Am J Clin Exp Immunol 2025;14(5):262-266
www.ajcei.us /ISSN:2164-7712/AJCEI0166683

Original Article

Cell-free DNA fragmentomics as a non-invasive tool for predicting pathologic response in colorectal cancer

Wenshi Hu[1*], Guoyan Li[2*], Wanyu Dong[1], Yaojuan Lu[1,3,4], Zhou Li[5,6], Qiping Zheng[1,3,4,7]

[1]Department of Hematological Laboratory Science, Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, Jiangsu, China; [2]Department of Anesthesiology, Dongzhimen Hospital Beijing University of Chinese Medicine, No. 5 Haiyuncang, Dongcheng District, Beijing 100700, China; [3]Shenzhen International Institute for Biomedical Research, Shenzhen 518110, Guangdong, China; [4]Shenzhen Walgenron Bio-Pharm Co., Ltd., Shenzhen 518118, Guangdong, China; [5]Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing 100084, China; [6]School of Biomedical Engineering, Tsinghua University, Beijing 100084, China; [7]The Molecular Oncology Laboratory, Department of Orthopedic Surgery and Rehabilitation Medicine, The University of Chicago Medical Center, Chicago, Illinois 60637, USA. [*]Equal contributors.

Received June 10, 2025; Accepted October 9, 2025; Epub October 25, 2025; Published October 30, 2025

Abstract: Despite advances in screening and therapy, colorectal cancer (CRC) remains a leading cause of cancer-related mortality worldwide, underscoring the need for early detection and for predicting treatment efficacy. This review highlights circulating cell-free DNA (cfDNA) fragmentomics as a promising non-invasive approach for tumor detection and disease monitoring. We focus on fragmentomic features - such as fragment size distributions, fragment-end motifs, and epigenetic signals - which, when integrated into machine-learning models, have shown strong performance in distinguishing patients with CRC from healthy controls. Emerging evidence indicates that, these signatures may support early-stage detection, track disease progression, and predict pathologic complete response (pCR), thereby enabling more personalized treatment strategies. We also discuss the potential role of fragmentomics in non-operative management, including "watch-and-wait" approaches. However, important gaps remain in clinical translation; prospective trials and standardized assays/analysis pipelines are required to validate these findings and define their real-world utility.

Keywords: Cell-free DNA, fragmentomics, colorectal cancer, non-invasive

Introduction

Colorectal cancer (CRC) remains a major global health burden given its high incidence and mortality, often related to delayed diagnosis and the complex biology of the tumor microenvironment. Despite Despite advances in endoscopy and imaging that have improved CRC management, significant challenges persist in early detection, accurate staging, and prediction of treatment response [1, 2]. Emerging evidence also Emerging evidence also highlights the interplay among tumor-associated macrophages, stress-response signaling pathways, and cytokine networks in CRC progression and immune evasion, Together, these factors complicate both diagnosis and therapy and underscore the need for sensitive, non-invasive methods to evaluate tumor burden and microenvironmental dynamics [3]. To address this gap, cfDNA fragmentomics has emerged as a promising modality for real-time tumor surveillance, providing a viable route to surmount these obstacles.

To meet this need, cfDNA fragmentomics has emerged as a promising molecular approach for detecting cancer-specific features-including fragment size distributions, DNA end motifs, and epigenetic signatures. Unlike traditional cfDNA assays that focus primarily on concentration levels or mutation hotspots, fragmentomics captures broader genomic alterations, making it particularly valuable for colorectal cancer (CRC), whose biology is influenced by processes such as angiogenesis, hypoxia, and cancer stem cell dynamics [2, 4, 5]. Incorporating cfDNA fragmentomic profiles into diag-

https://doi.org/10.62347/YSQL3793

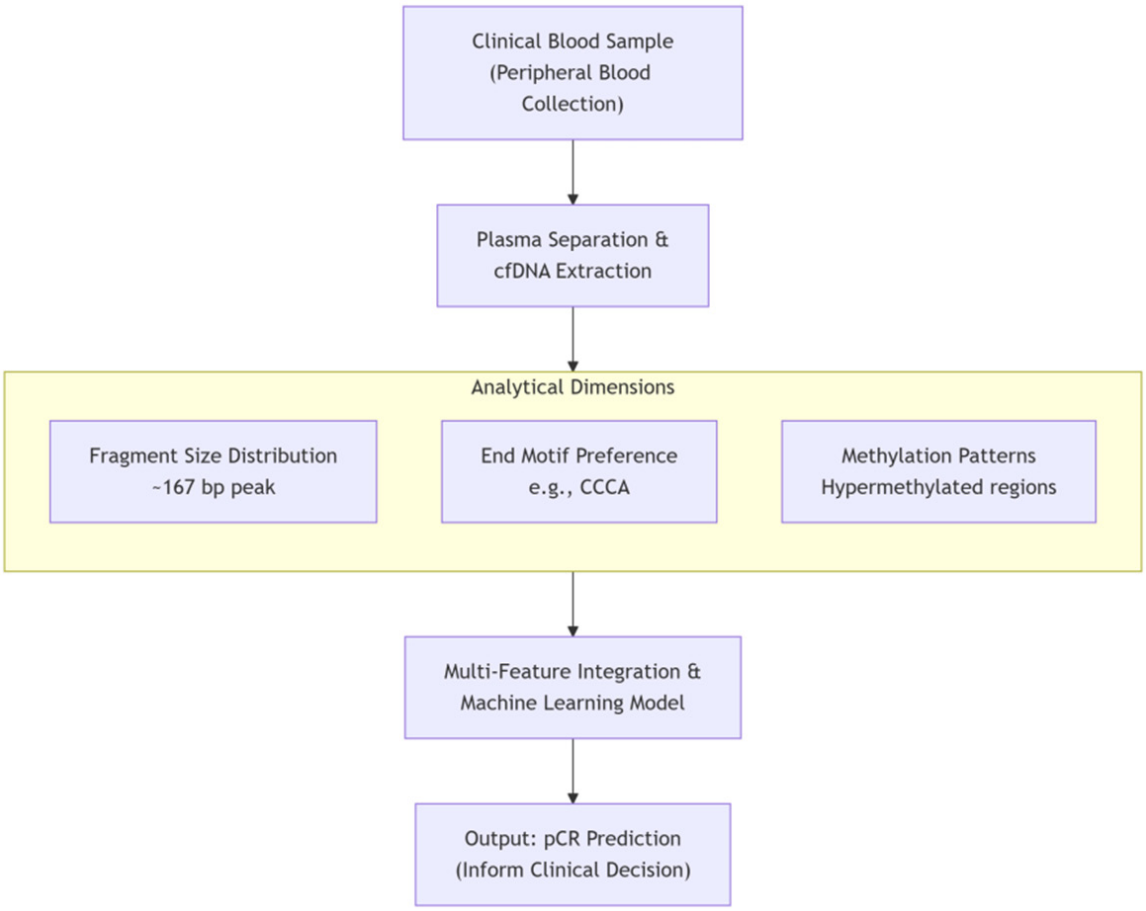

Figure 1. Schematic workflow of predicting pathological complete response (pCR) using cfDNA fragmentomics in colorectal cancer.

nostic workflows could enhance early detection, refine response prediction, and improve staging, ultimately informing precision medicine strategies and non-operative treatments such as the "watch-and-wait" approach in rectal cancer patients who achieve pCR. A potential workflow for using cfDNA fragmentomics to predict treatment response is summarized in **Figure 1**.

cfDNA concentration correlates with tumor burden

Multiple studies demonstrate that cfDNA levels increase with disease severity in CRC. Patients with stage IV CRC show significantly higher cfDNA concentrations than those with earlier-stage disease or healthy controls ($P = 0.049$) [6]. Using simple fluorescence-based cfDNA quantification, Bosque et al. effectively distinguished metastatic CRC from localized diseases and from healthy individuals [5]. Beyond stage correlation, cfDNA levels track treatment response: they typically spike immediately after surgery and decline within three months post-resection, a pattern also reported by Zhong et al. [6]. This dynamic aligns with clinicopathologic markers-higher cfDNA concentration and integrity index are associated with advanced TNM stage and elevated CEA, with both measures decreasing after therapy [7]. Collectively, these findings indicate that effective treatment-surgery-surgery or chemoradiation-should reduce tumor-derived cfDNA in plasma [8].

Fragmentomics enhances detection

Beyond cfDNA quantity, fragmentation patterns provide superior diagnostic value. Cao et al. developed a machine-learning model based on five fragmentomic features - including fragment size distributions and end-motif signatures - that achieved 98% specificity and 94.9% sen-

sitivity (AUC = 0.986) for early-stage CRC, with sensitivity increasing alongside disease progression [9]. Using a complementary strategy, Zhang et al. performed genome-wide cfDNA methylation profiling to capture epigenetic fragmentomic changes [10]. Methylation-based fragmentomics has identified hypermethylated regions in genes such as *PRDM14* and *TMEM132E* when incorporated into logistic models, these methylation markers augmented size-based features, underscoring the advantage of multi-dimensional cfDNA analyses over single-parameter tests [11]. The strength of fragmentomics lies in detecting global alterations that emerge early in tumorigenesis-an asset for early diagnosis. Nevertheless, translating these high-performance models into routine remains challenging due to limited standardization of sequencing protocols, bioinformatic pipelines, and machine-learning methods across laboratories, as well as uncertainties about generalizability from narrowly sampled cohorts; broader validation in diverse, multi-center populations is needed [12].

cfDNA genomics confirms tumor content

Whole-exome sequencing (WES) of cfDNA yields clinically relevant insights into tumor mutations and mechanisms of drug resistance. In CRC patients receiving EGFR-targeted therapy, Lee et al. applied cfDNA WES to track emergent variants-such as ADAMTS20 p.S1597P and TTN p.R7415H-alongside recurrent alterations in canonical CRC genes (*APC*, *TP53*, *KRAS*, and *SMAD4*) [13]. Although mutation-based cfDNA assays can lose sensitivity at low tumor burden, integrating genomic profiling with fragmentomics features substantially enhances the detection of minimal residual disease (MRD) [14, 15].

Analytic simplicity of cfDNA fragmentomics

Notably, advanced sequencing is not always necessary for cfDNA fragmentomics. S Several studies show that simple laboratory methods - such as electrophoresis and fluorometric quantification - can deliver reliable diagnostic performance [5]. Unlike customized ctDNA assays that reuire prior tumor genotyping, fragmentomics can be implemented without tumor tissue, because it targets general features of tumor DNA shedding. Cost-effective, scalable techniques-including fragment length analysis and methylated DNA immunoprecipitation sequencing (MeDIP-seq)-have demonstrated clinical utility [10]. Collectively, these findings indicate that fragmentomics can be both powerful and practical - yielding actionable information with less technical complexity. Nonetheless, a one-size-fits-all strategy is unlikely; selecting between sophisticated next-generation sequencing (NGS) and simpler fluorometric approaches should be guided by clinical context, balancing the need for multidimensional data against cost and turnaround time [16].

Conclusion

The multidimensional profile of cfDNA-including concentration, fragmentation patterns, and epigenetic markers-provides a robust framework for predicting treatment response in colorectal cancer. Multifeature fragmentomic models have demonstrated high accuracy in distinguishing CRC from healthy controls, with utility even at low tumor burden [9]. In the neoadjuvant setting, these approaches could noninvasively predict pathological complete response, supporting a safe “watch-and-wait” strategy. This paradigm embodies personalized medicine by enabling tailored tumor monitoring that extends beyond the capabilities of conventional imaging. Looking ahead, priorities include rigorous validation of cfDNA fragmentomic biomarkers for pCR and their integration into prospectiveclinical trials, with the broader goal of advancing organ-preserving therapies and improving patient outcomes in CRC.

Disclosure of conflict of interest

None.

Address correspondence to: Yaojuan Lu, Department of Hematology and Hematological Laboratory Science, School of Medicine, Jiangsu University, Zhenjiang 212013, Jiangsu, China. E-mail: luyaojuan191@outlook.com; Zhou Li, Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing 100084, China. E-mail: li_zhou@tsinghua.edu.cn; Qiping Zheng, Department of Hematology and Hematological Laboratory Science, School of Medicine, Jiangsu University, Zhenjiang 212013, Jiangsu, China; The Molecular Oncology Laboratory, Department of Orthopedic Surgery and Rehabilitation Medicine, The University of Chicago Medical Center, Chicago, Illinois 60637, USA. E-mail: qp_zheng@hotmail.com

References

[1] Liu Z, Jian C, Yuan W, Jia G, Cheng D, Liu Y, Zhang Y, Zhang B, Zhou Z and Zhao G. Epinephrine promotes tumor progression and M2 polarization of tumor-associated macrophages by regulating the TRIM2-NF-κB pathway in colorectal cancer cells. Genes Dis 2024; 11: 101092.

[2] Xie Z, Zheng G, Niu L, Du K, Li R, Dan H, Duan L, Wu H, Ren G, Dou X, Dai S, Feng F, Zhang J and Zheng J. SPP1 (+) macrophages in colorectal cancer: markers of malignancy and promising therapeutic targets. Genes Dis 2025; 12: 101340.

[3] Heitzer E, Haque IS, Roberts CES and Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019; 20: 71-88.

[4] Jamshidi A, Liu MC, Klein EA, Venn O, Hubbell E, Beausang JF, Gross S, Melton C, Fields AP, Liu Q, Zhang N, Fung ET, Kurtzman KN, Amini H, Betts C, Civello D, Freese P, Calef R, Davydov K, Fayzullina S, Hou C, Jiang R, Jung B, Tang S, Demas V, Newman J, Sakarya O, Scott E, Shenoy A, Shojaee S, Steffen KK, Nicula V, Chien TC, Bagaria S, Hunkapiller N, Desai M, Dong Z, Richards DA, Yeatman TJ, Cohn AL, Thiel DD, Berry DA, Tummala MK, McIntyre K, Sekeres MA, Bryce A, Aravanis AM, Seiden MV and Swanton C. Evaluation of cell-free DNA approaches for multi-cancer early detection. Cancer Cell 2022; 40: 1537-1549, e1512.

[5] Bosque J, Guirao C, Ferrández A, Suarez N, Castillejo MI, Anguita D, Pamies M, Moya A, Soto JL and Gallego Plazas J. Cell-free circulating tumor DNA in colorectal cancer: a proof of concept with simplified methodology. Clin Transl Oncol 2022; 24: 1924-1931.

[6] Pu W, Xiao L, Zhou C, Zhong F, Wu Y, Gong W, Lv X, Zhuang Z, Wang H, Bian H, Wang J, Zhang R, Li K and Xing C. Cancer stage-dependent alterations in cell-free DNA in patients with colorectal cancer. J BUON 2021; 26: 109-115.

[7] Zhong Y, Zhou Q, Zhang Y, Zhou S, Zhang G, Jiang C, Zhang Z, Zhang X, Xu J, Jin C, Cao L and Chen L. Cell-free DNA as a biomarker for colorectal cancer: a retrospective analysis in patients before and after surgery. Cell Mol Biol (Noisy-le-grand) 2020; 66: 135-141.

[8] Tie J, Cohen JD, Lahouel K, Lo SN, Wang Y, Kosmider S, Wong R, Shapiro J, Lee M, Harris S, Khattak A, Burge M, Harris M, Lynam J, Nott L, Day F, Hayes T, McLachlan SA, Lee B, Ptak J, Silliman N, Dobbyn L, Popoli M, Hruban R, Lennon AM, Papadopoulos N, Kinzler KW, Vogelstein B, Tomasetti C and Gibbs P. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. N Engl J Med 2022; 386: 2261-2272.

[9] Cao Y, Wang N, Wu X, Tang W, Bao H, Si C, Shao P, Li D, Zhou X, Zhu D, Yang S, Wang F, Su G, Wang K, Wang Q, Zhang Y, Wang Q, Yu D, Jiang Q, Bao J and Yang L. Multidimensional fragmentomics enables early and accurate detection of colorectal cancer. Cancer Res 2024; 84: 3286-3295.

[10] Zhang X, Li T, Niu Q, Qin CJ, Zhang M, Wu GM, Li HZ, Li Y, Wang C, Du WF, Wang CY, Zhao Q, Zhao XD, Wang XL and Zhu JB. Genome-wide analysis of cell-free DNA methylation profiling with MeDIP-seq identified potential biomarkers for colorectal cancer. World J Surg Oncol 2022; 20: 21.

[11] Mathios D, Johansen JS, Cristiano S, Medina JE, Phallen J, Larsen KR, Bruhm DC, Niknafs N, Ferreira L, Adleff V, Chiao JY, Leal A, Noe M, White JR, Arun AS, Hruban C, Annapragada AV, Jensen S, Ørntoft MW, Madsen AH, Carvalho B, de Wit M, Carey J, Dracopoli NC, Maddala T, Fang KC, Hartman AR, Forde PM, Anagnostou V, Brahmer JR, Fijneman RJA, Nielsen HJ, Meijer GA, Andersen CL, Mellemgaard A, Bojesen SE, Scharpf RB and Velculescu VE. Detection and characterization of lung cancer using cell-free DNA fragmentomes. Nat Commun 2021; 12: 5060.

[12] Zviran A, Schulman RC, Shah M, Hill STK, Deochand S, Khamnei CC, Maloney D, Patel K, Liao W, Widman AJ, Wong P, Callahan MK, Ha G, Reed S, Rotem D, Frederick D, Sharova T, Miao B, Kim T, Gydush G, Rhoades J, Huang KY, Omans ND, Bolan PO, Lipsky AH, Ang C, Malbari M, Spinelli CF, Kazancioglu S, Runnels AM, Fennessey S, Stolte C, Gaiti F, Inghirami GG, Adalsteinsson V, Houck-Loomis B, Ishii J, Wolchok JD, Boland G, Robine N, Altorki NK and Landau DA. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. Nat Med 2020; 26: 1114-1124.

[13] Lee JW, Park YS, Choi JY, Chang WJ, Lee S, Sung JS, Kim B, Lee SB, Lee SY, Choi J and Kim YH. Genetic characteristics associated with drug resistance in lung cancer and colorectal cancer using whole exome sequencing of cell-free DNA. Front Oncol 2022; 12: 843561.

[14] Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, Le Quesne J, Moore DA, Veeriah S, Rosenthal R, Marafioti T, Kirkizlar E, Watkins TBK, McGranahan N, Ward S, Martinson L, Riley J, Fraioli F, Al Bakir M, Grönroos E, Zambrana F, Endozo R, Bi WL, Fennessy FM, Sponer N, Johnson D, Laycock J, Shafi S, Czyzewska-Khan J, Rowan A, Chambers T, Matthews N, Turajlic S, Hiley C, Lee SM,

Forster MD, Ahmad T, Falzon M, Borg E, Lawrence D, Hayward M, Kolvekar S, Panagiotopoulos N, Janes SM, Thakrar R, Ahmed A, Blackhall F, Summers Y, Hafez D, Naik A, Ganguly A, Kareht S, Shah R, Joseph L, Marie Quinn A, Crosbie PA, Naidu B, Middleton G, Langman G, Trotter S, Nicolson M, Remmen H, Kerr K, Chetty M, Gomersall L, Fennell DA, Nakas A, Rathinam S, Anand G, Khan S, Russell P, Ezhil V, Ismail B, Irvin-Sellers M, Prakash V, Lester JF, Kornaszewska M, Attanoos R, Adams H, Davies H, Oukrif D, Akarca AU, Hartley JA, Lowe HL, Lock S, Iles N, Bell H, Ngai Y, Elgar G, Szallasi Z, Schwarz RF, Herrero J, Stewart A, Quezada SA, Peggs KS, Van Loo P, Dive C, Lin CJ, Rabinowitz M, Aerts H, Hackshaw A, Shaw JA, Zimmermann BG and Swanton C. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017; 545: 446-451.

[15] Wang B, Wu S, Huang F, Shen M, Jiang H, Yu Y, Yu Q, Yang Y, Zhao Y, Zhou Y, Pan B, Liu T and Guo W. Analytical and clinical validation of a novel amplicon-based NGS assay for the evaluation of circulating tumor DNA in metastatic colorectal cancer patients. Clin Chem Lab Med 2019; 57: 1501-1510.

[16] Mouliere F, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, Mair R, Goranova T, Marass F, Heider K, Wan JCM, Supernat A, Hudecova I, Gounaris I, Ros S, Jimenez-Linan M, Garcia-Corbacho J, Patel K, Østrup O, Murphy S, Eldridge MD, Gale D, Stewart GD, Burge J, Cooper WN, van der Heijden MS, Massie CE, Watts C, Corrie P, Pacey S, Brindle KM, Baird RD, Mau-Sørensen M, Parkinson CA, Smith CG, Brenton JD and Rosenfeld N. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Transl Med 2018; 10: eaat4921.