn N, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. Gut. 2014;63(2):317–25.
34. Zhao G, Li H, Yang Z, Wang Z, Xu M, Xiong S, Li S, Wu X, Liu X, Wang Z, et al. Multiplex methylated DNA testing in plasma with high sensitivity and specificity for colorectal cancer screening. Cancer Med. 2019;8(12):5619–28.
35. Gallardo-Gómez M, Moran S, Páez de la Cadena M, Martínez-Zorzano VS, Rodríguez-Berrocal FJ, Rodríguez-Girondo M, Esteller M, Cubiella J, Bujanda L, Castells A, et al. A new approach to epigenome-wide discovery of non-invasive methylation biomarkers for colorectal cancer screening in circulating cell-free DNA using pooled samples. Clin Epigenet. 2018;10:53.
36. Mo S, Dai W, Wang H, Lan X, Ma C, Su Z, Xiang W, Han L, Luo W, Zhang L, et al. Early detection and prognosis prediction for colorectal cancer by circulating tumour DNA methylation haplotypes: a multicentre cohort study. EClinicalMedicine. 2023;55: 101717.
37. Wu X, Zhang Y, Hu T, He X, Zou Y, Deng Q, Ke J, Lian L, He X, Zhao D, et al. A novel cell-free DNA methylation-based model improves the early detection of colorectal cancer. Mol Oncol. 2021;15(10):2702–14.
38. Sui J, Wu X, Wang C, Wang G, Li C, Zhao J, Zhang Y, Xiang J, Xu Y, Nian W, et al. Discovery and validation of methylation signatures in blood-based circulating tumor cell-free DNA in early detection of colorectal carcinoma: a case-control study. Clin Epigenetics. 2021;13(1):26.
39. Wang W, Zhang X, Zhu X, Cui W, Ye D, Tong G, Huang D, Zhou J, Lai X, Yan G, et al. Seven DNA methylation biomarker prediction models for monitoring the malignant progression from advanced adenoma to colorectal cancer. Front Oncol. 2022;12: 827811.
40. Wang BG, Huang HY, Chen YC, Bristow RE, Kassauei K, Cheng CC, Roden R, Sokoll LJ, Chan DW, Shih Ie M. Increased plasma DNA integrity in cancer patients. Can Res. 2003;63(14):3966–8.
41. Zhu F, Ma J, Ru D, Wu N, Zhang Y, Li H, Liu X, Li J, Zhang H, Xu Y, et al. Plasma DNA integrity as a prognostic biomarker for colorectal cancer chemotherapy. J Oncol. 2021;2021:5569783.
42. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol Off J Am Soc Clin Oncol. 2014;32(6):579–86.
43. Umetani N, Kim J, Hiramatsu S, Reber HA, Hines OJ, Bilchik AJ, Hoon DS. Increased integrity of free circulating DNA in sera of patients with colorectal or periampullary cancer: direct quantitative PCR for ALU repeats. Clin Chem. 2006;52(6):1062–9.
44. Bedin C, Enzo MV, Del Bianco P, Pucciarelli S, Nitti D, Agostini M. Diagnostic and prognostic role of cell-free DNA testing for colorectal cancer patients. Int J Cancer. 2017;140(8):1888–98.
45. Lo YM, Tein MS, Pang CC, Yeung CK, Tong KL, Hjelm NM. Presence of donor-specific DNA in plasma of kidney and liver-transplant recipients. Lancet. 1998;351(9112):1329–30.
46. Zviran A, Schulman RC, Shah M, Hill STK, Deochand S, Khamnei CC, Maloney D, Patel K, Liao W, Widman AJ, et al. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. Nat Med. 2020;26(7):1114–24.
47. Ladabaum U, Mannalithara A, Weng Y, Schoen RE, Dominitz JA, Desai M, Lieberman D. Comparative effectiveness and cost-effectiveness of colorectal cancer screening with blood-based biomarkers (Liquid Biopsy) vs fecal tests or colonoscopy. Gastroenterology. 2024;167(2):378–91.
48. Qi T, Pan M, Shi H, Wang L, Bai Y, Ge Q. Cell-free DNA fragmentomics: the novel promising biomarker. Int J Mol Sci. 2023;24(2):1503.
49. Chung DC, Gray DM 2nd, Singh H, Issaka RB, Raymond VM, Eagle C, Hu S, Chudova DI, Talasaz A, Greenson JK, et al. A cell-free DNA blood-based test for colorectal cancer screening. N Engl J Med. 2024;390(11):973–83.
50. Song P, Wu LR, Yan YH, Zhang JX, Chu T, Kwong LN, Patel AA, Zhang DY. Limitations and opportunities of technologies for the analysis of cell-free DNA in cancer diagnostics. Nature Biomed Eng. 2022;6(3):232–45.

Publisher's Note