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Mini Review

Advances and Challenges in Targeted Therapy for Colorectal Cancer: A Focus on Adenomatous Polyposis Coli and Kirsten Rat Sarcoma Virus Mutations

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Abstract

The global burden of colorectal cancer (CRC) is a pressing concern, with a substantial impact on public health. Despite advancements in understanding the molecular mechanisms of CRC development, challenges remain in translating this knowledge into effective clinical interventions. Key genetic mutations, notably in the adenomatous polyposis coli (APC) and Kirsten rat sarcoma virus (KRAS) genes, are central to CRC initiation and progression. Current CRC treatments include surgery and chemotherapy, often combined with targeted agents. However, resistance and heterogeneity within CRC patients limit the effectiveness of these therapies. Promisingly, research has focused on targeting APC and KRAS mutations for therapy. Small molecules inhibiting the Wnt pathway and antibodies targeting specific components are under investigation. Targeting KRAS itself is challenging due to its conserved structure, but disrupting its membrane interactions and subcellular localization are potential therapeutic strategies. To address the limitations of single-drug therapy, combination approaches are gaining traction. Combination therapy not only minimizes off-target effects but also tackles drug resistance and diverse genetic alterations within tumors. The intricate interplay of mutations and pathways in CRC necessitates multifaceted therapeutic strategies. Although progress has been made in understanding CRC genetics and developing targeted therapies, there is still work to be done to translate these insights into effective clinical treatments for CRC patients. This review provides crucial information for novel combination treatments for CRC.

Keywords: Colorectal cancer; Adenomatous polyposis coli; Kirsten rat sarcoma virus; Combination therapy; CRC treatment; Drug resistant.

Abbreviations: 5-FU, 5-fluorouracil; APC, adenomatous polyposis coli; CEB, CCAAT enhancer binding protein; CRC, colorectal cancer; CtBP, C-terminal binding protein; EGFR, growth factor receptor; FZD, frizzled receptors; GDP, guanosine diphosphate; GTP, guanosine triphosphate; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PI3K, phosphatidylinositol 3-kinase; PORCN, porcupine O-Acyltransferase; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; TCF, T-cell factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WNT, wingless-related integration site.

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Introduction

Colorectal cancer (CRC) is a malignant tumor that originates in the colon or rectum. CRC is a significant global health concern, as demonstrated by statistics from 2020, where approximately 150,000 individuals worldwide received a CRC diagnosis, resulting in 53,200 fatalities.[1] Among these patients, 17,930 individuals under the age of 50 were diagnosed with CRC, leading to 3,640 deaths in this age group.[1] Gender differences are apparent, with CRC being more prevalent in males than in females, as evidenced by data from the World Health Organization database. Furthermore, variations in CRC incidence rates are evident globally. Countries such as Australia, New Zealand, Europe, and North America experience higher rates of the disease, while Africa and South-Central Asia exhibit lower rates (Global Burden of Disease Cancer Collaboration). These disparities may stem from factors such as dietary habits, environmental influences, and genetic variations.[2] The rising trend of CRC incidence is particularly evident in China, where the burden on the healthcare system has been steadily increasing, especially in developed regions. A similar

scenario has been observed in Hong Kong, where CRC remains a common form of cancer, as highlighted by 5,634 new cases reported in 2018. Furthermore, the mortality rate for males was 37 per 100,000, while for females, it was 22.2 per 100,000 (Centre for Health Protection 2020).

CRC is not solely attributed to a single genetic mutation; instead, it emerges from intricate molecular signaling pathways characterized by a complex interplay of mutations and disruptions. This process involves a gradual transition from adenoma to carcinoma and eventually to metastatic disease—a multistep journey driven by gene mutations and irregular pathways.[3] Recent advances in genome-wide sequencing have unveiled a comprehensive array of nearly 80 mutated genes implicated in CRC. Notably, among these are adenomatous polyposis coli (APC), Kirsten rat sarcoma 2 viral oncogene homolog (KRAS), and p53.[4] The APC gene mutation, occurring in 70–80% of CRC cases, plays a pivotal role within the Wnt/beta-catenin signaling pathway is significant.[5] In addition to APC, another recurrently observed mutation involves the RAS gene family, especially KRAS, a commonly altered oncogene affecting 30–50% of CRC patients.[6] The p53 gene, functioning as a tumor suppressor, influences the cell cycle, apoptosis, genetic stability, and angiogenesis control.[7] While specific mutations initiate tumorigenesis, it is important to recognize that the progression and development of tumors involve the intricate interplay of multiple genes.[8] Additionally, epigenetic factors such as DNA methylation, histone modifications, chromatin remodelers, and noncoding RNAs have emerged as significant contributors to the advancement and growth of CRC.[9]

This review explores APC and KRAS mutations in colorectal cancer, discusses prevailing treatment challenges, and outlines emerging combination therapies. We aim for this review to enhance comprehension of colorectal cancer's mutational landscape and therapeutic strategies, thereby fostering research and implementation of innovative combination therapies.

APC mutations in CRC

The APC gene holds substantial importance as a frequently mutated tumor suppressor gene within CRC.[10] Situated on chromosome 5q21-q22, this gene spans 8535 nucleotides and comprises 21 exons encoding a 310 kDa protein containing 2843 amino acids. A pivotal site for both germline and somatic mutations of APC lies within exon 15, encompassing 75% of the gene's coding sequence.[11] This finding is consistent with the central role of APC in governing the influence of the Wnt pathway on the proliferation and differentiation of gastrointestinal tract cells.[12] Mechanistically, APC plays a pivotal role in inhibiting β-catenin/T-cell factor (TCF)-dependent transcription through complex breakdown. This process involves stimulating the phosphorylation and subsequent ubiquitin-dependent degradation of β-catenin.[13] APC bolsters this degradation mechanism by promoting Axin multimerization and stabilizing the Axin complex.[14] Additional regulatory mechanisms include reducing nuclear β-catenin levels through the promotion of β-catenin export, direct binding to β-catenin to impede TCF interactions,[15] and facilitating C-terminal binding protein (CtBP)-mediated repression of Wnt-target genes through direct interaction with a repression complex.[16,17] Alterations in APC result in the activation of β-catenin/TCF transcriptional activity due to β-catenin accumulation. This attenuation of CtBP-mediated inhibition within the repression complex leads to elevated levels of downstream targets, including cyclin D1 and c-myc. These factors significantly influence tumor cell proliferation, apoptosis, and cell cycle regulation (Fig. 1).[18,19] Evidently, APC intricately interacts with critical signaling pathways and biological processes implicated in CRC development.[10] Recent investigations have shown that restoring APC functionality can promote tumor regression and restore crypt homeostasis in CRC, suggesting that the Wnt pathway is a promising therapeutic target for CRC treatment.[20]

KRAS mutation in CRC

KRAS is one of the most commonly mutated genes in human cancer and has significant implications for CRC treatment. Within this context, various forms of KRAS mutations have been categorized into three main groups based on the altered codon: G12 (mutations at codon 12), G13 (mutations at codon 13), and Q61 (mutations at codon 61).[21] Notably, KRAS mutations are prevalent in approximately 30–50% of CRC cases.[6] Among these mutations, 15 distinct point mutations are found to be particularly significant: G12A, G12D, G12F, G12K, G12N, G12S, G12V, G12Y, G12C, G12E, G12I, G12L, G12R, G12T, and G12W. Of these, G12D and G12V are the predominant subtypes, accounting for approximately 41% and 28%, respectively, of all G12 mutations.[22] Clinical investigations consistently indicate that CRC patients carrying KRAS mutations tend to experience reduced survival rates compared to those without such mutations.[23] Moreover, within the realm of KRAS mutations, G12D and G12V mutations have been associated with the poorest prognoses among CRC patients.[24] Additionally, research findings demonstrate that individuals with G13 mutations in CRC patients experience significantly lower survival rates when diagnosed at stage I or II than when diagnosed with wild-type KRAS.[6,25] Furthermore, for CRC patients harboring mutations at codon 12, the 5-year overall survival rate is notably lower than that for those carrying codon 13 mutations or wild-type KRAS.[26]

KRAS functions as a pivotal sensor that initiates a cascade of signaling molecules, facilitating the transmission of signals from the cell surface to the nucleus. This activation process significantly influences essential cellular functions, including cell differentiation, growth, chemotaxis, and apoptosis. Notably, KRAS plays a critical role in regulating key signaling pathways such as the PI3K-Akt and RAS-RAF-MAPK pathways, which play pivotal roles in cell proliferation.[27–29] KRAS functions as a downstream component of the epidermal growth factor receptor (EGFR) pathway. Upon EGFR activation, the intracellular tyrosine kinase phosphorylates and activates KRAS, subsequently triggering the RAS-RAF-MAPK pathway. After activation, KRAS transitions to its activated state, KRAS-GTP, which is later hydrolyzed by GTPase, returning to the inactive KRAS-GDP state. This dynamic equilibrium involves alternating between its active (KRAS-GTP) and inactive (KRAS-GDP) forms. However, mutations within KRAS lead to the abnormal activation of downstream pathways, such as RAS-RAF-MAPK or phosphoinositide 3-kinase (PI3K), regardless of EGFR activation status (Fig. 2).[30,31] Persistently active KRAS results in irregular and uncontrolled cell growth, cellular transformation, heightened cancer metastasis, and increased resistance to chemotherapy and EGFR-targeted therapies across various cancer types, including CRC.[32,33]

Clinical challenges

Surgery stands as the primary curative approach for patients with nonmetastatic CRC, while chemotherapy offers an alternative therapeutic avenue. Notable drugs utilized for CRC treatment include

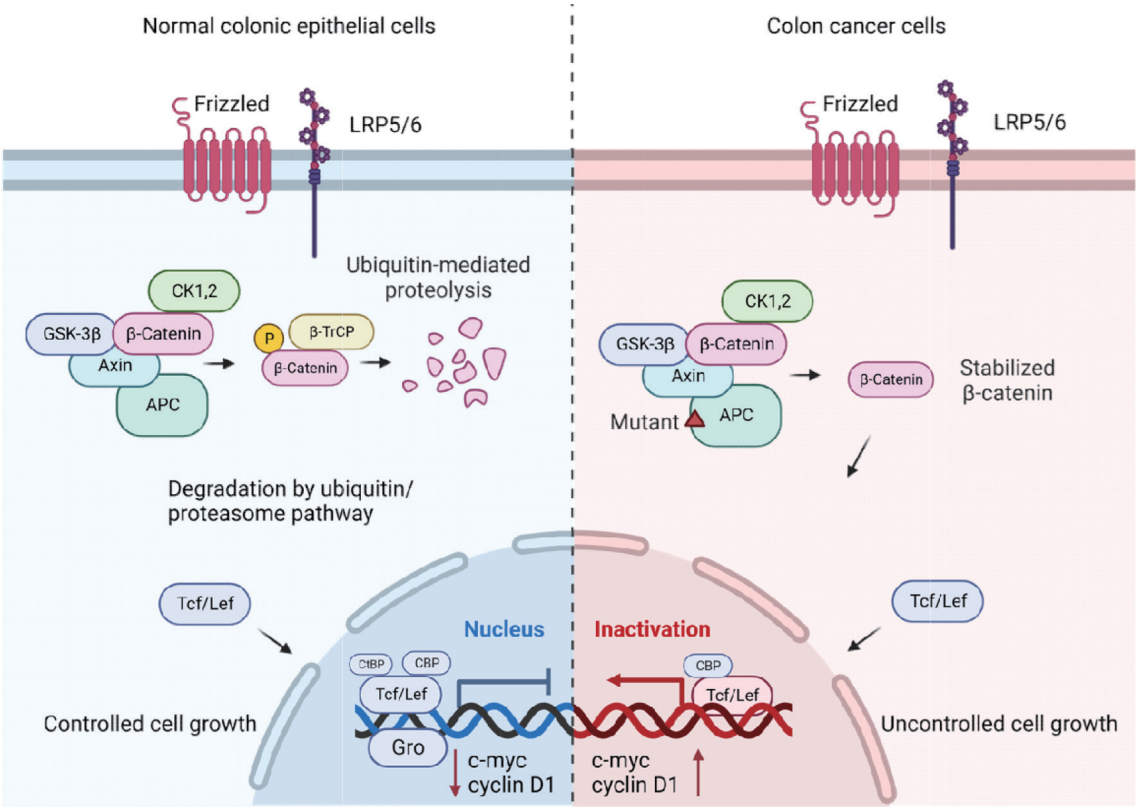

Fig. 1. A schematic diagram showing the Wnt signaling pathway in normal colonic epithelial cells and colon cancer cells. APC, adenomatous polyposis coli; CBP, cAMP-response element binding protein; CIBP, calcium- and integrin-binding protein; CK, creatine kinase. CtBP, C-terminal binding protein; GRO, growth-regulated oncogene alpha; GSK, glycogen synthase kinase LRP5/6, low-density lipoprotein receptor-related protein (LRP)5 and 6; Tcf/Lef, T-cell factor/lymphoid enhancer factor.

5-fluorouracil (5-FU), capecitabine, irinotecan, oxaliplatin, cetuximab, and panitumumab.[34] In addition to conventional chemotherapy, targeted agents play a role in treating metastatic CRC. For example, cetuximab, the first FDA-approved targeted drug for CRC, targets EGFR. Additionally, bevacizumab, focusing on VEGF, has gained approval. Other drugs like panitumumab, regorafenib, and ramucirumab, all targeting VEGF/VEGFR, have also been approved for CRC treatment. Notably, recent years have seen the approval of immune checkpoint inhibitors such as pembrolizumab, nivolumab, and ipilimumab.[35] However, the landscape of CRC is complex and characterized by multifaceted processes marked by a sequence of genetic alterations.[36] Notably, the pronounced occurrence of tumor heterogeneity in CRC, stemming from chromosomal instability or microsatellite instability,[37] collectively influences the efficacy of targeted therapies.

Despite these promising avenues, drugs specifically targeting APC and/or KRAS mutations have yet to receive FDA approval. CRC frequently involves APC and KRAS mutations, rendering them attractive therapeutic targets. However, it is important to note that medications aimed at targeting the APC/WNT/beta-catenin signaling pathways are currently in the preclinical development phase (Table 1).[38,39–46]

Over the past decade, a dedicated pursuit has aimed to advance therapeutic strategies against the APC/WNT/beta-catenin signaling pathway in CRC patients. This endeavor has led to the discovery of a range of small molecules that effectively inhibit this pathway by targeting various signaling molecules.[38,47,48] Notably, phase 1 and 2 clinical trials have been conducted for these inhibitors, including WNT974, ETC-1922159, RXC004, and CGX1321, which are PORCN inhibitors; OTSA101-DTPA-90Y, which functions as an FZD10 antagonist; OMP-18R5, a monoclonal antibody targeting FZD receptors; and PRI-724, a CEB/beta-catenin antagonist.[49] Despite these promising efforts, none have yet secured FDA approval for CRC treatment. The exceptional complexity of the APC/WNT/beta-catenin pathway plays a significant role in this process. Beyond APC mutations, beta-catenin can be further activated through alternate signaling pathways.[50–53] Numerous studies suggest that these supplementary regulatory processes contribute to the observed limitations in achieving satisfactory clinical outcomes with these inhibitors and antibodies. Moreover, the potential toxicity of these inhibitors on the intestinal epithelium, coupled with the risk of off-target effects, might have hindered their progress in clinical applications (Table 2).[54]

Presently, there is a lack of approved drugs specifically targeting KRAS for CRC treatment. Instead, approvals have been directed toward inhibitors of downstream signaling cascades, such as the RAF and MEK pathways (Table 1).[55] For example, selumetinib (AZD6244), functioning as a MEK 1/2 inhibitor, is designed to hinder the MEK enzyme within the RAS/MAPK pathway. Additionally, trametinib, a potent and selective ATP-independent inhibitor of MEK1/2 kinases, falls within this category.[56] Another example is GDC-0623, a MEK inhibitor that enhances BIM expression, which is currently under investigation in a phase I clinical trial.[57] However, concentrating solely on downstream cascades unrelated

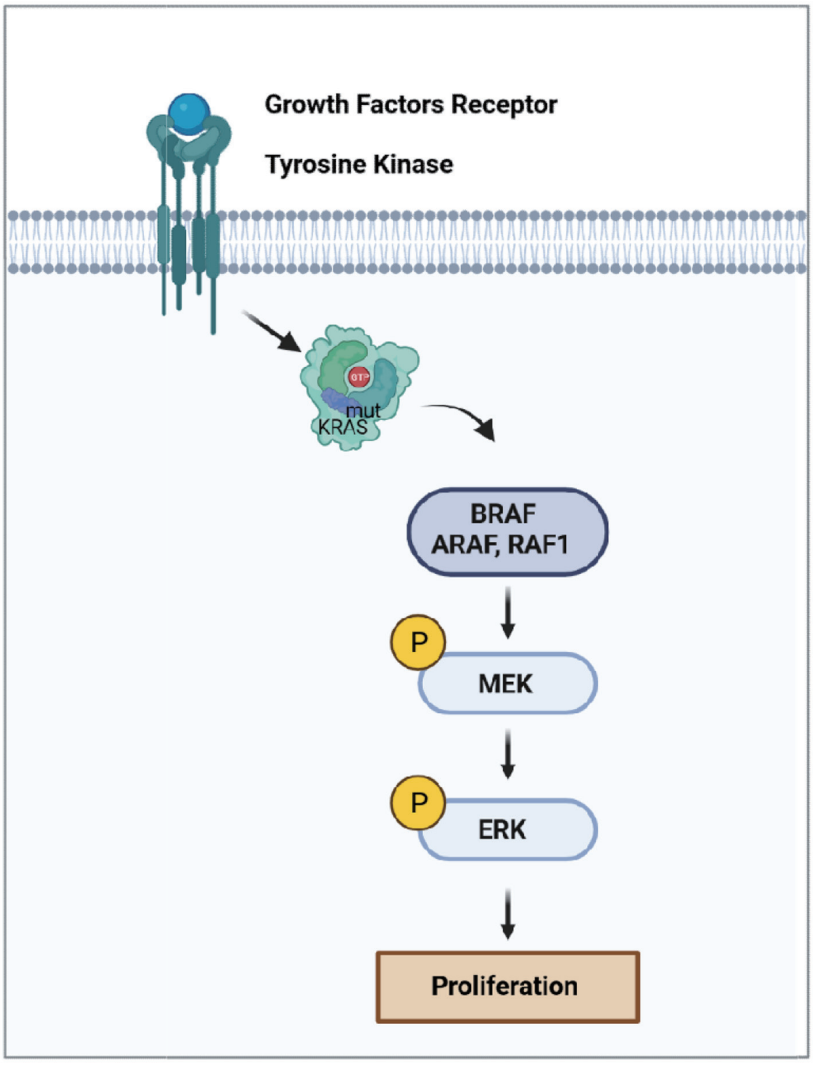

Fig. 2. A schematic diagram showing oncogenic signaling pathways associated with mutated KRAS. ARAF, serine/threonine-protein kinase A-Raf; BRAF, B-Raf proto-oncogene, serine/threonine kinase; ERK, extracellular signal-regulated kinase; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; MEK, mitogen-activated protein kinase kinase; RAF1, rapidly accelerated fibrosarcoma 1.

to KRAS may not yield the desired effectiveness in cancer treatment. This challenge might arise from the inherent difficulty in pharmaceutically targeting KRAS.[58] Research has highlighted several obstacles in the quest for KRAS-targeted treatments (Table 2). These include the highly conserved nature of the GTPase catalytic domain on KRAS proteins, the competitive binding issues faced by small molecule drugs with substrates, and the limited number of binding sites on the KRAS protein surface for small molecule inhibitors.[59–64] Nevertheless, strategies aimed at disrupting KRAS-membrane interactions and altering KRAS subcellular localization continue to hold promise. Recent insights into functionally significant posttranslational modifications of the KRAS protein, including phosphorylation and ubiquitylation, introduce novel prospects for inhibiting KRAS activity.

Development of novel drug combinations for CRC treatment

The inception of combination therapy dates back to 1965 when Emil Frei and colleagues pioneered the inaugural utilization of combination chemotherapy in pediatric patients afflicted with acute leukemia.[65] The resounding success of this innovative therapeutic paradigm ushered in a transformative era within clinical oncology.[66] Subsequently, cancer research has increasingly focused on the exploration of combination therapies designed to concurrently target disparate molecular pathways, resulting in favorable anticancer outcomes. Concurrently, progress in cancer cell genomics, epigenomics, transcriptomics, and proteomics has facilitated the identification of novel molecular targets, underpinning the development of highly selective targeted anticancer interventions.[67] These targeted therapies have substantially diversified the arsenal of combinational anticancer modalities, capable of integration with other targeted therapies or conventional chemotherapeutic agents.[68]

The efficacy of single-drug therapy often encounters limitations, leading to the emergence of drug resistance.[69] In fact, resistance to 5-FU treatment occurs in approximately half of all CRC patients.[70] Recently, there has been a growing focus on combining

Table 1. Selected targeted therapy trials for colorectal cancer

Treatment	Trail	Sample size	Study groups	Response rate	Side effects	Reference
WNT974	Phase 1	94	BRAF-mutant CRC, BRAF-mutant CRC with RNF43 mutation and/or RSPO fusion	N.A	Dysgeusia, Decreased appetite, and Nausea	39
ETC-1922159	Phase 1	20	Metastatic solid tumors	N.A	Dysgeusia, β-CTX increase, Fatigue, Constipation, and Nausea	40
RXC004	Phase 2	20	RNF43 or RSPO aberrated, metastatic, microsatellite stable colorectal cancer	Ongoing	Ongoing	41
CGX1321	Phase 1	77	colorectal cancer or small bowel cancer carrying RSPO or RNF43 alterations	N.A	Dysgeusia, Bone resorption	42
OTSA101-DTPA-90Y	Phase 1	20	Progressive advanced Synovial Sarcomas	N.A	Reversible hematological disorders	43
OMP-18R5	Phase 1	18	Advanced solid tumors	N.A	Fatigue, Vomiting, Abdominal pain, Constipation, Diarrhea and Causea	44
PRI-724	Phase 1	18	Advanced solid tumors	N.A	Hyperbilirubinemia, Diarrhea, Bilirubin elevation, Hypophosphatemia, Nausea, Fatigue, Anorexia, Thrombocytopenia and Alkaline phosphatase elevation.	45
GDC-0623	phase 1	45	Advanced solid tumors	N.A	Rash, Gastrointestinal symptoms and Visual disturbance	46

BRAF, B-Raf proto-oncogene, serine/threonine kinase; CRC, colorectal cancer; RNF, ring finger protein; RSPO, R-spondin; β-CTX, serum C-terminal telopeptide of type I collagen.

drugs to leverage synergistic interactions. Combination therapy offers notable advantages. First, it allows for reduced drug dosages, thereby decreasing the risk of off-target side effects.[71] Second, this approach targets multiple facets, effectively curbing the development of drug resistance.[72] These attributes hold particular importance when addressing heterogeneous cancers such as CRC.[73] The intrinsic heterogeneity of CRC is well documented. In some cases, patients with the same tumor may display distinct genetic alterations, and even cells within a tumor might carry varying genetic mutations. Resistance to a single chemotherapeutic agent, whether innate or acquired, can stem from factors such as suppressed apoptosis or enhanced DNA repair, leading to cancer relapse or treatment resistance. Therefore, combination therapy is especially advantageous because diverse drugs can target different pathways or genes. This approach substantially reduces the number of cancer cells that can withstand treatment, effectively delaying cancer recurrence and, optimally, achieving complete eradication.

The utilization of combination chemotherapy has evolved into the prevailing standard of care within the field of medical oncology. Considering the profusion of available chemotherapeutic and targeted anticancer agents, forecasting and developing innovative drug combinations presents a formidable challenge. Thus, it is imperative to explore the requisite methodologies for prognosticating combinations that exhibit synergistic anticancer efficacy.

Conclusion

CRC represents a significant global health challenge, with considerable variations in incidence rates across regions and gender differences. Among numerous genes that contribute to CRC development, APC and KRAS mutations are pivotal factors driving tumorigenesis. Current research efforts are focused on inhibiting the APC/Wnt/beta-catenin and KRAS pathways. While progress has been made in the field of small molecules and inhibitors, their clinical application has encountered hurdles due to the complexity of these pathways and the emergence of alternative signaling mechanisms. Combination therapy has emerged as a promising approach to address the complexity and heterogeneity of CRC. By targeting multiple facets and pathways simultaneously, combination therapies can potentially enhance treatment efficacy, mitigate drug resistance, and ultimately improve patient outcomes.

Acknowledgments

None.

Table 2. Hurdles of development of targeted therapies

Target	Obstacles
APC	Potential toxicity; Off-target effects.
KRAS	Highly conserved nature of the GTPase; Catalytic domain on KRAS proteins; Competitive binding issues; Limited binding sites.

APC, adenomatous polyposis coli; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; WNT, wingless-related integration site.

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Conflict of interest

All authors declared that there are no conflict of interests.

Author contributions

Conceptualization (GQC, SC, RHG), Data curation and original draft preparation (RHG, GQC, SC), Figures and Tables (RHG, GQC, SC), Review and editing (GQC, SC, RHG). All authors contributed to the article and approved the final manuscript.

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