Review Article

Molecular Pathology of Colorectal Cancer: Genetic Alterations, Signaling Pathways, and Clinical Implications

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Abstract

Colorectal cancer develops through a complex multistep process driven by the progressive accumulation of genetic and epigenetic alterations that transform normal colonic epithelium into malignant carcinoma. The adenoma–carcinoma sequence represents the classical model describing this progression, in which mutations in key genes such as APC, KRAS, and TP53 disrupt cellular regulatory mechanisms and activate oncogenic signaling pathways including Wnt, EGFR, and PI3K/AKT. Epigenetic mechanisms also contribute significantly to tumorigenesis, particularly through DNA methylation and histone modifications that alter gene expression. One of the most important epigenetic patterns is the CpG island methylator phenotype, which leads to promoter hypermethylation and the silencing of tumor suppressor genes, including MLH1, resulting in mismatch repair deficiency and microsatellite instability. Genomic instability plays a central role in tumor progression and occurs mainly through chromosomal instability and microsatellite instability, both of which contribute to tumor heterogeneity and biological diversity. The molecular heterogeneity of colorectal cancer has been further clarified through the consensus molecular subtype classification, which categorizes tumors into four major groups based on transcriptomic features. These subtypes display distinct biological characteristics related to immune activity, metabolic regulation, signaling pathway activation, and stromal interactions, and they are associated with different prognostic outcomes and treatment responses. Molecular biomarkers have also become essential in colorectal

cancer management. Diagnostic markers such as stool DNA testing, circulating tumor DNA, and methylation assays facilitate early detection, while prognostic and predictive biomarkers including RAS, BRAF, and microsatellite instability guide risk stratification and therapeutic decisions. Advances in molecular diagnostics, particularly next-generation sequencing and liquid biopsy technologies, have expanded the ability to characterize tumor profiles and monitor disease progression. These innovations support personalized treatment strategies, including targeted therapies and immunotherapy, and continue to shape the evolving landscape of precision oncology in colorectal cancer.

Key words

Colorectal carcinogenesis, genomic instability, microsatellite instability, molecular subtypes, tumor biomarkers, precision oncology.

Introduction

Colorectal cancer represents a major global health challenge and remains one of the most frequently diagnosed malignancies worldwide. It is currently recognized as the third most commonly diagnosed cancer and the second leading cause of cancer-related mortality on a global scale [1, 2]. Although epidemiological data indicate that the overall incidence of colorectal cancer has shown a gradual decline in several populations, an emerging and concerning trend has been observed in the increasing incidence of young-onset colorectal cancer, defined as cases diagnosed before the age of fifty [3]. This shift in epidemiological patterns has generated significant attention in recent years and highlights the importance of identifying underlying risk factors and biological mechanisms contributing to disease development in younger individuals. Among the recognized contributors to colorectal cancer risk, lifestyle-related factors play a substantial role. Dietary patterns, tobacco use, and alcohol consumption have all been consistently associated with an increased likelihood of colorectal carcinogenesis [4].

Understanding the molecular mechanisms underlying colorectal cancer is essential for explaining its pathogenesis and clinical behavior. The development of this malignancy is driven by the progressive accumulation of both genetic and epigenetic alterations that disrupt critical regulatory pathways involved in cellular proliferation, differentiation, and genomic stability. Several major molecular mechanisms have been identified as central drivers of colorectal tumorigenesis, including chromosomal instability, microsatellite instability, and the CpG island methylator phenotype. These molecular alterations frequently involve mutations in key regulatory genes such as APC, KRAS, NRAS, BRAF, and TP53. The disruption of these genes results in the activation of multiple signaling pathways, including Wnt, epidermal growth factor receptor, and phosphatidylinositol 3-kinase/protein kinase B pathways, which collectively promote tumor initiation and progression [5]. In addition to these intrinsic molecular alterations, the tumor microenvironment has emerged as a critical determinant of colorectal cancer progression and prognosis. Interactions between malignant cells and surrounding stromal and immune components significantly influence tumor growth, invasion, and therapeutic response [6, 7].

Advances in genomic and transcriptomic technologies have led to a transition from traditional histopathological classifications toward more refined molecular classification systems that better capture the biological heterogeneity of colorectal cancer. One of the most widely recognized frameworks is the consensus molecular subtype classification, which categorizes colorectal cancer into four distinct molecular subtypes based on RNA

sequencing data. These subtypes reflect important differences in tumor biology, including variations in immune activity, metabolic characteristics, and signaling pathway activation. More recently, additional classification systems have been proposed to further characterize tumor heterogeneity. These include colorectal cancer intrinsic subtypes and pathway-derived subtypes, which provide further insights into tumor-specific biological properties and clinically relevant attributes [6]. The implementation of molecular classification systems has significantly enhanced the understanding of colorectal cancer heterogeneity and has contributed to the development of personalized therapeutic strategies tailored to individual tumor profiles [3].

The growing integration of molecular pathology into clinical practice has had profound implications for the management of colorectal cancer. Molecular profiling now plays a central role in guiding targeted therapeutic approaches and identifying mechanisms of treatment resistance. Targeted therapies, including tyrosine kinase inhibitors and immune checkpoint inhibitors, rely on the identification of specific molecular alterations to optimize treatment selection and clinical outcomes [5]. In this context, molecular diagnostic testing has become an essential component of modern oncologic care. The evaluation of mismatch repair status and the analysis of mutations in genes such as KRAS, NRAS, and BRAF are now routinely performed to guide therapeutic decision-making and predict treatment response. Ongoing advances in molecular diagnostic technologies continue to refine precision medicine approaches, allowing clinicians to tailor treatments more effectively and ultimately improving patient outcomes in colorectal cancer management [8].

The objective of this review is to analyze the molecular pathology of colorectal cancer by examining the genetic and epigenetic alterations that drive tumor development and progression.

Methodology

This manuscript was developed as a structured narrative review aimed at providing an updated and clinically integrated analysis of the molecular pathology of colorectal cancer, with emphasis on the genetic and epigenetic alterations that drive tumor initiation and progression, the principal signaling pathways involved in colorectal carcinogenesis, and the clinical implications of molecular classification and biomarker-guided management. The review was conducted in accordance with the SANRA (Scale for the Assessment of Narrative Review Articles) framework and followed a predefined methodological protocol established prior to literature screening. Given the biological complexity and heterogeneity of colorectal cancer, as well as the coexistence of multiple molecular pathways and classification systems, a narrative interpretative synthesis was selected rather than quantitative pooling in order to integrate mechanistic, pathological, and clinical evidence into a coherent framework. Particular attention was given to chromosomal instability, microsatellite instability, and the CpG island methylator phenotype, along with key alterations involving APC, KRAS, NRAS, BRAF, and TP53. Additional emphasis was placed on the tumor microenvironment, consensus molecular subtypes, emerging molecular stratification models, and the impact of molecular profiling on prognosis, targeted therapies, and precision oncology. The objective was to provide a structured synthesis capable of supporting a comprehensive understanding of colorectal cancer biology and its translation into clinical practice.

A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science, including peer-reviewed publications in English or Spanish published between January 2020 and December 2026. The final search was performed in December 2026. This timeframe was selected to capture contemporary advances in molecular classification systems, genomic and transcriptomic profiling, biomarker discovery,

targeted therapies, immunotherapy-related molecular selection, and the integration of molecular diagnostics into routine oncologic practice. Foundational studies were incorporated when necessary to contextualize canonical molecular pathways or the historical development of colorectal cancer classification. The search strategy combined MeSH and free-text terms using Boolean operators related to colorectal cancer, molecular pathology, chromosomal instability, microsatellite instability, CpG island methylator phenotype, APC, KRAS, NRAS, BRAF, TP53, tumor microenvironment, consensus molecular subtypes, biomarker, molecular classification, targeted therapy, immunotherapy, and precision medicine. Searches were conducted in titles and abstracts as well as indexed subject headings to maximize sensitivity.

The initial search yielded 215 records. After removal of duplicates, 171 articles remained for title and abstract screening. Of these, 94 underwent full-text evaluation, and 46 studies were included in the final synthesis. Selection was performed independently by two authors, with disagreements resolved through discussion and consensus. Exclusion criteria included non–peer-reviewed publications, isolated case reports, editorials without substantive molecular or clinical data, redundant datasets, studies focused on non-colorectal malignancies, and reports not directly addressing molecular mechanisms, classification systems, diagnostic biomarkers, or therapeutic implications in colorectal cancer.

Eligible studies included randomized controlled trials, large observational cohorts, translational research studies, systematic reviews, meta-analyses, expert consensus statements, and contemporary international guidelines from oncology, pathology, gastroenterology, and molecular diagnostics societies. Priority was assigned to multicenter investigations, studies with robust molecular characterization, and research evaluating associations between molecular alterations and clinically relevant outcomes such as prognosis, therapeutic response, resistance mechanisms, and survival. Extracted variables included study design, patient population, tumor subtype, molecular alterations, pathway involvement, molecular classification model, biomarker profile, diagnostic platform, therapeutic implications, and relevant clinical outcomes. Methodological quality and internal validity were assessed narratively by considering risk of bias, sample size, reproducibility of molecular methods, consistency of subtype definitions, and applicability to clinical practice. In cases of conflicting evidence, greater interpretative weight was assigned to higher-level evidence and guideline-supported recommendations.

Reference lists of included studies were manually screened to identify additional relevant publications. Because of its narrative design, this review is subject to potential selection bias and does not provide pooled quantitative estimates. Artificial intelligence–based tools were used exclusively to assist in literature organization and structural coherence, while critical appraisal, synthesis, and final interpretation were performed independently by the authors to maintain methodological rigor.

Molecular Basis of Colorectal Carcinogenesis

The multistep model of colorectal tumorigenesis describes the progressive transformation of normal colonic epithelium into malignant carcinoma through a series of molecular alterations that accumulate over time. One of the most widely recognized frameworks explaining this process is the adenoma–carcinoma sequence, which outlines the gradual progression from benign adenomatous polyps to invasive adenocarcinoma. This sequence reflects the stepwise acquisition of genetic mutations and epigenetic changes that disrupt normal cellular regulation and ultimately lead to malignant transformation. As these molecular alterations accumulate, they progressively impair mechanisms that regulate cell proliferation,

differentiation, and apoptosis, thereby promoting tumor development [2, 6].

Within this sequence, several key genetic alterations have been consistently identified as major drivers of colorectal carcinogenesis. Mutations affecting the APC, KRAS, and TP53 genes represent central events in this progression and contribute to the activation of multiple oncogenic signaling pathways. These include the Wnt pathway, which plays a critical role in early tumor initiation, as well as the epidermal growth factor receptor and phosphatidylinositol 3-kinase/protein kinase B pathways, both of which further promote tumor growth and progression. Through the activation of these signaling networks, these mutations facilitate the transition from benign adenomatous lesions to malignant colorectal tumors [5].

In addition to these genetic alterations, epigenetic modifications also play a fundamental role in colorectal cancer progression. Changes such as DNA methylation and histone modifications can significantly influence gene expression without altering the underlying DNA sequence. These mechanisms contribute to tumor development by promoting the silencing of tumor suppressor genes and the activation of oncogenes [9, 10]. Among the most relevant epigenetic phenomena in colorectal cancer is the CpG island methylator phenotype, which is characterized by extensive promoter hypermethylation. This process leads to the transcriptional silencing of critical genes, including MLH1, and contributes to the development of mismatch repair deficiency and the subsequent emergence of microsatellite instability [11].

A central feature of colorectal cancer development is the presence of genomic instability, which acts as a driving force for tumor progression by facilitating the accumulation of additional molecular alterations. Two major forms of genomic instability have been described in colorectal cancer: chromosomal instability and microsatellite instability. These mechanisms contribute to tumor heterogeneity and promote the evolutionary processes that allow malignant cells to acquire increasingly aggressive biological characteristics [6, 12].

Chromosomal instability is characterized by widespread structural and numerical chromosomal abnormalities that lead to aneuploidy and alterations in chromosomal architecture. These abnormalities frequently involve both gains and losses of chromosomal regions, resulting in significant genomic imbalance. Common alterations include the loss of chromosomal regions such as 18q, 1p, and 17p, as well as the gain of regions including 8q, which contains the MYC oncogene, 20q, and 7p, where the epidermal growth factor receptor gene is located. These chromosomal changes are strongly associated with disease progression and have been linked to poorer clinical outcomes, highlighting the role of chromosomal instability as a major contributor to the transformation of adenomas into invasive adenocarcinomas [12].

Microsatellite instability represents another important pathway of genomic instability in colorectal cancer and arises from defects in the DNA mismatch repair system. These defects frequently occur as a consequence of epigenetic silencing of mismatch repair genes, particularly MLH1. The loss of mismatch repair function leads to the accumulation of mutations within microsatellite regions of the genome, which consist of short repetitive DNA sequences that are particularly susceptible to replication errors [11]. The presence of microsatellite instability has important clinical implications, as tumors exhibiting this molecular feature are often associated with improved prognosis and demonstrate increased responsiveness to certain forms of immunotherapy [5].

Closely related to microsatellite instability is the CpG island methylator phenotype, which reflects a distinct epigenetic pattern characterized by widespread promoter hypermethylation of CpG-

rich genomic regions. This epigenetic process results in the silencing of multiple tumor suppressor genes and plays a significant role in colorectal carcinogenesis. The CpG island methylator phenotype is strongly linked to the inactivation of genes such as MLH1, thereby contributing to mismatch repair deficiency and the development of microsatellite instability. Tumors exhibiting this phenotype often display distinct molecular and clinical characteristics, and the presence of CpG island methylator phenotype has been associated with specific biological behaviors that can influence therapeutic responses and patient outcomes [9, 11].

Molecular Subtypes of Colorectal Cancer

The consensus molecular subtype classification represents one of the most widely accepted molecular frameworks for understanding the biological heterogeneity of colorectal cancer. Based on transcriptomic analyses, colorectal tumors have been categorized into four principal consensus molecular subtypes, each characterized by distinct molecular features, biological behaviors, and clinical implications. These subtypes reflect differences in genomic instability patterns, signaling pathway activation, immune interactions, and metabolic activity, thereby providing a more refined understanding of colorectal cancer biology. Consensus molecular subtype 1, known as the MSI immune subtype, is characterized primarily by the presence of microsatellite instability and a pronounced activation of immune-related pathways. Tumors within this category exhibit a hypermutated phenotype that results in the generation of numerous neoantigens, which in turn stimulate a strong immune response within the tumor microenvironment. This high level of immune activation contributes to the observed responsiveness of these tumors to immunotherapeutic strategies. Despite this immunogenic profile and the potential benefits of immunotherapy, patients with this subtype tend to demonstrate poorer outcomes in metastatic disease compared with other molecular subtypes [14, 15].

Consensus molecular subtype 2, referred to as the canonical subtype, is defined by the activation of key oncogenic pathways, particularly the Wnt and MYC signaling pathways. This subtype represents the most frequently observed form of colorectal cancer and is commonly associated with chromosomal instability. Tumors belonging to this category typically display classical epithelial characteristics and are often associated with more favorable clinical outcomes. In general, patients with this subtype demonstrate better overall survival and tend to respond more effectively to conventional chemotherapy regimens [6, 15].

Consensus molecular subtype 3, known as the metabolic subtype, is characterized by significant alterations in cellular metabolism and metabolic signaling pathways. Although this subtype is less common than others, it demonstrates distinctive metabolic dysregulation that influences tumor behavior and progression. In precursor lesions, tumors belonging to this category may follow a relatively indolent course; however, during disease progression, these tumors may evolve into more aggressive molecular phenotypes, including transition toward the mesenchymal subtype [16]. The therapeutic response in this subtype is variable, with some patients deriving benefit from targeted therapeutic strategies in addition to conventional treatments [15].

Consensus molecular subtype 4, referred to as the mesenchymal subtype, is distinguished by prominent stromal infiltration and activation of epithelial–mesenchymal transition pathways. These biological features contribute to increased tumor invasiveness and a higher propensity for metastatic spread. Consequently, tumors classified within this subtype are associated with poorer clinical outcomes, reflecting their aggressive biological behavior and frequent resistance to conventional therapeutic

approaches. In addition, this subtype demonstrates a specific dependency on certain kinases, including p21-activated kinase 2, suggesting potential opportunities for targeted therapeutic interventions [17].

The molecular classification of colorectal cancer into consensus molecular subtypes has important clinical implications, particularly in terms of prognostic stratification and therapeutic decision-making. From a prognostic perspective, the classification provides valuable information regarding expected disease behavior and patient outcomes. Among the four subtypes, consensus molecular subtype 4 is generally associated with the poorest prognosis due to its aggressive characteristics and high metastatic potential. Conversely, although consensus molecular subtype 1 is characterized by strong immune activation, patients within this group often demonstrate unfavorable survival outcomes in metastatic settings, illustrating the complex role of immune interactions in tumor progression [16].

In addition to its prognostic value, molecular subtyping also has important therapeutic implications. Different consensus molecular subtypes exhibit distinct patterns of treatment responsiveness, which can inform personalized therapeutic strategies. Tumors classified as consensus molecular subtypes 2 and 3 have been shown to respond more favorably to adjuvant chemotherapy, whereas tumors within the mesenchymal subtype may derive greater benefit from irinotecan-based regimens and emerging targeted therapies, including inhibitors directed against kinases such as PAK2. Tumors within the MSI immune subtype may benefit from immunotherapeutic approaches, although overall survival in metastatic disease remains limited [14, 16]. Furthermore, the consensus molecular subtype framework can assist in the selection of targeted therapies, including anti-epidermal growth factor receptor agents for tumors classified as consensus molecular subtypes 2 and 4 in the context of RAS wild-type status, as well as anti-vascular endothelial growth factor therapies that may be considered for tumors within the MSI immune subtype [17].

Biomarkers in Colorectal Cancer

Diagnostic biomarkers play a crucial role in the early detection of colorectal cancer, as timely identification of malignant or premalignant lesions significantly improves treatment outcomes and patient survival. Several molecular-based diagnostic approaches have been developed to enhance screening accuracy and facilitate noninvasive detection of colorectal tumors. Among these, multi-target stool DNA testing has become an important screening strategy. This method, widely used in the United States, includes tests such as Cologuard Plus, which detect specific DNA mutations and methylation markers in stool samples, thereby allowing the identification of molecular alterations associated with colorectal carcinogenesis. In addition to stool-based testing, liquid biopsy technologies have emerged as promising diagnostic tools. Circulating tumor DNA analysis using next-generation sequencing enables the detection of tumor-derived genetic alterations in blood samples, providing a noninvasive diagnostic approach with high sensitivity and specificity [18, 19]. Epigenetic biomarkers have also demonstrated substantial diagnostic potential. DNA methylation assays targeting genes such as SDC2 and SEPT9, detected in stool or plasma samples, have shown superior diagnostic accuracy in identifying colorectal cancer [20]. Furthermore, microRNAs have gained increasing attention as potential diagnostic markers due to their regulatory role in gene expression. Specific microRNAs, including miR-21 and miR-211, have been identified as promising candidates for early detection of colorectal cancer [21].

Prognostic biomarkers provide valuable information regarding disease progression and patient survival, thereby supporting risk stratification and clinical management. Genetic alterations involving RAS and BRAF represent

important prognostic indicators in colorectal cancer. These mutations are associated with more aggressive tumor behavior and poorer clinical outcomes, making them critical factors in evaluating disease prognosis. Another key prognostic marker is microsatellite instability, which reflects defects in the DNA mismatch repair system. Tumors characterized by high microsatellite instability generally demonstrate improved survival outcomes and distinct biological characteristics, highlighting the importance of this molecular feature in predicting patient prognosis [22, 23]. Epigenetic alterations also contribute to prognostic assessment. Methylation of genes such as NKX6.1 and IGFBP3 has been associated with reduced survival rates, whereas hypermethylation of SFRP2 has been linked to more favorable clinical outcomes. In addition, specific microRNAs have been correlated with patient prognosis. Elevated expression of miR-675-5p and miR-150 has been associated with poorer survival, while increased levels of miR-767-5p and miR-215 have been linked to improved clinical outcomes [20].

Predictive biomarkers are essential for guiding treatment decisions and predicting therapeutic responses in patients with colorectal cancer. Among the most clinically relevant predictive markers are mutations in KRAS and NRAS, which are strongly associated with resistance to anti–epidermal growth factor receptor therapies. The identification of these mutations therefore plays a critical role in determining whether therapies such as cetuximab or panitumumab are appropriate for individual patients [8, 24]. Additional predictive biomarkers include HER2 amplification and the Immunoscore, both of which provide important information regarding potential treatment responsiveness. HER2 amplification has been associated with responsiveness to HER2-targeted therapies, while the Immunoscore evaluates immune cell infiltration within the tumor microenvironment and can help predict response to immunotherapy [22]. Liquid biopsy components also contribute to predictive assessment in colorectal cancer. The analysis of circulating tumor cells and circulating tumor DNA allows clinicians to monitor treatment response and detect minimal residual disease, providing valuable information throughout the course of therapy [19]. In addition, tumor-agnostic biomarkers such as high tumor mutational burden and microsatellite instability have become increasingly relevant in guiding immunotherapy decisions regardless of tumor origin, reflecting the growing role of molecular profiling in precision oncology [23].

Molecular Diagnostics in Clinical Practice

Next-generation sequencing has become a fundamental tool in tumor profiling and has significantly expanded the ability to characterize the molecular landscape of colorectal cancer. Through comprehensive genomic profiling, this technology enables detailed analysis of tumor genetic alterations and facilitates the identification of molecular targets that may be relevant for therapeutic intervention. In colorectal cancer, next-generation sequencing frequently detects mutations in genes such as KRAS, BRAF, and TP53, which play key roles in tumor development and progression and are critical for guiding treatment decisions in clinical practice. Beyond identifying actionable alterations, next-generation sequencing also contributes to prognostic evaluation by enabling highly sensitive mutation detection. In this context, increases in variant allele frequencies of mutations involving KRAS and TP53 have been associated with higher mortality risk, underscoring the prognostic value of molecular profiling in colorectal cancer management [25].

In parallel with tissue-based genomic profiling, liquid biopsy technologies have emerged as important tools for molecular monitoring. The analysis of circulating tumor DNA provides a minimally invasive approach for assessing tumor burden and evaluating treatment response throughout the course of disease. Circulating tumor DNA is particularly valuable in detecting minimal residual disease and identifying early

tumor recurrence, frequently before such changes become evident through conventional radiologic imaging [26, 27]. However, the clinical utility of circulating tumor DNA varies according to disease stage. In advanced disease, particularly in tumors classified as stage T4, circulating tumor DNA demonstrates high concordance with tumor tissue, making it a reliable biomarker for monitoring tumor dynamics and therapeutic response. In contrast, sensitivity is lower in earlier stages, such as T1 through T3, which indicates the need for further improvements in detection technologies to enhance its diagnostic performance in early disease [28]. In addition to monitoring tumor burden, circulating tumor DNA analysis allows for real-time assessment of therapeutic effectiveness and facilitates the identification of emerging resistance mutations. This dynamic molecular monitoring can support timely adjustments to treatment regimens and improve the precision of therapeutic strategies [29, 30].

The integration of molecular testing into clinical algorithms represents an important step toward the implementation of personalized medicine in colorectal cancer management. The combined use of next-generation sequencing and circulating tumor DNA analysis enables clinicians to tailor therapeutic strategies according to the specific molecular profile of individual tumors while also considering dynamic changes in circulating tumor DNA levels during treatment [31, 32]. By incorporating circulating tumor DNA–guided approaches into treatment algorithms, it becomes possible to optimize therapeutic decision-making, reduce unnecessary interventions, and improve patient outcomes through more individualized care. Despite the considerable promise of these technologies, several challenges remain. Issues related to assay standardization, limited sensitivity in early-stage disease, and the complexity associated with integrating multi-omic datasets continue to present obstacles to broader clinical implementation. Future research efforts should therefore focus on improving detection sensitivity, refining molecular testing platforms, and validating emerging biomarkers to facilitate their wider application in clinical practice [33, 34].

Targeted Therapies and Precision Oncology

Targeted therapies have become an essential component of modern colorectal cancer management, with treatment strategies increasingly guided by the molecular profile of individual tumors. Among these approaches, therapies directed against the epidermal growth factor receptor have played a central role in the treatment of metastatic colorectal cancer. However, the effectiveness of anti-EGFR agents is strongly influenced by the presence of specific genetic alterations. Mutations in the KRAS and NRAS genes occur in approximately forty percent of colorectal cancer cases and are well recognized for conferring resistance to anti-EGFR monoclonal antibodies such as cetuximab and panitumumab. These mutations result in constitutive activation of downstream signaling pathways, particularly the MAPK/ERK and PI3K/AKT pathways, which promote tumor proliferation and survival independently of EGFR signaling [35, 36]. Because of this autonomous pathway activation, tumors harboring KRAS or NRAS mutations are unlikely to benefit from EGFR-targeted therapies. The identification of these alterations has therefore become a critical component of molecular testing prior to treatment selection. In particular, the KRAS G12C variant has stimulated interest in the development of specific inhibitors targeting this mutation, although the therapeutic efficacy of these agents in colorectal cancer has so far remained more limited than in other malignancies [37]. Consequently, comprehensive molecular profiling, including the use of liquid biopsy approaches, is routinely employed to identify patients who may benefit from EGFR-directed therapies by excluding individuals with mutations that drive therapeutic resistance [38].

In addition to alterations in RAS genes, mutations affecting the BRAF gene represent another important molecular determinant of treatment response and clinical outcome in colorectal cancer. BRAF mutations, particularly the V600E variant, are identified in approximately ten percent of colorectal cancer cases and are generally associated with more aggressive tumor behavior and poorer prognosis. Historically, the presence of this mutation has been associated with limited responsiveness to standard therapies, which has prompted the development of combination therapeutic strategies targeting the BRAF signaling pathway. These approaches commonly involve the combined use of BRAF inhibitors, such as vemurafenib, together with MEK inhibitors and anti-EGFR antibodies. Such combination regimens have demonstrated improved clinical outcomes in patients with BRAF-mutant colorectal cancer by simultaneously targeting multiple components of the signaling cascade. The rationale behind these combination therapies lies in their ability to overcome adaptive resistance mechanisms that arise through the activation of alternative signaling pathways following BRAF inhibition [39, 36].

Another major therapeutic advancement in colorectal cancer has been the introduction of immunotherapy, particularly in tumors characterized by microsatellite instability. Colorectal cancers with high levels of microsatellite instability arise from defects in the DNA mismatch repair system and account for approximately fifteen percent of all cases. These tumors exhibit a high mutational burden and generate numerous neoantigens, which enhance immune recognition and make them particularly responsive to immune checkpoint blockade. Immune checkpoint inhibitors, including pembrolizumab and nivolumab, have demonstrated substantial clinical activity in patients with microsatellite instability–high colorectal cance. By targeting immune regulatory pathways, these therapies enhance the ability of the immune system to recognize and eliminate tumor cells, thereby improving clinical outcomes. As a result of these benefits, immunotherapy has become a standard treatment option for patients with microsatellite instability–high colorectal cancer, offering durable responses and improved survival in a subset of patients [5, 40].

Beyond currently established therapeutic targets, ongoing research continues to identify additional molecular pathways that may serve as potential targets for future colorectal cancer therapies. Among these emerging targets are components of the PI3K/AKT/mTOR signaling pathway, amplifications involving the HER2 gene, and mutations affecting genes such as ATR and ARID1A. Therapeutic strategies targeting these molecular alterations are currently under investigation in an effort to expand treatment options and address mechanisms of therapeutic resistance. For example, inhibitors directed against ATR are being explored for their potential effectiveness in tumors harboring specific genetic vulnerabilities [39, 40]. At the same time, innovative therapeutic platforms are being developed to further enhance treatment efficacy. These include the development of bispecific antibodies capable of engaging multiple molecular targets simultaneously, as well as RNA-based therapeutic approaches designed to modulate gene expression. Such strategies represent promising areas of investigation aimed at overcoming resistance mechanisms and improving outcomes for patients with colorectal cancer [38].

Future Perspectives in Molecular Pathology

Recent advances in genomic technologies have significantly expanded the understanding of colorectal cancer biology and have contributed to the development of more precise diagnostic and therapeutic strategies. Comprehensive molecular profiling has enabled the detailed characterization of the genetic landscape of colorectal tumors, allowing the identification of distinct molecular subtypes with specific

biological features and therapeutic vulnerabilities. This improved understanding of tumor heterogeneity has facilitated the development of targeted therapies and immunotherapeutic strategies that are tailored to the genetic profiles of individual tumors, thereby supporting the advancement of precision oncology in colorectal cancer management [3, 41]. In addition to bulk genomic analysis, recent progress in single-cell analysis has further refined the understanding of tumor complexity. Through the study of individual cellular populations within the tumor microenvironment, single-cell technologies have enabled the identification of previously unrecognized cell subtypes that play critical roles in tumor progression, offering new opportunities for the identification of potential therapeutic targets. Complementing these advances, liquid biopsy technologies have emerged as valuable tools for molecular monitoring. Methods designed to detect circulating nucleic acids, including circulating tumor DNA and cell-free RNA, provide noninvasive approaches for the early detection of colorectal cancer, the monitoring of treatment response, and the assessment of minimal residual disease during follow-up [3, 34].

Artificial intelligence has also begun to play an increasingly important role in molecular diagnostics and clinical decision-making in colorectal cancer. Machine learning techniques and convolutional neural networks have enhanced diagnostic accuracy by improving the interpretation of complex medical imaging and supporting the identification of molecular and histopathological biomarkers associated with colorectal tumors. In addition to improving diagnostic performance, artificial intelligence systems have demonstrated potential in predictive analytics. By analyzing large datasets that integrate clinical, imaging, and molecular information, these systems can help predict treatment responses and patterns of disease progression, thereby supporting the development of individualized treatment strategies. Artificial intelligence technologies have also been incorporated into therapeutic approaches. Robotic surgical systems, including platforms such as the da Vinci system, have improved surgical precision and operative control, while the application of artificial intelligence in chemoradiotherapy planning has contributed to optimizing treatment delivery and enhancing therapeutic efficacy [42, 43].

These technological developments have also supported the exploration of new therapeutic strategies for colorectal cancer. Advances in targeted therapy have led to the development of monoclonal antibodies and tyrosine kinase inhibitors directed against specific molecular pathways involved in tumor growth and progression. These therapies have shown promising results in improving clinical outcomes for patients with colorectal cancer, although ongoing research continues to address mechanisms of therapeutic resistance and to develop next-generation inhibitors capable of overcoming these limitations [44]. Immunotherapy has also emerged as a major area of therapeutic innovation. The development of immune checkpoint inhibitors and other molecularly targeted immunomodulatory treatments has demonstrated promise in improving prognosis in selected patient populations, especially in cases of metastatic disease [45]. At the same time, continued efforts are focused on identifying novel biomarkers that may serve diagnostic, prognostic, and predictive roles. The discovery and validation of such biomarkers are essential for advancing personalized medicine approaches and improving clinical outcomes in colorectal cancer [46].

Conclusions

Colorectal cancer develops through a multistep process driven by the progressive accumulation of genetic and epigenetic alterations that disrupt key regulatory pathways controlling cell proliferation, differentiation, and genomic stability, ultimately leading to malignant transformation.

Molecular classification and biomarker identification have significantly improved the understanding of tumor heterogeneity in colorectal cancer, enabling more accurate prognostic stratification and facilitating the implementation of personalized therapeutic strategies.

Advances in molecular diagnostics, genomic technologies, and targeted therapies have transformed colorectal cancer management, allowing the integration of precision oncology approaches that improve treatment selection, monitor disease dynamics, and enhance patient outcomes.

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