International Journal of Pathology and Biomarkers
https://ijpb.org/

The Role of Pathogenic Mutations in Colorectal Cancer

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DOI: 10.71428/IJPB.2025.0102

ABSTRACT

One of the most common cancers in the world, colorectal cancer (CRC) is influenced by both environmental and genetic factors. Pathogenic mutations are among the genetic factors that are crucial to the development, course, and response to treatment of colorectal cancer. Unchecked cell proliferation, apoptosis evasion, and metastasis result from key mutations in genes like APC, KRAS, TP53, PIK3CA, and SMAD4 that disrupt important signalling pathways like Wnt/β-catenin, MAPK, PI3K/AKT, and TGF-β. Furthermore, germline mutations in the APC gene and DNA mismatch repair genes, respectively, are the cause of hereditary syndromes such as Lynch syndrome and Familial Adenomatous Polyposis (FAP). We now have a better understanding of these mutations thanks to developments in genomic profiling, which enables more individualized treatment strategies. The main pathogenic mutations linked to colorectal cancer (CRC) are highlighted in this review along with their biological significance, diagnostic implications, and potential as therapeutic targets.

Keywords: Pathogenic mutations; Colorectal cancer; APC gene.

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer death worldwide. Understanding the genetic alterations that underpin these cancers can help researchers develop better diagnostic and treatment strategies, contributing to improved patient outcomes (Morgan *et al*.,2023; Baidoun *et al*.,2021; Hossain *et al*.,2022).

CRC cases are broadly divided into hereditary and sporadic diseases. Although causative mutations have been identified for most hereditary cases, the underlying molecular defects in many sporadic cases remain unclear. Recent whole-exome trinucleotide mutational spectra analysis indicates that relatively few CRC tumours are driven by the well-known cancer predisposition genes. Instead, these tumours often exhibit sequence-like signatures, reflecting a distinct mechanism for tumour initiation and progression (Zhao *et al*.,2022).

Received: April 20, 2025. Accepted: June 18, 2025. Published: July 1, 2025

Pathogenic mutations—genetic alterations that contribute to the development or progression of disease—can be classified as point mutations, insertions/deletions (indels), and copy number variations (CNVs). As described by (Grant *et al.*, 2021), point mutations involve changes of a single nucleotide, such as substitutions or transitions; indels correspond to the insertion or deletion of one or more nucleotides; and CNVs refer to the duplication or deletion of segments of DNA ranging from a few kilobases to entire chromosomes. Underlining their importance in CRC oncogenesis, mutations also indirectly influence tumor growth and spread by modulating the physiological microenvironment (Sina, 2023; Liu *et al.*,2024).

2. Overview of Colorectal Cancer

Colorectal cancer ranks among the cancers with the highest incidence and mortality worldwide and is a leading malignancy affecting Chinese males. Its high morbidity and mortality can be attributed to a combination of factors such as unhealthy dietary habits and an aging population. The initiation and progression of colorectal cancer result from a combination of both internal and external factors. Externally, irregular diet, smoking, alcohol consumption, and a sedentary lifestyle can contribute to the disease's development. Internally, intestinal polyps, ulcerative colitis, Crohn's disease, and diabetes are considered risk factors for colorectal cancer. When combined, these risk factors increase the likelihood of colorectal cancer. Although the pathogenesis of colorectal cancer is not yet fully understood, it is widely accepted that tumorigenesis and progression arise from the accumulation of genetic mutations in oncogenes, tumor suppressor genes, and DNA mismatch repair genes. These genes include KRAS, BRAF, NRAS, PIK3CA, APC, TP53, PTEN, SMAD4, and BRCA1/2 (He & Gan, 2023; Bugter *et al.*, 2021).

Germline mutations in BRCA1 and BRCA2 are associated with breast, ovarian, and prostate cancers. The relationship between BRCA1/2 mutations and colorectal cancer remains unclear, prompting an investigation into whether pathogenic or likely pathogenic BRCA1/2 mutations increase colorectal cancer risk. The establishment of next-generation sequencing technology has facilitated the molecular-level evaluation of BRCA1/2 germline mutations. However, a bias in the region and race of subjects remains evident in existing studies. Given that the Han population accounts for nearly 92% of the Chinese population, it is particularly important to consider the ethnic and regional characteristics of the Chinese population (Vietri *et al.*,2022; Feng *et al.*,2023).

3. Genetic Basis of Colorectal Cancer

Colorectal cancer (CRC) arises from the accumulation of somatic mutations in the colon and rectal epithelium that can occur sporadically or be inherited (Whiffin & S. Houlston, 2014). Characterizing the genes containing these variants remains a challenge; however, advances in genome-wide association studies (GWASs) and next-generation sequencing have enhanced understanding of these mutations. To date, 10% of CRCs are attributed to Mendelian inheritance patterns (Hryhorowicz *et al.*,2022; Rebuzzi *et al.*, 2023).

In 3–5% of CRC cases, the risk is elevated due to mutations in single, highly penetrant genes that result in familial cancer syndromes, including Lynch syndrome, Familial Adenomatous Polyposis (FAP), and Polyposis syndromes. Lynch syndrome (hereditary nonpolyposis colorectal cancer) predisposes to HNPCC-type tumors through germline mutations in genes of the mismatch repair (MMR) pathway—namely, MSH2, MLH1, MSH6, PMS2, and EpCAM. Juvenile Polyposis syndrome is caused by

mutations in SMAD4 and BMPR1A, while Peutz-Jeghers syndrome is associated with mutations in STK11; all these genes encode tumor suppressors with autosomal dominant inheritance. Pathogenic mutations in these genes confer a 45-80% lifetime risk of CRC for affected individuals (Alzahrani *et al*.,2021; Weledji, 2024).

CRC-predisposing, highly penetrant mutations in the APC and MMR genes have been identified in less than 10% of cases; 70% of the familial risk remains unexplained and is not characterized by clear segregation patterns. Recent models predict that an inherited polygenic component accounts for a substantial proportion of the remaining risk, consisting of multiple genetic variants linked to relatively modest increases in disease susceptibility. Inherited susceptibility is therefore considered to result from a continuous index of risk alleles that spans from extremely common, low-penetrance to rare, high-penetrance variants. The latter are likely responsible for a large fraction of the remaining genetic risk (Terradas *et al*., 2020).

4. Pathogenic Mutations: Definition and Significance

Pathogenic mutations are defined as gene alterations that induce protein structure or function changes leading to disease phenotypes. These mutations link genotype and phenotype, sometimes synergistically with other disease-associated gene variants, as observed in cystic fibrosis. For instance, the sea salt substitution mutation D1275N in α1-syntrophin/dystrophin complex has been implicated in long-QT syndrome (LQT). Characterization of pathogenic mutations and indirect cofactors synergistically affecting phenotypic traits provides crucial insights into underlying physiological mechanisms. Moreover, pathogenic mutations associated with inherited diseases suggest drug targets if the mutated gene encodes a druggable protein or participates in a druggable pathway or upstream/downstream network. The identification of such mutations is instrumental in prioritizing drug target candidates. The OMIM database documents pathogenically relevant gene variants. Approximately one-fifth of known genomic loci are implicated in human disease, emphasizing the significance of pathogenic mutations (Nakayama & Oshima, 2018). The notion of pathogenic mutations differs fundamentally from published disease-associated mutations or polymorphisms. The pathogenic-variant test integrates this definition, systematically investigating links between genomic loci, disease phenotypes, and mutation-driven protein structure-function relations (Kim & Bodmer, 2021; Terradas *et al*.,2021; Young *et al*.,2024).

5. Types of Pathogenic Mutations

Pathogenic mutations in genes such as APC, RNA-dependent RNA polymerase (POLR2A), and TP53 are crucial to colorectal cancer (CRC) development. Significant disruption to the APC gene, which activates WNT signaling, has been identified in approximately 80% of CRCs (Noh Hong, 2018). Mutant APC protein cannot degrade CTNNB1, causing its accumulation and subsequent activation of oncogenes such as MYC. Loss of heterozygosity on chromosome 18q is another frequent genetic alteration. Deficiency in nucleotide excision repair (NER) and increased microsatellite instability (MSI) further degrade genomic and chromosomal integrity. POLR2A assists in global genome NER (GG-NER). Because the POLR2A gene is located close to TP53 on chromosome 17, it is often co-deleted (Nakayama & Oshima, 2018). Germ-line and somatic mutations of TP53 appear in approximately 50% of human malignancies. Mutations clustered in the DNA-binding domain of p53 affect its ability to transactivate target

genes and also promote the acquisition of oncogenic functions (Timmerman *et al*.,2021).

5.1. Point Mutations

Somatic protein-coding mutations include single-base, double-base, and frameshift mutations. Many of these are non-randomly distributed in primary colorectal tumours, particularly in the genes APC, TP53, KRAS, and PIK3CA. The APC gene is mutated in most cases; mutational inactivation of TP53 and activation of KRAS probably represent important steps in adenoma progression. PIK3CA is frequently mutated in colorectal cancer, and mutations have been associated with progression and advanced stage. The current somatic mutation spectrum is changing as recent large-scale analyses of colorectal tumours, including adenomas, have provided a much clearer picture of the pathogenesis of colorectal cancer (Wassenaar *et al*.,2024; Ewing *et al*.,2025).

More than 30,000 missense, nonsense, insertions, deletions, and unclassified variants have been listed in the dbSNP database; however, the contribution of all these variants to colorectal cancer disease development is not entirely known. Classifying a variant as pathogenic or as a neutral SNP is not always straightforward. Several mutations associated with colorectal cancer are not part of the Genes Dis database. Many of these mutants are classified as having an 'unknown role' due to the many possible combinations of co-existing mutations. The role and combined effect of these co-existing mutations in colorectal cancer progression are not well understood but require further elucidation (Yildiz *et al*., 2025; Household *et al*.,2022).

5.2. Insertions and Deletions

Insertions and deletions (indels) encompass a spectrum of genetic alterations ranging from one to several base pairs up to megabase pair variations with consequential effects on protein levels, structures, and functions. Indels occur more frequently than point mutations in colorectal cancer and arise from DNA polymerase slippage during replication, especially in microsatellite regions. These microsatellites are short tandem repeats that become particularly unstable in the absence of mismatch repair, leading to frameshift mutations in tumor suppressor and other protein-coding genes (Zhang *et al.*, 2017). Insertions are a specific type of indel where extra nucleotides are introduced into the DNA sequence. Depending on their size and location, insertions can cause frameshifts, disrupting the reading frame of the gene, or add amino acids without altering the downstream sequence. Retrotransposon insertions have been predicted to initiate approximately 1% of CRCs. Analysis of 5,072 insertions revealed that 33% were located within protein-coding genes, predominantly in introns. Recurrent insertions were observed in 333 genes, including 15 listed in the Cancer Gene Census, such as LRP1B, DLG2, PTPRD, and LSAMP, all situated in intronic regions without clustering. The most frequently affected genes not classified as cancer-associated included RCN1, COL25A1, ARAP2, and ZNF251. Seventy-two insertions were detected in exons, notably in PIK3CA and APC; the two APC insertions disrupted the protein reading frame, implicating a role in early tumorigenesis. These findings support a contributory role for retrotransposon insertions in CRC development (Cajuso *et al*., 2019).

5.3. Copy Number Variations

Genes involved in the WNT signaling pathway

are highly conserved across species, and somatic mutations have been reported in key pathway components such as APC and CTNNB1 in many cancer types. In colorectal neoplasia, mutations in APC constitute one of the earliest events and are predominantly truncating, affecting the WNT pathway. APC emerged as the most frequently mutated gene in adenomas, with alterations in other pathway members being relatively infrequent. While APC and CTNNB1 function as oncogenes, AMER1 acts as a tumor suppressor in the context of the WNT pathway. Additional mutated genes in colorectal adenomas include BCL2, FBXW7, KRAS, TP53, and NOTCH1, all of which contribute to tumorigenesis by providing potential selective advantages. Structural variations such as deletions, insertions, duplications, and large-scale copy number variations (CNVs) influence gene expression in both normal and cancer tissues. An increase in the overall load of CNVs was observed from low-grade dysplasia to high-grade dysplasia and subsequently to malignant samples, further linking genomic instability to colorectal cancer development. Rare CNVs may elevate colorectal cancer risk by affecting chromatin-related or olfaction-related genes. Notably, CNVs were detected not only in tumor tissues but also in the normal colon and blood samples of patients. Mutation load progressively increased from low-grade to high-grade adenomas, with carcinoma components exhibiting mutation frequencies comparable to low-grade adenomas. The number of CNVs rose in parallel from low-grade dysplasia to carcinoma. Many genetic alterations, particularly those targeting the WNT signaling pathway, represent early contributors to colorectal carcinogenesis; however, only a few genes were mutated in a substantial proportion of cases, and most mutations affected single instances only (Karczmarski *et al.*, 2019; Jungwirth *et al*.,2022; Matas *et al*.,2022).

6. Key Genes Involved in Colorectal Cancer

Cancer develops through multiple stages when normal cells start to grow abnormally and invade by way of tumour initiation, tumour promotion, and tumour progression. Numerous genes have been identified as key players in the initiation, promotion, and progression of cancer, many of which have been investigated and validated via extensive experimental approaches. Of the several hundred genes associated with colorectal cancer (CRC), four main driver genes—TP53, FBXW7, APC, and ARID1A—are particularly significant (Abdullah & Azlan Nor Muhammad, 2018). Ajuba family proteins regulate the Hippo pathway and promote colorectal tumorigenesis by repressing p53-mediated mechanisms. Mutations in APC or its modifiers are among the earliest defects noted during colon carcinogenesis. Molecular driver mutations in genes such as ERBB3 are critical theranostic targets for CRC patients, offering insights into therapy and strategies to overcome drug resistance. ARID1A loss impairs enhancer-mediated gene regulation and drives colon tumorigenesis in mouse models. Decreased HLA-A expression in CRC tissues is significantly correlated with prognosis. The p53 and DNA mismatch repair (dMMR) genes are major types of pathogenic CRC mutations (H. Zaidi *et al.*, 2020). The gut microbiota continually produces physiological, metabolic, and immunological functions, and bacterial metabolites from the gut microbiota are increasingly recognized as playing a vital role in colorectal carcinogenesis (Zhang *et al*.,2021).

6.1. APC Gene

Colorectal cancer (CRC), a prevalent and often fatal

solid tumor, involves the interplay of numerous genetic factors, among which APC mutations are highly significant. The APC gene—a crucial transcriptional regulator within the Wnt/β-catenin signaling pathway that influences cell division, signal transduction, and tumor suppression—harbors mutations in more than two thirds of CRC patients, with alterations also documented in other tumor types (Li *et al.,* 2017). A novel p.1125Val>Ala mutation in APC has been reported in familial adenomatous polyposis (FAP) and sporadic CRC cases, while the single-nucleotide polymorphism rs11954856 correlates with increased expression of APC, β-catenin, TCF7L1, TCF7L2, LEF1, and GSK-3β, thereby underscoring APC's central role in CRC pathogenesis (Olkinuora *et al*.2021; Zhang *et al*.2023).

The APC gene, situated on chromosome 5q, negatively regulates the beta-catenin/WNT pathway that promotes cell growth and differentiation. Although most APC mutations are somatic, FAP—an autosomal dominant inherited syndrome caused by germline APC mutations—induces early formation of premalignant polyps. FAP variants such as attenuated FAP, Gardner Syndrome, and Turcot Syndrome are all linked to APC malfunction; the latter associates with hereditary non-polyposis colorectal cancer and the emergence of colonic and brain tumors (Noe *et al.,* 2021). APC inactivation is a frequent occurrence in colorectal cancers, yet targeted therapies remain limited and confer minimal survival advantage. Somatic APC mutation initiates approximately 80 % of all CRCs, but the frequency of APC alterations is significantly reduced in early-onset CRCs (Grant *et al.,* 2021). APC forms part of a destruction complex that, in response to WNT stimulation, inhibits β-catenin degradation; loss of APC function sustains constitutive WNT activation in colorectal epithelial cells, thereby preserving the stem cell compartment. Early-onset CRCs lacking APC mutations exhibit alternative molecular mechanisms, and in mismatch repair-deficient CRCs with microsatellite instability, APC mutations are similarly infrequent while BRAF mutations assume a prominent role. Comparative analyses of molecular profiles in microsatellite-stable CRCs with and without APC mutations seek to elucidate APC-independent pathways driving tumor progression (Mzoughi *et al*.,2025; Herranz-Montoya *et al*.,2025).

6.2. KRAS Gene

KRAS is one of the most frequently mutated oncogenes, involved in signal transduction that controls cell proliferation, differentiation, and apoptosis (Zhou *et al.,* 2024). It is present in approximately 40% of colorectal cancer (CRC) patients. KRAS mutations have been linked to diminished progression-free and overall survival in metastatic CRC, with liver metastases particularly affected. Right-sided colon cancers exhibit a higher mutation rate; however, the mutation portends a worse prognosis in left-sided colon cancer. The TMEM16A protein, implicated in tumorigenesis, shows a positive correlation with mutation status. Patients harboring multiple KRAS mutations appear to experience improved prognoses and may derive greater benefit from immunotherapy. Mutation prevalence by codon is 73.3% for codon 12, 20% for codon 13, and 6.67% for codon 61. G12C is the most common mutation, yet G12D associates with the poorest survival outcomes. Novel inhibitors targeting G12D have recently emerged and demonstrate therapeutic potential. Beyond tumorigenesis, KRAS mutations modulate tumor progression and the microenvironment by influencing immune modulation, metabolic

reprogramming, and angiogenesis. (Singhal *et al.*, 2024; Bożyk *et al*.,2021)

6.3. TP53 Gene

TP53 gene, also located on the short arm of chromosome 17 (17p13.1), is one of the most frequently altered genes in human cancers, and its protein product p53 is known to regulate cell cycle arrest, apoptosis, and genomic stability. Mutation of TP53 is commonly found in the late stage of the adenoma–carcinoma sequence: the TP53 protein accumulates in the nucleus and can be detected by immunohistochemistry during the malignant transformation from adenoma to carcinoma. Genetic testing looking for biallelic inactivation of the TP53 gene can be used as a helpful diagnostic for distinguishing whether an adenoma has progressed to becoming an invasive carcinoma (Marbun *et al.*, 2022; Chen *et al*., 2022; Hondelink *et al*., 2023).

Given that the TP53 protein is mutated with a frequency of 40%–50% in colorectal cancer, mutation of non-coding miRNA genes, including miR-34a and miR-34b/c, is also frequently detected in colorectal cancer tissues. CRC samples show decreased expression of some TP53-regulated miRNA genes, including miR-15a, miR-16-1, miR-143, and miR-145 (Kim *et al.*2021).

6.4. PIK3CA Gene

The PIK3CA gene encodes the p110α catalytic subunit of PI3K and is frequently mutated in colorectal cancer (CRC), occurring in approximately 14–16% of cases (Manceau *et al.*, 2015). Hotspot mutations at codons 542, 545, and 1047 enhance PI3K enzymatic activity and drive tumorigenesis, elevating cell proliferation, decreasing apoptosis, promoting angiogenesis, and increasing invasion. The PI3K–AKT–mTOR pathway is a central regulator of cellular growth and survival that is commonly deregulated in human cancers. Mutations in PIK3CA therefore represent potential therapeutic targets, and also influence responsiveness to certain drugs, such as aspirin (Li *et al.*, 2020). In mismatch repair-deficient CRC, PIK3CA mutations occur at a frequency comparable to that in microsatellite-stable tumours. Surveying 5763 Chinese CRC cases, Fu et al. confirmed this trend and illustrated that mutations are clustered predominantly in exons 9 and 20, encompassing 58 distinct types (Fu *et al*., 2021). Diagnostic strategies that combine the high sensitivity of high-resolution melting assays with the robustness of Sanger sequencing optimise the detection of PIK3CA mutations in clinical samples. A retrospective study of microsatellite-stable stage-I-III CRC found that PIK3CA mutations correlate with a better prognosis, a result subsequently validated in a prospective cohort. (Di *et al*.2024)

7. Mechanisms of Tumorigenesis

Tumorigenesis—a complex, multistep process—can develop through disruption of many interlinked pathways. The major tumorigenic pathways in colorectal cancer have been termed chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylation phenotypes (CIMP). Each of these pathways emphasizes a specific aspect of genetic instability that can precipitate colorectal tumor development. CIN is a state of genetic instability that accelerates the loss or gain of whole or large portions of chromosomes. MSI represents a hypermutable phenotype caused by germline mutations of genes responsible for mismatch repair. MSI status can also be the result of germline epimutations, somatic mutations, or methylation of mis-

match repair genes. The CIMP pathway is characterized by specific hypermethylation, and thereby transcriptional silencing, of normally unmethylated CpG islands in the promoter regions of tumor suppressor genes. These pathways are not strictly independent; for example, hypermethylation-induced transcriptional silencing can contribute to MSI, and the CIN phenotype can also arise from defects in other DNA damage response genes (Fard & Mahmoodzadeh, 2024; Angius *et al*.,2021).

When these pathways are disturbed, they allow for the accumulation of molecular alterations in key oncogenes and tumor suppressor genes, which ultimately drive cellular transformation and progression to carcinoma. Individual cancers most commonly move through all three pathways to varying degrees, with some tumors showing heavy influence of a particular pathway. These tumorigenic pathways—either through genetic or epigenetic dysfunction—ultimately produce the diagnostic changes in microsatellite stability and the associated hypermutation phenotype (Li *et al*.2024).

8. Role of the Microenvironment

Colorectal cancer (CRC) is composed of endothelial, stromal, and immune cells, all of which contribute to tumor growth by secreting pro-angiogenic and pro-inflammatory signals (Hanus *et al*., 2021). The immune system, microbiota, and microbial metabolites participate in the CRC environment through a complex and unresolved triad that affects the initiation and/or progression of the tumor. High microbial diversity may be associated with extended disease-free survival, and mucosal samples have shown better microbial signatures compared with fecal samples (B. Burns *et al*., 2018). Stable microbial communities are associated with host cell mutational profiles; loss-of-function mutations in cancer-related genes and pathways such as MAPK and Wnt signaling correlate with defined microbial taxa in the tumor microenvironment (B. Burns *et al*., 2016). Germ-line inactivation of components of the signaling network and mutations induced by deamination can contribute to the disruptive modulation of bacterial populations. Bacterial signals and microbial metabolites, especially short-chain fatty acids, interact with tumor and microenvironmental cells during the carcinogenic process (Zhou *et al*.2024).

9. Genomic Testing and Its Implications

Genomic testing, increasingly available for clinical use, typically sequences a panel of clinically relevant genes or the exome or genome, allowing variant identification in thousands of genes exome-wide or genome-wide. Testing for monogenic variants that confer susceptibility to CRC is broadly indicated for patients with cancer or colorectal polyposis, especially when the clinical presentation is suggestive of an underlying hereditary cancer predisposition syndrome. Certain high-risk individuals without CRC or colorectal polyposis may also benefit from genetic risk assessment. Identifying pathogenic variants indicative of CRC carrier status provides cancer risk assessment, informs treatment planning, enables screening, risk-reducing interventions, and prognosis, and allows assessment and management of risk for relatives. Noncarriers of known pathogenic variants can also be reassured. Numerous expert groups have developed recommendations for the clinical use of germline genomic testing. (Biscaglia *et al*.,2022)

Therapeutic responses in CRC may vary according to the presence or absence of certain molecu-

lar characteristics. The effects of some aspects of tumor genomics on therapeutic response have received sufficient attention to be incorporated into guidelines, clinicians' decision-making, and US Food and Drug Administration drug labeling, indicating the level of evidence necessary to justify the use of tumor genomics in guiding therapy. Tumors impaired in DNA mismatch repair are less responsive to chemotherapeutic agents that damage DNA through inducing methylation, such as 5-fluorouracil. In advanced disease, tumors that lack repair proteins due to the inability to perform mismatch repair because of loss of function in one or more of the mismatch repair genes MLH1, MSH2, MSH6, and PMS2, or through epigenetic silencing of MLH1, are much more susceptible than tumors that have proficient mismatch repair to immunotherapeutic agents that target the immune checkpoint proteins PD-1 and PD-L1. Tumors that harbor BRAF p.Val600Glu show a poor response to standard chemotherapy and monoclonal antibody therapies targeting the EGFR receptor but respond better than do BRAF p.Val600Glu-negative tumors to newer therapeutic regimens involving BRAF inhibitors (Xu et al., 2023; Chabanon *et al*.,2021).

10. Clinical Impact of Pathogenic Mutations

Cancer is rare in the normal population and usually occurs following the accumulation of mutations in key genes important for cell division: homeostatic signalling, cell cycle regulation, proliferation/apoptosis balance, etc. Oncogenes are mutated in a dominant or gain-of-function manner; one copy of the gene is sufficient for tumour formation. Tumour suppressor genes are mutated recessively or loss-of-function; both copies of the gene must be inactivated for tumour generation—examples include APC, TP53, and FBXW7. (Li *et al*.,2023)

In practice, it is difficult to classify some genes as oncogenes or tumour suppressor genes (e.g., oncogenes mutated in a recessive manner or tumour suppressor genes mutated in a dominant manner). Moreover, some genes whose mutation confers chemoresistance exist, although these are generally not required for tumour proliferation. Pathogenic mutations are also linked to prognosis, and it is likely that pathogenic mutations in clinically relevant genes will be part of clinical care. (Zhao *et al*.,2022)

10.1. Diagnosis

In the clinic, multiple diagnostic technologies exist for identifying germline pathogenic mutations associated with hereditary colorectal cancer syndromes in early-onset cases, including Lynch syndrome, familial adenomatous polyposis, and Li-Fraumeni syndrome (Zhang *et al*., 2017). These approaches rely on a stepwise strategy. First, germline variants known to be associated with hereditary colorectal cancer syndromes are selected and searched for evidence of pathogenicity in relevant databases. Subsequently, novel rare variants within the set are evaluated with mutation prediction software; those resulting in protein truncation, non-synonymous missense at conserved positions with a deleterious prediction, or a high CADD score are prioritized. Segregation of these pathogenic mutations at the family level remains crucial, particularly in patients without a recorded family history of colorectal cancer (Gimeno-Valiente *et al*.,2023).

10.2. Prognosis

The prognostic relevance of KRAS and BRAF mutations in colorectal carcinoma has been widely investigated with conflicting results, sug-

gesting for the most part an association between mutation status and prognosis. However, a recent prospective study performed on a community-based cohort of more than 1,200 patients with clearly defined follow-up with curative surgery confirms the association of RAS or BRAF mutations with a higher probability of relapse and cancer death in nonmetastatic colorectal carcinoma. This study also evidenced novel associations between KRAS mutation and specific metastatic sites, which could underlie the link between KRAS mutation and prognosis in colorectal carcinoma. Those data indicate that both KRAS- and BRAF-mutant tumors, compared with wild-type tumors, are associated with an increased likelihood of peritoneal, brain, and lung metastases. AKT1 mutations are a cause of resistance to anti-EGFR therapy and significantly reduce survival. Moreover, data obtained in a large collection of stage I colorectal carcinomas indicate that information on the KRAS/PIK3CA status may contribute to identifying the subset of patients with a worse prognosis that would benefit from additional treatments (Domingo *et al*., 2018; Reggiani Bonetti *et al*., 2018).

10.3. Treatment Strategies

Therapy of colorectal cancer and screening for hereditary colorectal cancer syndromes are linked: If a hereditary cause is found, like Lynch syndrome, affected patients might enter a surveillance program for the early detection of additional cancers. Screening for hereditary causes might also lead to the identification of other family members at risk. For therapy, both germline and somatic mutations need to be considered. For example, an activating mutation of the BRAF gene (often somatic) is associated with poor prognosis, whereas, for patients with colorectal cancer stage III, germline variants in the 5-fluorouracil metabolic route play a role (Tan *et al*.2022; Xu *et al*.2023).

The advancing understanding of carcinogenesis and tumor progression has led to potential new therapeutic modalities. Patients with metastatic colorectal cancer harboring pathogenic mutations in RAS can be treated with a combination of epidermal growth factor receptor (EGFR) antibodies and 5-fluorouracil. Furthermore, immune checkpoint inhibition therapy can be used for tumors with a mismatch repair-deficient status. Large-scale genome studies increasingly convey the message that in the near future not only somatic, but also germline pathogenic variants will guide therapy (Yildirim *et al*.2025).

11. Emerging Therapies Targeting Pathogenic Mutations

However, the relevance of these mutations to therapeutic targeting remains elusive, in part because driver genes elude direct pharmacological inhibition. The secondary effects of pathogenic driver mutations on downstream signaling pathways provide an alternative means to identify therapeutic targets. A multidisciplinary systems approach has been directed toward this problem, exemplified in colorectal cancer (CRC). Global gene expression data from 582 patients were interrogated in 18 Gene Expression Omnibus series. The differential expression of all genes was examined in wild-type and mutant cases of each of the 14 most frequent disruptive mutations. Each disruptive mutation was correlated with the up-regulation of a large number of genes. A survival analysis was conducted in 2,100 patients, and up-regulated genes associated with worse recurrence-free survival were filtered for potentially actionable drug targets. A total of 426 dis-

ruptive mutation-associated upregulated genes were identified, of which 95 were linked to worse prognosis. Based on multiple druggability criteria, 37 potentially actionable targets were revealed, and the seven most promising were validated in 171 colorectal cancer specimens (Menyhart *et al.*, 2019).

12. Ethical Considerations in Genetic Testing

Certain pathogenic germline sequence variants are characteristic of hereditary colorectal cancer (CRC) and may affect diagnostics and targeted treatment of patients with CRC. Next-generation sequencing facilitates simultaneous analysis of large panels of cancer-associated genes and allows identification of new high-risk susceptibility genes, which contribute to an increased understanding of inherited CRC risk. Known inherited cancer syndromes with increased CRC risk include Lynch syndrome, familial adenomatous polyposis, MUTYH-associated polyposis, Peutz–Jeghers syndrome, and juvenile polyposis syndrome (Hassanin *et al*.2023).

Patients with an earlier age-of-onset of CRC may be subjected to hereditary CRC assessment by gene panel-based testing, but such testing raises important ethical questions and often reveals variants of uncertain significance with unclear clinical implications. Germline exome-wide gene panel study of early onset and familial cases of CRC and high-risk polyposis identified a diverse set of high-probability variants distributed across biological pathways affecting CRC risk (Djursby *et al*., 2020). Eighteen variants classified as pathogenic or likely pathogenic were identified in well-established hereditary CRC genes, including APC, POLE, MSH6, PMS2, and CHEK2. Only 50–60% of patients with familial or early onset CRC carry pathogenic variants, reflecting the limited genetic understanding of inherited CRC (Rebuzzi *et al.*, 2023).

13. Future Directions in Research

The molecular and clinical characteristics of CRC have been extensively studied. In the following section, potential paths for further exploration of the underlying CRC genetics, tumor pathogenesis, and clinical management are proposed (Guo *et al*.,2023).

Molecular profiling of cancer tissue at a clinical level continues to expand and is increasingly important for guiding prognosis and treatment options. However, the pathogenesis of biologically and clinically distinct subsets of CRC remains only partially understood. Early-stage efforts are underway to define and characterize changes in DNA methylation, chromatin modification, mRNA and noncoding RNA expression, and protein expression. The complex interactions among dietary and environmental agents, gut microbiome, and inflammation associated with increased CRC risk are also poorly understood. Advances in CRC care therefore, depend on the establishment of molecular subtypes based on genetic alterations, gene profiling, or proteome, and the development of targeted therapies that address specific tumorigenesis mechanisms (Hinoi, 2021). Secondary and germline findings from molecular studies require careful handling to formulate appropriate clinical recommendations. Continued research and clinical efforts are expected to produce further insights into the molecular changes that underlie CRC and to identify means of improving CRC prevention and treatment (Li *et al*.2024).

Molecular profiling methods remain incomplete, and analytical methods require refinement. Much of the evidence on which current CRC-related

mutation sets and associated clinical interpretations are based derives from investigations of single-nucleotide variants (SNVs) and small insertion-deletions (indels). Yet structural variants (SVs) and copy-number alterations can impact larger genomic regions and the formation of fusion genes, which can also drive colorectal tumorigenesis. Moreover, the clinical significance of many newly discovered mutation variants remains unclear (H. Zaidi *et al*., 2020); thus, the ability to correctly classify mutations and the resulting inferences concerning tumor susceptibility or prognosis remain limited (Xu *et al*., 2023).

14. Case Studies of Pathogenic Mutations in Colorectal Cancer

Large-scale studies in colorectal cancer (CRC) have characterized the prevalence and functional impact of somatic mutations in both germline and sporadic tumors. Next-generation sequencing resources enable systematic investigation of somatic single-nucleotide variants and small insertions or deletions. Exome sequencing of 1,134 CRC tumors—alongside targeted sequencing of 1,058 additional tumors—identified 73 genes enriched for non-silent mutations. Mutation load associated with age and cigarette smoking history, as well as other exposures; hypermutated tumors displayed distinct mutation spectra (H. Zaidi *et al.*, 2020).

A sequential strategy examines germline mutations through a targeted panel encompassing 14 CRC susceptibility genes. Inclusion of mismatch repair genes, APC, MUTYH, POLE, POLD1, NTHL1, and CHEK2, enables the detection of a wide spectrum of CRC predisposition alleles. The Geneva panel effectively identifies germline variants in early-onset CRC patients from various ethnic backgrounds; application to 36 unselected Chinese CRC cases found pathogenic variants in 20. Concordance between molecular inversion probe sequencing and Sanger sequencing substantiated robustness; the approach demonstrated sensitivity limitations for copy number variations (Zhang *et al.*, 2017).

15. Public Health Implications

Colorectal cancer (CRC) is one of the most common cancers worldwide and is recognised as a global healthcare problem. Pathogenic germline mutations have been proven to contribute to hereditary CRC in a subset of patients. However, the role of pathogenic germline mutations in sporadic CRC remains unexplored. A genetic test based on next-generation sequencing was established in a patient cohort from the Irish population. Significant associations between Lynch syndrome and familial adenomatous polyposis-associated mutations and CRC were identified. The results indicate a protective role of the homozygous NTHL1 p.Q90* mutation in affected Lynch syndrome patients. (Xie *et al*.2022)

Several genes contain multiple types of risk variants that can cause various inborn conditions or diseases. Pathogenic germline mutations contribute to hereditary colorectal cancer (CRC) in only a small subset of patients. The role of pathogenic germline mutations in sporadic CRC remains less investigated. While a genetic test based on next-generation sequencing has been carried out in an Irish patient cohort, a much larger patient group from the TCGA database was examined. Lynch syndrome variants and familial adenomatous polyposis-associated variants in the AXIN2 gene are significantly associated with CRC risk. Interestingly, the homozygous NTHL1 p.Q90* mutation may protect patients with Lynch syndrome. (Hassanin *et al*.2023; Kim & Bodmer,

2022)

16. Patient Education and Awareness

The importance of patient education and awareness, as well as the role of pathogenic mutations, in colorectal cancer (CRC) is another area of interest. CRC is one of the most commonly diagnosed types of cancer worldwide, accounting for more than 9% of the global cancer incidence. Although the majority of CRC cases have a sporadic origin, between 5% and 10% of affected patients carry a high-risk pathogenic germline mutation associated with a hereditary CRC syndrome. The identification of a pathogenic mutation therefore can help in both the early identification of affected patients and at-risk individuals within their families (Rebuzzi *et al*., 2023).

17. Conclusion

Colorectal cancer (CRC) represents the second most common malignancy and the second leading cause of cancer-related mortality worldwide. It arises through a multistep process driven by the acquisition of successive genetic alterations that disrupt normal homeostasis and elicit a malignant phenotype (Nakayama & Oshima, 2018). The DCC protein has been implicated in epithelial/mesenchymal interactions that regulate proliferation and differentiation, and it is considered a tumour suppressor given that its expression is reduced in over 50% of CRC and its gene reversion can revert the tumour phenotype (Arvelo *et al*., 2015). The TP53 gene encodes the tumour suppressor p53, a major driver of cancer development. Certain p53 missense mutations induce the acquisition of oncogenic functions that influence colorectal tumourigenesis. Studies in human and mouse models have highlighted the complex role of p53 mutations, which can encompass loss of wild-type function and gain of oncogenic properties. The p53 protein normally maintains genomic integrity by controlling the cell cycle, mediating DNA repair, and inducing apoptosis. Consistent with its pivotal tumour suppressor role, p53 is overexpressed and mutated in most colorectal tumours. In the paradigm of multistep tumour progression proposed by Fearon and Vogelstein, mutation of p53 occurs late in colorectal tumourigenesis and correlates with the transition from adenoma to carcinoma. The elucidation of carcinogenic pathways and the intense characterisation of key molecular events are likely to contribute to improvements in the management of colorectal carcinoma (Yang *et al*.2024; Parmar & Easwaran, 2022).

Conflict of interest: NIL

Funding: NIL

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