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CURRENT STATUS OF CIRCULATING TUMOR DNA APPLICATION IN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the most common oncological diseases worldwide, characterized by high incidence and mortality rates. With the advancement of modern technologies, new approaches to the diagnosis and treatment of malignant tumors are being actively developed across the globe. The development and implementation of minimally invasive diagnostic methods, as well as tools for early disease detection, represent key objectives in contemporary oncology.

Over the past decade, there has been growing interest in the minimally invasive liquid biopsy technology, which is based on the analysis of circulating tumor DNA (ctDNA) in biological fluids. This method, allowing for the assessment of ctDNA level dynamics, contributes to improving diagnostic accuracy and selecting more effective treatment strategies. Moreover, ctDNA enables the timely detection of minimal residual disease (MRD) and the prevention of disease recurrence.

Modern analytical techniques such as digital PCR (dPCR) and next-generation sequencing (NGS) ensure rapid and precise detection of driver mutations. Analysis of diagnostic and therapeutic studies in CRC demonstrates that ctDNA is among the most significant biomarkers. Promising evidence suggests that ctDNA may serve as a potential tool for CRC screening, although further validation is still required. The implementation of this method into clinical practice will enable preventive and personalized treatment approaches, which, in turn, may reduce disease incidence and mortality rates.

Key words: colorectal cancer; liquid biopsy; ctDNA; mutations; diagnosis

INTRODUCTION

Oncological diseases remain one of the most significant global healthcare challenges. CRC ranks third in prevalence among malignant neoplasms: about 2 million new cases are diagnosed annually, and this figure is projected to rise to 3.2 million by 2040 [1]. In terms of mortality, CRC holds the second position worldwide: in 2020 alone, the disease claimed approximately 1 million lives, and by 2040, the number of deaths may reach 1.6 million [2, 3]. In Kazakhstan, colorectal cancer also ranks third in both incidence (9.3%) and mortality (10.6%) among the population [4].

The increasing incidence of CRC requires the development of new approaches to the diagnosis and treatment of patients. The primary methods for diagnosing CRC today are instrumental techniques such as colonoscopy, sigmoidoscopy, MRI, and CT of the abdominal and pelvic organs [5]. Laboratory methods, including the fecal occult blood test (FOBT), fecal immunochemical test (FIT), and tumor biomarker studies, serve as practical screening tools [6]. Nowadays, determining the molecular biomarkers of a tumor is a crucial step in selecting the most effective treatment option in clinical practice [7, 8, 9]. Tissue biopsy remains the conventional method for obtaining molecular characteristics of a tumor, including cancer type, gene mutations, levels of gene expression, and data for screening.

Nevertheless, this approach has several limitations: it requires invasive surgical procedures that may cause pain, discomfort, and potential clinical risks or complications [10]. Furthermore, certain tumors are in anatomically challenging areas, making tissue sampling difficult or, in some cases, unfeasible. In addition, a biopsy may carry a risk of promoting metastasis [11]. These challenges underscore the need for minimally invasive biomarkers that can detect and monitor disease progression at various stages of treatment, with the advantage of allowing repeated assessments over time. A promising alternative to tissue biopsy is the application of liquid biopsy.

In this review, we aimed to systematize information on the prospects for using ctDNA as a biomarker in clinical oncology.

Liquid biopsy in oncology

Liquid biopsy represents a minimally invasive diagnostic approach that examines various biological fluids – most commonly blood, but also urine, saliva, or cerebrospinal fluid – to identify molecular markers associated with disease, particularly cancer. Rather than relying on conventional tissue sampling through surgery or biopsy, this technique detects circulating tumor cell-free nucleic acids, primarily circulating cell-free DNA (cfDNA) and cell-free RNA (cfRNA), as well as circulating tumor cells (CTCs), exosomes, and other tumor-derived biomarkers present in the bloodstream. cfDNA was first described in 1948 [12], and its clinical significance became evident decades later with its introduction into prenatal diagnostics [13, 14]. The incidental detection of cancer during prenatal testing demonstrated the potential of cfDNA in oncology [15], leading to the development of this method for tumor mutation analysis. Today, liquid biopsy is regarded as an essential complementary tool in cancer diagnosis and monitoring [16, 17, 18]. This diagnostic method is vital for several reasons. First, it enables the confirmation of a primary tumor, which is particularly valuable since approximately 30% of cancer patients (most commonly with lungs, liver, or pancreatic cancer) cannot undergo invasive procedures due to the inaccessible location of the tumor or severe clinical condition. Second, it is used for prognosis and disease stratification [19]. Third, it allows the assessment of tumor sensitivity to therapy, as resistance to chemotherapy or radiotherapy may develop during treatment [18, 20]. In addition, this approach contributes to personalized therapy and facilitates the detection of metastases and recurrences.

Liquid biopsy has already been implemented in clinical practice in several countries, including the USA, EU, China, Japan, and South Korea (Table 1). However, its use depends on the type of tumor and the analytical method employed. For example, in the USA [21], Singapore [22] and China [23], liquid biopsy is widely used for the diagnosis and treatment of EGFR-driven non-small cell lung cancer (NSCLC). In Germany, France, the United Kingdom [24], Japan and South Korea [22] liquid biopsy is applied to identify mutations for targeted therapy and to predict treatment response in patients with CRC. The technology of liquid biopsy has not yet been introduced into clinical practice in Kazakhstan. Furthermore, ongoing efforts in this area remain at the research and exploration stage.

Circulating cell-free DNA

cfDNA represents a fraction of nucleic acids circulating in body fluids, predominantly as short double-stranded fragments (100-200 bp) or larger fragments (up to 1000 Kb) [26]. cfDNA is a general term that encompasses all DNA fragments circulating in body fluids (primarily in the blood). These fragments may originate from normal cells of the body because of biological processes such as apoptosis and necrosis of blood, liver, and other cells, as well as from mitochondria, or from microorganisms (e.g., bacteria and viruses) present in the host organism [27]. Regarding the mechanisms of cfDNA release, numerous studies employing various preclinical *in vitro* and *in vivo* models have demonstrated that its liberation is modulated by a combination of apoptotic processes, cellular senescence, and their respective inhibitors. Notably, the study by A. Rostami et al. reported that senescence may counteract the release of cfDNA.

Furthermore, it has been demonstrated that exposure to ionizing radiation results in necrosis being the predominant mode of cell death, contributing to cfDNA release, whereas apoptosis plays a comparatively minor role in specific tumor types [28].

Within the total pool of cfDNA, a tumor-specific fraction is referred to as ctDNA. The application of cfDNA in oncology has gained wider popularity due to its significantly elevated concentration in cancer patients, reaching levels up to 100 times higher than in healthy individuals. This fraction harbors mutations that mirror those found in the primary tumor [29]. Consequently, ctDNA can provide comprehensive biological insights, including mutational changes that emerge in response to therapy.

Although the first studies on cfDNA were primarily focused on oncology, today there is considerable interest in cfDNA from researchers in many other fields of medicine, including prenatal diagnostics [30] and transplantation [31].

Cell-free DNA detection methods

ctDNA is typically present in biological samples at very low concentrations, which for a long time limited its detection by conventional sequencing methods, such as Sanger sequencing, due to their insufficient sensitivity to rare mutant variants. As a result, mutation-specific digital PCR remained the only approach capable of providing adequate accuracy and specificity for identifying weak tumor-derived signals [32]. Multiple interlaboratory and validation studies report that dPCR can reliably detect mutant allele fractions down to the 0.01-0.1% range (and, under optimized conditions, even lower), depending on DNA input, assay design, and partition number [33]. dPCR assays demonstrate excellent specificity for single-nucleotide variants and small indels, which explains their widespread use for targeted mutation monitoring. For example, in a study of 77 patients using cfDNA from the peripheral blood of lung cancer patients, dPCR analysis demonstrated superior sensitivity in detecting *EGFR* mutations in the blood [34].In a study conducted by a research team from the King Faisal Specialist Hospital and Research Center in Riyadh, the *BRAF* V600 mutation was analyzed in the ctDNA fraction present in the plasma of patients

Table 1 – Regulatory Approvals of Liquid Biopsy Tests by Country

Country/ Region	Regulatory Authority	Approved Test(s)	Notes on Indication
United States	FDA (Food and Drug Administration)	Guardant360 CDx, FoundationOne® Liquid CDx	First FDA-approved NGS-based liquid biopsy companion diagnostic for EGFR mutations in metastatic NSCLC. Also expanded to include other indications (e.g. KRAS G12C, HER2 mutations) [21]
European Union	IVDR (EU *in vitro* Diagnostic Regulation)	Guardant360 CDx	IVDR certification in 2024 permitting use across solid tumors; companion diagnostic indications in NSCLC, breast cancer etc [24]
Japan	MHLW (Ministry of Health, Labour and Welfare)	Guardant360 CDx, OncoBEAM RAS CRC Kit	Approved as a companion diagnostic for multiple indications including tumor mutation profiling for advanced solid tumors; NSCLC, MSI-High CRC etc. Also, reimbursement approved [22]
Singapore	Guardant360 CDx	Approved by Singapore's Health Sciences Authority (HSA)	Companion diagnostic (CDx) for advanced solid cancers; mutation profiling; also approved for NSCLC patients with EGFR alterations for osimertinib (Tagrisso) [22]
India	FoundationOne® Liquid CDx	Launched in India by Roche after FDA-approval; available in Indian market for advanced solid tumors as pan-tumor test	Genomic profiling from cfDNA across >300 genes, including MSI and tumor mutational burden — used when tissue biopsy is not feasible or as a complementary option [22]
China	AmoyDx Super-ARMS EGFR Mutation Test (plasma)	Governmental regulation / CFDA (NMPA)	Companion diagnostic for EGFR-mutation in NSCLC patients [23]
South Korea	Multiple ctDNA / PCR / NGS-based mutation panels (e.g., EGFR, KRAS, ALK, etc.)	National accreditation under Korea Ministry of Food and Drug Safety (MFDS); several domestic ctDNA tests are officially accredited	Used for molecular diagnostics, mutation testing in NSCLC and possibly other solid tumors; some NGS-based panels, others PCR-based [22]

with papillary thyroid carcinoma (PTC) using dPCR. The findings demonstrated that the assay could detect even minimal ctDNA levels (ranging from 0 to 2.07%). This high analytical sensitivity enabled accurate assessment of disease status in PTC patients based on ctDNA concentration fluctuations in plasma (expressed in copies/mL), achieving a sensitivity of 86% and a specificity of 90% [35].

Nevertheless, other methods are also available for detecting ctDNA. A variety of approaches are applied for the analysis of cfDNA, including BEAMing (Beads, Emulsion, Amplification, and Magnetics) [36], Amplification Refractory Mutation System (ARMS) [37], which relies on allele-specific primers designed to amplify target mutations selectively, and advanced sequencing strategies such as whole-genome sequencing (WGS), whole-genome bisulfite sequencing (WGBS-Seq), whole-exome sequencing (WES), tagged-amplicon deep sequencing (TAm-Seq), and cancer personalized profiling by deep sequencing (CAPP-Seq) [38]. Direct comparative analyses indicate that dPCR generally demonstrates superior sensitivity and quantitative accuracy relative to many single-target PCR methods, while also offering a faster and more cost-efficient option than broad NGS assays when monitoring a limited number of predefined variants. By contrast, targeted NGS platforms — particularly tumor-informed or ultra-

deep sequencing strategies, afford more extensive genomic coverage and enable the simultaneous detection of multiple alterations [21, 22, 38]. Thus, the choice between dPCR and NGS depends on the clinical objective, whether the aim is to discover novel variants or to conduct focused surveillance of known mutations. Comparative studies and systematic reviews have highlighted the trade-offs among sensitivity, multiplexing capacity, cost, and turnaround time.

Application of ctDNA in early diagnosis of CRC

ctDNA has emerged as a promising noninvasive biomarker for the early detection of CRC. Unlike conventional diagnostic tools such as colonoscopy or fecal occult blood testing, ctDNA analysis enables the identification of tumor-associated molecular alterations that can appear long before clinical or radiological evidence of malignancy [39]. Advances in NGS and digital PCR technologies have significantly improved the sensitivity and specificity of detecting somatic mutations, aberrant methylation patterns, and other epigenetic changes characteristic of early-stage CRC.

Several large-scale studies have demonstrated that ctDNA methylation signatures, including those of *SEPT9* and *SDC2* [40], possess high diagnostic accuracy and may serve as practical screening tools, particularly in populations at an increased risk of CRC [41]. The integration of ctDNA-based assays into routine clinical screening protocols has the potential to enhance early cancer detection, increase participation in screening, and ultimately reduce CRC-related mortality [42].

Nevertheless, despite recent advances, the sensitivity of this approach as a biomarker for early cancer detection remains suboptimal in the context of solid tumors. Data from a study involving 123 patients with locally advanced rectal cancer (LARC) indicate that total cfDNA at diagnosis has moderate prognostic value: patients with cfDNA levels above the 75th percentile exhibited poorer disease-free survival (DFS) and a higher risk of recurrence compared to those below this threshold (HR 2.48; 95% CI, 1.3 – 4.8; p=0.007) [43].

Application of ctDNA in the assessment of metastatic CRC treatment effectiveness

Recent studies have revealed that ctDNA is recognized as a sensitive and specific biomarker for monitoring the effectiveness of anticancer therapy. The dynamics of ctDNA levels enable the assessment of early tumor response to treatment and the prediction of disease recurrence long before clinical or radiological evidence becomes apparent [44]. A decrease in ctDNA concentration following surgical resection or systemic therapy correlates with a favorable therapeutic response and improved prognosis, whereas persistence or reappearance of mutant DNA fragments in plasma indicates therapeutic resistance or early disease progression [45].

ctDNA has emerged as a promising biomarker for monitoring response to targeted therapies in CRC. Quantitative and qualitative changes in ctDNA reflect tumor dynamics and can reveal early molecular indicators of treatment efficacy or resistance before radiographic progression becomes evident [46]. For example, ctDNA analysis enables detection of *RAS, BRAF*, and *EGFR* pathway alterations associated with acquired resistance to anti-*EGFR* monoclonal antibodies [47], such as cetuximab and panitumumab [48]. Serial ctDNA profiling, therefore, provides a real-time, non-invasive approach for assessing therapeutic response, guiding treatment modification, and optimizing patient outcomes in CRC.

In addition to predicting response to chemotherapy and/or targeted therapy, another potential clinical application of ctDNA-guided monitoring lies in the early identification of patients who are unlikely to benefit from immunotherapy. The predictive potential of ctDNA for assessing response to immunotherapy has been demonstrated in the INSPIRE study, a prospective Phase II trial that followed 94 patients with 25 different types of advanced solid tumors treated with pembrolizumab, accompanied by serial ctDNA measurements. An increase in ctDNA levels at six weeks, coinciding with tumor burden growth, was observed in 42% of patients and predicted a lack of clinical response with 100% accuracy. ctDNA clearance was achieved in 16% of patients undergoing immunotherapy, all of whom exhibited 100% overall survival (OS) with a median follow-up exceeding 25 months from the first clearance event. Furthermore, 98% of patients who experienced ctDNA elevation by the initiation of cycle three did not achieve an objective response, suggesting that ineffective treatment could potentially be avoided in this subgroup [49].

Monitoring of MRD

Minimal/Measurable Residual Disease (MRD) refers to a small number of malignant cells that remain in the patient's body after the achievement of complete clinical remission. The clinical importance of MRD lies in its potential to cause disease relapses, as the residual tumor cells may survive treatment and subsequently proliferate [50].

Extensive international studies have shown that patients with MRD-positive status have a significantly higher risk of relapse compared with those who are MRD-negative. Therefore, the evaluation of MRD serves as a crucial prognostic marker and a tool for risk stratification. Identifying patients with a higher probability of relapse enables clinicians to adjust treatment intensity, accordingly, ultimately improving overall outcomes and survival rates [51]. Furthermore, periodic monitoring of MRD levels provides valuable information for long-term management. Sustained MRD negativity confirms ongoing remission, whereas a rising MRD level may indicate an impending relapse, allowing for early therapeutic intervention [52].

In the context of CRC, the term MRD means Molecular residual disease and often refers to ctDNA detected after curative-intent surgery. ctDNA has emerged as a robust biomarker capable of predicting which CRC patients are at increased risk of recurrence, reflecting the presence of occult or persistent disease [53]. Due to its short half-life, ranging from minutes to a few hours, ctDNA allows for precise and dynamic assessment of tumor burden in real-time. This property makes ctDNA a valuable tool for monitoring molecular residual disease and for the early detection of relapse. For example, in a cohort of 231 patients with resected stage II colorectal cancer, the presence of ctDNA in postoperative plasma samples was strongly associated with disease recurrence. Among patients who did not receive adjuvant chemotherapy, ctDNA was detectable in 14 of 178 cases; 11 of these patients (79%) experienced relapse during a median follow-up of 27 months (HR 18; 95% CI, 7.9 – 40; p<0.001). A similar trend was observed among patients who received adjuvant chemotherapy: ctDNA was detected in 3 of 44 patients after treatment, and all three subsequently relapsed within 11 months after therapy (HR 11; 95% CI, 1.8 – 68; p=0.001). These associations remained significant regardless of «low risk» or «high-risk» clinical stratification based on pathological features [54].

CONCLUSION

Thus, ctDNA is considered a noninvasive molecular biomarker with high potential in oncology; however, its clinical application remains limited by several factors, including its low concentration in the bloodstream, technical challenges in detection and analysis, and the lack of standardized methodologies. The use of ctDNA as a biomarker has already been implemented in clinical practice across several developed countries, where it has demonstrated substantial diagnostic and prognostic value in CRC. Both completed and ongoing clinical trials emphasize the importance of ctDNA testing in the postoperative setting for therapy personalization and improvement of patient outcomes. Although current tumor-derived molecular approaches still show modest sensitivity, ctDNA-based strategies hold significant potential for further advancement -particularly given the decreasing costs of sequencing and the integration of multi-omics data to address existing challenges. Emerging technologies and integrative analytical frameworks are expected to enable earlier and more precise detection of malignant diseases soon.

Authors contributions:

A. Aitkulova, D. Begimbetova, A. Akilzhanova, D. Sarbassov – concept development, collection and analysis of literature data, writing and preparation of the manuscript.

T. Kadenova, A. Gabdulkayum, D. Yerezhepov – scientific supervision, methodological consulting, and approval of the final version of the manuscript.

S. Rakhimova, D. Samatkyzy, D. Begimbetova, U. Kozhamkulov – scientific supervision, methodological consulting, and approval of the final version of the manuscript.

A. Aitkulova – technical formatting and preparation of the manuscript for publication.

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СОВРЕМЕННЫЕ ВОЗМОЖНОСТИ ПРИМЕНЕНИЯ ЦИРКУЛИРУЮЩЕЙ ОПУХОЛЕВОЙ ДНК ПРИ КОЛОРЕКТАЛЬНОМ РАКЕ

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Колоректальный рак (КРР) является одним из наиболее распространенных онкологических заболеваний в мире, характеризующимся высокой заболеваемостью и смертностью. С развитием технологий во всем мире активно разрабатываются новые подходы к диагностике и лечению злокачественных опухолей. Разработка и внедрение минимально инвазивных методов диагностики, а также инструментов для раннего выявления заболевания является ключевой задачей современной онкологии. За последнее десятилетие возрос интерес к малоинвазивной технологии жидкостной биопсии, основанной на анализе циркулирующей опухолевой ДНК (ctDNA) в биологических жидкостях. Этот метод, позволяющий оценивать динамику изменений уровня ctDNA, способствует повышению точности диагностики и выбору более эффективных стратегий лечения. Кроме того, ctDNA позволяет своевременно диагностировать минимальную остаточную болезнь (MRD) и предотвратить рецидив заболевания. Современные методы, такие как цифровая ПЦР (dPCR) и секвенирование нового поколения (NGS), обеспечивают быстрое и точное выявление драйверных мутаций. Анализ диагностических и терапевтических исследований при КРР показывает, что ctDNA является наиболее значимым биомаркером. Обнадеживающие данные свидетельствуют о том, что ctDNA может использоваться как перспективный инструмент для скрининга КРР, хотя требуется дальнейшая валидация. Внедрение данного метода в клиническую практику позволит реализовать профилактические и персонализированные подходы к лечению, что в свою очередь может снизить показатели заболеваемости и смертности.

Ключевые слова: колоректальный рак; жидкостная биопсия; ctDNA; мутации; диагностика

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ТОҚ ІШЕК ЖӘНЕ ТІК ІШЕК ҚАТЕРЛІ ІСІГІНДЕ АЙНАЛЫМДАҒЫ ІСІК ДНҚ-СЫН ҚОЛДАНУДЫҢ ҚАЗІРГІ ЖАҒДАЙЫ

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Тік ішек және тоқ ішек обыры (КРР) – әлемдегі ең кең таралған онкологиялық аурулардың бірі, жоғары сырқаттанушылық және өлім-жітім көрсеткіштерімен сипатталады. Қазіргі технологиялардың дамуына байланысты қатерлі ісіктерді ерте анықтау мен емдеудің жаңа тәсілдері белсенді түрде әзірленіп, енгізілуде. Минималды инвазивті диагностикалық әдістер мен ауруды ерте кезеңде анықтауға арналған құралдарды әзірлеу және енгізу – қазіргі онкологияның негізгі міндеттерінің бірі болып табылады. Соңғы онжылдықта биологиялық сұйықтықтардағы айналымдағы ісік ДНҚ-сын (ctDNA) талдауға негізделген сұйық биопсияның аз инвазивті технологиясына қызығушылық артты. Бұл әдіс ctDNA деңгейінің динамикасын бағалауға мүмкіндік беріп, диагностикалық дәлдікті арттыруға және неғұрлым тиімді емдеу стратегияларын таңдауға ықпал етеді. Сонымен қатар, ctDNA минималды қалдық ауруды (MRD) уақытылы диагностикалауға және аурудың қайталануын болдырмауға мүмкіндік береді. Цифрлық ПТР (dPCR) және жаңа буын секвенирлеу (NGS) сияқты заманауи әдістер драйверлік мутацияларды жылдам және дәл анықтауды қамтамасыз етеді. КРР бойынша диагностикалық және терапиялық зерттеулердің талдауы ctDNA-ның ең маңызды биомаркерлердің бірі екенін көрсетті.

Қуантарарлық деректер ctDNA-ны КРР скринингі үшін болашағы зор құрал ретінде пайдалануға болатынын айғақтайды, дегенмен бұл тәсілді қосымша валидациялау қажет. Аталған әдісті клиникалық практикаға енгізу алдын алу және дербестендірілген емдеу тәсілдерін жүзеге асыруға мүмкіндік береді, бұл өз кезегінде аурушаңдық пен өлім-жітім көрсеткіштерін төмендетуге ықпал етеді.

Кілт сөздер: тоқ ішек және тік ішек қатерлі ісігі; сұйықтықтық биопсия; cfDNA; мутациялар; диагностика