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Advancements and Challenges in Biomarkers for Colorectal Cancer Detection: A Comprehensive Review

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Abstract: This study provides an overview of the current landscape of biomarkers for colorectal cancer (CRC) detection, focusing on genetic, proteomic, circulating microRNA (miRNA), and metabolomic biomarkers. CRC remains a significant global health challenge, ranking among the most prevalent cancers worldwide and being a leading cause of cancer-related deaths. Despite advancements in screening methods such as colonoscopy, sigmoidoscopy, and fecal occult blood tests (FOBT), the asymptomatic nature of early-stage CRC often results in late diagnoses, negatively impacting patient outcomes. Genetic biomarkers like *APC*, *KRAS*, *TP53*, and microsatellite instability (MSI) play critical roles in CRC pathogenesis and progression. These biomarkers, detectable through polymerase chain reaction, next-generation sequencing, and other advanced techniques, guide early detection and personalized treatment decisions. Proteomic biomarkers such as CEA, CA 19-9, and novel signatures offer insights into CRC's physiological changes and disease status, aiding prognosis and treatment response assessments through enzyme-linked immunosorbent assay and mass spectrometry. Circulating miRNAs, including *miR-21* and *miR-92a*, present promising non-invasive biomarkers that can be detected in blood and stool samples, reflecting CRC presence, progression, and therapeutic response. Metabolomic biomarkers, encompassing amino acids, lipids, and TCA cycle intermediates, provide further insights into CRC-associated metabolic alterations, which are crucial for early detection and treatment monitoring using mass spectrometry and nuclear magnetic resonance. Despite these advancements, challenges such as biomarker validation, standardization, and clinical utility remain. Future research directions include integrating multi-omics approaches and leveraging technologies like liquid biopsies and AI for enhanced biomarker discovery and clinical application. By addressing these challenges and advancing research in biomarker development, CRC screening and management could potentially be revolutionized, improving patient outcomes and reducing the global burden of this disease.

Keywords: Colorectal cancer; Biomarker; Diagnosis; Detection

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1. Introduction

Colorectal cancer (CRC) is one of the most significant global health challenges, ranking as the third most common cancer and the second leading cause of cancer-related deaths. The burden of CRC is substantial, with over 1.9 million new cases and approximately 935,000 deaths estimated worldwide in 2020 [1]. The incidence of CRC varies by geographic region, reflecting differences in risk factors such as diet, lifestyle, and access to healthcare services [2].

Early detection of CRC is crucial for improving patient outcomes, as the prognosis is significantly better when the disease is diagnosed at an early stage. The five-year survival rate for localized CRC exceeds 90% but drops to less than 15% for metastatic cases [3]. Despite the clear benefits of early detection, many CRC cases are diagnosed at advanced stages due to the asymptomatic nature of early-stage disease and limitations in current screening methods [4]. Traditional screening techniques, including colonoscopy, sigmoidoscopy, and fecal occult blood tests (FOBT), such as fecal immunochemical tests (FIT), are commonly used. While colonoscopy is considered the gold standard for CRC detection and prevention, it is invasive, expensive, and often associated with patient discomfort and low compliance rates [5]. FOBT and FIT, though non-invasive and more cost-effective, have variable sensitivity and specificity, leading to false positives and negatives [6].

In recent years, there has been growing interest in developing biomarkers for CRC detection. Biomarkers are biological molecules found in blood, body fluids, or tissues that indicate a normal or abnormal process, or the presence of a disease [7]. They offer the potential for non-invasive, accurate, and early detection of CRC, thereby improving patient compliance and screening efficacy [8].

The term “biomarker” encompasses a wide range of biological entities, including genetic mutations, epigenetic alterations, protein expression patterns, metabolites, and circulating tumor components. These biomarkers can be detected in various biological samples such as blood, stool, urine, and tissue biopsies [9]. The ideal biomarker for CRC detection should have high sensitivity and specificity, be easily accessible, cost-effective, and applicable across diverse populations [10].

This review aims to provide a comprehensive update on the current state of biomarkers for CRC detection. It will explore various types of biomarkers, including genetic, epigenetic, proteomic, metabolomic, and circulating biomarkers, and discuss their roles in early detection and diagnosis. Furthermore, the review will examine the clinical utility of these biomarkers, the challenges associated with their implementation, and future perspectives in the field.

By offering an overview of the latest advancements and ongoing research in CRC biomarkers, this review seeks to highlight the potential of these novel approaches to transform CRC screening and improve patient outcomes. Understanding the current landscape of CRC biomarkers is essential for researchers, clinicians, and policymakers aiming to reduce the global burden of CRC through innovative and effective screening strategies [11].

2. Genetic biomarkers

Genetic biomarkers are pivotal for understanding the molecular underpinnings of CRC and play a significant role in early detection and personalized treatment strategies. CRC arises from the accumulation of genetic mutations that drive the transformation of normal colonic epithelium into adenocarcinoma [12]. Several key genetic biomarkers have been identified, each contributing to different stages of CRC development and progression. This section delves into the primary genetic biomarkers associated with CRC, including *APC*, *KRAS*, *TP53*, and microsatellite instability (MSI), discussing their roles, detection methods, and clinical implications [13-16].

2.1. APC mutations

The adenomatous polyposis coli (*APC*) gene is a tumor suppressor that plays a crucial role in the Wnt signaling pathway. Mutations in *APC* are considered one of the earliest events in colorectal tumorigenesis and are present in approximately 80% of sporadic CRC cases [17]. These mutations lead to the loss of APC protein function, resulting in the accumulation of β-catenin in the nucleus, where it activates transcription of genes involved in cell proliferation and survival [18,19].

2.2. KRAS mutations

The *KRAS* gene, encoding a GTPase involved in the EGFR signaling pathway, is another critical genetic biomarker in CRC. Mutations in *KRAS* occur in approximately 35%–45% of CRC cases, typically in codons 12 and 13. These mutations lead to the constitutive activation of the KRAS protein, promoting cell proliferation, survival, and metastasis [20,21].

2.3. TP53 mutations

The *TP53* gene, which encodes the tumor suppressor protein p53, is often referred to as the "guardian of the genome" due to its role in maintaining genomic stability. *TP53* mutations are present in approximately 50% of CRC cases and often occur at later stages of tumorigenesis, marking the transition from adenoma to carcinoma [22,23].

2.4. Microsatellite instability

MSI is a form of genomic instability resulting from defects in the DNA mismatch repair (MMR) system. MSI is characterized by the accumulation of insertion or deletion errors in microsatellite regions of the genome. Approximately 15% of CRC cases exhibit high-level MSI (MSI-H), which is commonly associated with Lynch syndrome (hereditary nonpolyposis CRC, HNPCC) and some sporadic CRC cases [24,25].

2.5. Emerging genetic biomarkers

In addition to well-established genetic biomarkers, ongoing research continues to identify novel genetic alterations with potential clinical utility in CRC detection and management. Emerging biomarkers include:

(1) *BRAF* mutations: *BRAF* V600E mutations occur in approximately 10% of CRC cases and are associated with poor prognosis and resistance to certain targeted therapies. Detection of *BRAF* mutations can guide treatment decisions and identify patients who may benefit from BRAF inhibitors combined with other therapeutic agents [26,27].

(2) *PIK3CA* mutations: Mutations in the *PIK3CA* gene, encoding a subunit of the PI3K enzyme, are found in about 15%–20% of CRC cases. These mutations drive tumor growth and resistance to therapies, making them valuable targets for novel therapeutic strategies [28,29].

(3) *NRAS* mutations: *NRAS* mutations, though less common than *KRAS* mutations, are present in a subset of CRC cases. Like *KRAS*, *NRAS* mutations can impact response to anti-EGFR therapies, underscoring the importance of comprehensive RAS testing in CRC [30,31].

2.6. Detection methods and clinical implications

The detection of genetic biomarkers in CRC utilizes a variety of advanced techniques to ensure accurate identification and subsequent clinical action. *APC* mutations, early events in CRC tumorigenesis, can be detected through polymerase chain reaction (PCR), next-generation sequencing (NGS), and digital droplet PCR (ddPCR), with non-invasive stool DNA testing, such as the Cologuard test, offering a practical option [32,33].

KRAS mutations, present in about 35%–45% of CRC cases, are identified using PCR-based methods, NGS, and allele-specific oligonucleotide PCR, with liquid biopsy offering a minimally invasive alternative [34,35]. *TP53* mutations, found in roughly 50% of CRC cases, are detected through Sanger sequencing, NGS, immunohistochemistry (IHC), and circulating tumor DNA (ctDNA) analysis [22,36]. MSI, associated with defects in the MMR system, is assessed using PCR to analyze microsatellite markers and IHC for MMR protein expression, with NGS panels providing comprehensive profiling [25,37,38].

Clinically, these biomarkers are crucial for guiding CRC management and treatment. *APC* mutation identification aids in early detection, particularly in individuals with familial adenomatous polyposis (FAP), enabling preventive measures [32,33]. *KRAS* mutation status is critical for determining eligibility for anti-EGFR therapies, such as cetuximab and panitumumab, as patients with these mutations do not benefit from such treatments [34,35]. The presence of *TP53* mutations, often associated with aggressive disease and poorer prognosis, informs the need for therapies targeting DNA repair pathways or reactivating mutant p53 [22,36]. MSI testing, essential for all CRC patients, identifies those likely to respond to immune checkpoint inhibitors and helps in screening for Lynch syndrome [25,37]. Emerging biomarkers like *BRAF* and *PIK3CA* mutations also provide insights into therapy resistance and potential targets for novel treatments, while NRAS mutations underscore the importance of comprehensive RAS testing to optimize anti-EGFR therapy decisions [25,30].

3. Proteomic biomarkers

Proteomic biomarkers, which involve studying the complete set of proteins expressed by a genome, cell, tissue, or organism, hold great promise for the detection and management of CRC. Unlike genetic biomarkers, which provide information about the potential for cancer development, proteomic biomarkers reflect real-time physiological changes and disease states. The dynamic nature of the proteome makes it a rich source for identifying disease-specific alterations that can aid in early detection, prognostication, and therapeutic targeting [39,40].

This section explores key proteomic biomarkers in CRC, including carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA 19-9), and novel proteomic signatures, highlighting their detection methods and clinical implications [41-43].

3.1. Carcinoembryonic antigen

CEA is one of the most extensively studied and widely used proteomic biomarkers in CRC. CEA is a glycoprotein involved in cell adhesion, and its expression is significantly elevated in colorectal tumors compared to normal tissues [41,44].

3.2. Cancer antigen 19-9

CA 19-9 is another glycoprotein that serves as a tumor marker in various gastrointestinal cancers, including CRC. Although CA 19-9 is more commonly associated with pancreatic cancer, it can also be elevated in a subset of CRC patients [41,45].

3.3. Novel proteomic signatures

Advances in proteomics technologies, such as mass spectrometry and protein microarrays, have facilitated the discovery of novel proteomic signatures that can improve CRC detection and management. These signatures often comprise multiple protein biomarkers that together enhance diagnostic accuracy and provide insights into the molecular mechanisms of CRC [42,43].

3.4. Circulating tumor proteins

Circulating tumor proteins, released by tumor cells into the bloodstream, represent another important category of proteomic biomarkers. These proteins can be detected in blood samples, providing a minimally invasive approach to CRC detection and monitoring [42,43].

3.5. Detection methods and clinical implications

Proteomic biomarkers for CRC, such as CEA, CA 19-9, novel proteomic signatures, and circulating tumor proteins, are detected using advanced techniques and have significant clinical implications. CEA and CA 19-9 levels are typically measured using enzyme-linked immunosorbent assays (ELISAs) or chemiluminescent immunoassays (CLIAs) in serum samples, providing a non-invasive method for monitoring disease progression and treatment response [41,44]. While CEA is valuable for postoperative monitoring and detecting recurrence, CA 19-9, though less specific to CRC, can complement CEA in assessing disease status [45].

Novel proteomic signatures, identified through high-throughput platforms like mass spectrometry (MS) and protein microarrays, offer comprehensive profiling that enhances diagnostic accuracy and provides insights into CRC's molecular mechanisms [42,43]. These signatures are crucial for early detection, patient stratification, and identifying therapeutic targets. Circulating tumor proteins, detected through techniques like ELISA, bead-based multiplex assays, and MS, provide a minimally invasive approach for early detection and monitoring. Elevated levels of these proteins in blood samples can indicate tumor presence, progression, and treatment response, aiding in timely CRC management [42,43].

Integrating these proteomic biomarkers into clinical practice enhances early detection, informs prognosis, guides personalized therapy, and ultimately improves patient outcomes.

4. Circulating microRNAs

Circulating microRNAs (miRNAs) have emerged as promising non-invasive biomarkers for CRC detection, prognosis, and monitoring. These small, non-coding RNA molecules, typically 19–25 nucleotides in length, regulate gene expression post-transcriptionally and can be found in various body fluids, including blood, serum, plasma, and stool [46,47]. The stability of miRNAs in circulation, owing to their protection within exosomes, microvesicles, or protein complexes, makes them attractive candidates for clinical applications. This section explores key circulating miRNAs, their detection methods, and their clinical implications in CRC.

4.1. Key circulating miRNAs

(1) *miR-21*: One of the most extensively studied miRNAs in CRC, *miR-21* is frequently overexpressed in CRC tissues and detectable at elevated levels in the blood of CRC patients [48,49].

(2) *miR-92a*: Part of the *miR-17-92* cluster, *miR-92a* is upregulated in CRC and is associated with tumor growth and metastasis [50,51].

(3) *miR-29a*: This miRNA is involved in the regulation of apoptosis and cell proliferation, with increased levels observed in the blood of CRC patients [52,53].

(4) *miR-17-3p* and *miR-20a*: Both are part of the *miR-17-92* cluster and are implicated in CRC progression and metastasis [54,55].

(5) *miR-145*: Generally downregulated in CRC, *miR-145* acts as a tumor suppressor, and its reduced expression correlates with more advanced disease stages [56,57].

4.2. Detection methods and clinical implications

Circulating miRNAs are detected using techniques such as quantitative PCR (qPCR), ddPCR, NGS, microarrays, and bead-based multiplex assays. These methods enable the sensitive and specific quantification of miRNAs in body fluids [58,59]. Clinically, circulating miRNAs hold significant potential for early CRC detection, prognosis, and monitoring of treatment response. Elevated levels of miRNAs, such as *miR-21*, *miR-92a*, and *miR-29a*, in blood samples can indicate the presence of CRC, even at early stages, facilitating timely intervention [53,60]. Furthermore, the expression profiles of specific miRNAs provide prognostic information, correlating with disease progression and patient outcomes [49,61]. Changes in miRNA levels during and after treatment can reflect therapeutic efficacy and help detect recurrence, aiding in the development of personalized treatment strategies.

The non-invasive nature of circulating miRNA testing offers a patient-friendly alternative to traditional biopsies, making routine monitoring more accessible and less burdensome. Integrating miRNA profiles into clinical practice enhances the precision of CRC diagnosis, prognosis, and treatment, ultimately improving patient outcomes through personalized medicine.

5. Metabolomic biomarkers

Metabolomics, the comprehensive study of metabolites in biological systems, has become an invaluable approach for identifying biomarkers in CRC [62]. Metabolites are small molecules involved in various metabolic pathways, reflecting the physiological state of cells, tissues, and organisms [63]. Changes in metabolite levels can indicate alterations in metabolic processes associated with cancer development and progression. This section explores key metabolomic biomarkers in CRC, their detection methods, and their clinical implications.

5.1. Key metabolomic biomarkers

(1) Amino acids: Altered levels of amino acids, such as tryptophan, glutamine, and arginine, have been observed in CRC patients. These changes reflect disruptions in amino acid metabolism, which is crucial for tumor growth and survival [64,65].

(2) Lipid metabolites: Abnormal lipid metabolism is a hallmark of cancer. Elevated levels of certain phospholipids, sphingolipids, and free fatty acids are commonly found in CRC patients [66,67].

(3) Carbohydrate metabolites: Changes in carbohydrate metabolism, including elevated levels of glucose and lactate, are indicative of the Warburg effect—a phenomenon where cancer cells preferentially utilize glycolysis for energy production even in the presence of oxygen [68,69].

(4) Bile acids: Altered bile acid profiles have been linked to CRC. Elevated levels of primary and secondary bile acids can reflect changes in gut microbiota and hepatic function [70,71].

(5) Tricarboxylic acid cycle intermediates: Disruptions in the tricarboxylic acid (TCA) cycle, such as altered levels of citrate, succinate, and fumarate, indicate metabolic reprogramming in cancer cells [72,73].

5.2. Detection methods and clinical implications

Metabolomic biomarkers in CRC are detected using advanced analytical techniques, such as MS, nuclear magnetic resonance (NMR) spectroscopy, capillary electrophoresis-mass spectrometry (CE-MS), Fourier transform infrared (FTIR) spectroscopy, and high-performance liquid chromatography (HPLC) [63,65]. These methods enable the sensitive and specific identification and quantification of metabolites in biological samples, providing a detailed metabolic profile of both the tumor and the host. Clinically, metabolomic biomarkers

have significant implications for CRC management. They facilitate early detection through the identification of specific metabolic changes associated with cancer onset [64,69]. Certain metabolites, such as altered amino acids, lipids, carbohydrates, bile acids, and TCA cycle intermediates, offer prognostic value by correlating with disease stage, progression, and patient outcomes [70,72]. Metabolomic biomarkers are also valuable for monitoring treatment response and detecting recurrence, as changes in metabolite levels can reflect therapeutic efficacy and disease status [66,73]. Furthermore, metabolomic profiling can guide personalized therapy by identifying metabolic vulnerabilities that can be targeted with specific treatments [65,68]. The non-invasive nature of metabolomic testing, using fluids such as blood, urine, and stool, enhances patient comfort and facilitates routine monitoring. Integrating metabolomic data with other omics data provides a comprehensive understanding of CRC, leading to improved disease management and patient outcomes through personalized medicine.

6. Challenges and future directions

Biomarker research for CRC faces several significant challenges that must be addressed to realize its full potential in clinical practice. One critical challenge is the standardization and validation of biomarkers. Biomarker discovery often involves diverse methodologies and sample types across different studies, leading to variability in results. Standardizing protocols for biomarker identification, validation, and clinical implementation is essential to ensure reproducibility and reliability across various research settings and populations.

Another key hurdle is demonstrating the clinical utility of biomarkers and integrating them into routine clinical practice. While biomarkers show promise in research, their adoption in clinical settings requires robust evidence of their effectiveness in improving patient outcomes. Biomarkers must demonstrate clear benefits in terms of sensitivity, specificity, cost-effectiveness, and impact on clinical decision-making to justify their incorporation into screening and diagnostic algorithms.

The heterogeneity of CRC presents another challenge. CRC includes various molecular subtypes and clinical manifestations, requiring biomarkers that can accurately reflect this diversity. Biomarkers must be validated across different patient populations to ensure their efficacy in stratifying patients for personalized treatment strategies.

Ethnic and geographic variations also influence biomarker performance. Genetic, lifestyle, and environmental factors can affect biomarker expression and efficacy across different ethnic groups and geographic regions. Developing biomarkers that are effective and reliable across diverse populations is crucial for their global applicability and adoption in clinical practice.

Additionally, the transition from single biomarkers to multimodal biomarker panels represents a promising future direction in CRC research. Single biomarkers may lack sufficient sensitivity or specificity for accurate CRC detection and prognosis. Combining multiple types of biomarkers, such as genetic, proteomic, and metabolomic markers, could enhance diagnostic accuracy and reliability, paving the way for more effective screening and personalized treatment approaches.

Looking ahead, advancements in multi-omics approaches—including genomics, epigenomics, proteomics, and metabolomics—hold great potential for improving biomarker discovery and validation. Integrating data from multiple omics layers can provide a more comprehensive understanding of CRC biology and facilitate the development of robust biomarker panels. Moreover, leveraging technologies such as liquid biopsies and artificial intelligence (AI) for biomarker detection and analysis could further enhance the clinical utility and predictive power of biomarkers in CRC management.

Addressing these challenges and pursuing these future directions will be instrumental in advancing biomarker research for CRC. By overcoming these obstacles, biomarkers have the potential to revolutionize CRC screening, diagnosis, and treatment, ultimately improving patient outcomes and reducing the global burden of this disease.

7. Conclusion

Biomarkers present a promising pathway for the early detection and management of CRC. Genetic, proteomic, circulating miRNA, and metabolomic biomarkers have demonstrated potential in enhancing screening accuracy and improve patient outcomes. However, significant challenges remain in the standardization, validation, and clinical integration of these biomarkers. Future research should focus on developing robust, multimodal biomarker panels and utilizing technological advancements to strengthen CRC detection and screening programs. With continued efforts, biomarker-based screening could become a cornerstone in the fight against CRC, ultimately reducing its global impact.

Disclosure statement

The authors declare no conflict of interest.

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