Journal of Clinical and Nursing Research, 2026, Volume 10, Issue 4
http://ojs.bbwpublisher.com/index.php/JCNR
Online ISSN: 2208-3693
Print ISSN: 2208-3685

Research on Drug Resistance Mechanisms and Countermeasures of Targeted Therapy for Colorectal Cancer Based on Next-Generation Sequencing

Mengyi Li

Henan Provincial Hospital of Traditional Chinese Medicine, Zhengzhou 450002, Henan, China

Abstract: Colorectal cancer, a malignant gastrointestinal tumor with high morbidity and mortality, has long been plagued by difficulties in treatment and high recurrence rates, seriously threatening patients' lives and health. With the continuous development of molecular biology technology, targeted therapy has emerged and shown great value in improving the survival and prognosis of patients with colorectal cancer. However, drug resistance is common in clinical practice, which directly impairs therapeutic efficacy. Next-Generation Sequencing (NGS), a high-efficiency and high-throughput molecular detection technology, can deeply analyze the genomic characteristics of tumors, accurately identify relevant molecular targets, and provide important support for the study of drug resistance mechanisms and the formulation of individualized countermeasures. This paper discusses the drug resistance mechanisms of targeted therapy for colorectal cancer based on NGS and the corresponding countermeasures, aiming to provide reference for relevant practitioners.

Keywords: Next-generation sequencing; Colorectal cancer; Targeted therapy; Drug resistance mechanism; Countermeasures

Online publication: Apr 30, 2026

1. Introduction

Colorectal cancer is one of the most common malignant tumors worldwide, characterized by high incidence and mortality. The advent of targeted therapy has transformed the treatment of colorectal cancer from traditional surgery and chemotherapy to individualized targeted therapy, which has gradually become an important approach for the treatment of advanced colorectal cancer [1]. At present, clinical targeted drugs for colorectal cancer mainly include EGFR, VEGF and BRAF inhibitors, which can effectively prolong patients' progression-free survival and overall survival. Nevertheless, drug resistance is a key factor limiting their therapeutic efficacy. The application of NGS can accurately and rapidly detect gene mutations, copy number

variations and other molecular alterations in tumor genes, providing precise molecular evidence for the study of drug resistance mechanisms and the formulation of individualized treatment strategies [2]. Therefore, it is of great practical significance to explore the drug resistance mechanisms of targeted therapy for colorectal cancer based on NGS and the corresponding countermeasures.

2. Common drugs for targeted therapy of colorectal cancer and classification of drug resistance

2.1. Common drugs for targeted therapy of colorectal cancer

2.1.1. EGFR inhibitors

Targeted therapy against the epidermal growth factor receptor (EGFR) in colorectal cancer has seen widespread clinical application of several drugs. These agents primarily function as monoclonal antibodies, aiming to block the EGFR signaling pathway and thereby inhibit tumor growth. Currently, EGFR-targeted therapy for colorectal cancer mainly relies on two monoclonal antibodies: Cetuximab and Panitumumab. Both drugs specifically bind to EGFR, preventing ligand binding and subsequently inhibiting the activation of downstream signaling pathways.

Cetuximab: As a chimeric monoclonal antibody, Cetuximab is widely used in the treatment of RAS wild -type, left-sided metastatic colorectal cancer (mCRC) and is one of the preferred targeted therapies. Its efficacy has been validated in multiple Phase III clinical trials, demonstrating significant improvements in both progression-free survival (PFS) and overall survival (OS).

Panitumumab: As a fully humanized monoclonal antibody, Panitumumab has a mechanism of action similar to that of Cetuximab. It is also an important treatment option in clinical practice, particularly for patients with specific molecular subtypes.

2.1.2. VEGF inhibitors

Such agents mainly cut off the nutrient supply of tumors by blocking VEGF expression or binding, thereby effectively inhibiting tumor angiogenesis. Common ones include ramucirumab and bevacizumab [4]. Bevacizumab is the most widely used in targeted therapy of colorectal cancer and can be combined with chemotherapy in first- and second-line treatment. In addition, the efficacy of these drugs is not affected by RAS gene status, but drug resistance still occurs.

2.1.3. BRAF inhibitors

These drugs are mainly used for patients with BRAF-mutated colorectal cancer. BRAF is a key node in the RAS-RAF-MEK-ERK pathway, and its mutation leads to continuous activation of the pathway [5]. Common drugs include encorafenib and vemurafenib. Among them, encorafenib combined with cetuximab has been approved for the treatment of advanced colorectal cancer with BRAF V600E mutation. However, in clinical practice, most patients develop drug resistance after a period of administration, affecting long-term efficacy.

2.2. Classification of drug resistance in targeted therapy of colorectal cancer

According to the occurrence time and mechanism, drug resistance in targeted therapy of colorectal cancer can be divided into primary resistance and acquired resistance.

Primary resistance refers to the existence of drug-resistant molecular alterations in tumor cells before treatment, resulting in no obvious efficacy after treatment. It is mainly related to inherent molecular characteristics of tumors such as RAS and BRAF gene mutations, so it is also called innate resistance [6].

Acquired resistance refers to significant initial efficacy followed by tumor recurrence or progression after 6–12 months of treatment, mainly due to adaptive changes of tumor cells under drug selection pressure.

In addition, based on resistance mechanisms, drug resistance can be divided into target-dependent and target-independent resistance. In summary, both can be distinguished by NGS, providing important information for subsequent treatment and avoiding ineffective therapy.

3. Analysis of drug resistance mechanisms of targeted therapy for colorectal cancer based on NGS

3.1. Drug resistance mechanisms related to target abnormalities

Target abnormality is the most dominant mechanism of drug resistance, which mainly includes the following aspects.

First, drug resistance caused by target gene mutations. As a key target in colorectal cancer targeted therapy, the development of resistance to EGFR is directly associated with specific mutations. For instance, approximately 10% of patients with microsatellite-stable colorectal cancer harbor the EGFR variant III (EGFRvIII) mutation, a deletion mutant receptor that correlates with poor response to cetuximab treatment. Furthermore, BRAF gene mutations are common in colorectal cancer, particularly the BRAF V600E mutation , which is often closely linked to poor patient prognosis and resistance to conventional chemotherapy.

Second, drug resistance mediated by target gene amplification. Overexpression of the target protein impairs effective inhibition by the drug, thereby inducing resistance. For example, HER2 gene amplification leads to cross-activation of the EGFR pathway, and MET gene amplification activates the PI3K-AKT pathway, both are important drivers of resistance.

Third, drug resistance resulting from target gene deletion. This mainly refers to the loss of target protein expression, rendering the drug without a functional target. For instance, deletions of EGFR and VEGF can cause primary resistance to the corresponding inhibitors, respectively. Such events are relatively rare in clinical settings.

3.2. Drug resistance mechanisms related to abnormal activation of signaling pathways

Abnormal reconfiguration or cross-activation of signaling pathways is an important cause of drug resistance. Specifically, it is manifested in the following aspects:

First, the RAS-RAF-MEK-ERK pathway is abnormally activated. For this pathway, it is the most frequently abnormally activated pathway in colorectal cancer. The main reason for this is the mutation of the RAS gene (accounting for 40–50% of colorectal cancer patients), which also leads to resistance to EGFR inhibitors (primary) [8]. At the same time, for those patients who have received EGFR inhibitor treatment, they may also face the problem of acquired RAS mutations, thereby keeping the pathway in an activated state. The BRAF gene mutation may lead to the activation of the pathway, resulting in inhibitor resistance and cross-resistance of other inhibitors.

Second, the PI3K-AKT-mTOR pathway is abnormally activated. This pathway has cross-dialogue with the first pathway. The main reasons for this are the mutation of the PI3KCA gene and the deletion or mutation of the PTEN gene, both of which make tumor cells resistant to related inhibitors. The over-activation of the PLK1-MYC-CDC7 pathway is associated with chemotherapy resistance in colorectal cancer, and combining PLK1 inhibitors can enhance the efficacy of oxaliplatin [9].

Third, other signaling pathways may also be abnormally activated, such as the ET pathway (activated

mutations, high expression of HGF ligand) and the cross-activation with the EGFR pathway, the abnormal activation of the FGFR pathway promotes angiogenesis, and the abnormal activation of the TGF-β pathway promotes EMT, all of which led to drug resistance. Second-generation sequencing can comprehensively detect related genetic alterations.

3.3. Drug resistance mechanisms related to tumor microenvironment changes

Changes in the tumor microenvironment affect the drug resistance of tumor cells, mainly reflected in:

First, abnormal activation of cancer-associated fibroblasts (CAFs). As the major stromal cell type, activated CAFs secrete cytokines including TGF-β and IL-6, which in turn activate resistance pathways [10]. Next-generation sequencing has revealed high expression of genes such as FAP in CAFs, which promotes their activation, EMT, and tumor angiogenesis, ultimately leading to resistance.

Second, abnormal immune cell infiltration. In drug-resistant patients, tumor tissues show reduced $CD8^+$ T-cell infiltration and increased infiltration of Tregs and M2-type macrophages. The former facilitates immune evasion, while the latter supports tumor survival and resistance. Furthermore, KRAS-mutant colorectal cancer often exhibits a “cold tumor” phenotype, further elevating drug resistance.

Third, abnormal cytokines and extracellular matrix. For example, aberrant expression of IL-8 and IL-6 in some patients activates resistance pathways. Meanwhile, excessive collagen deposition impedes drug penetration, resulting in resistance [11].

3.4. Drug resistance mechanisms related to abnormal epigenetic regulation

Abnormal epigenetic regulation directly causes disordered gene expression, leading to drug resistance:

First, abnormal DNA methylation. Hypermethylation of genes such as MLH1 and p16 leads to gene silencing and enhanced resistance. For example, MLH1 hypermethylation causes DNA mismatch repair deficiency, conferring resistance to related chemotherapies and inhibitors.

Second, abnormal histone modification. High expression of genes such as HDAC disturbs histone modification, activating oncogenes and silencing tumor suppressors. HDAC inhibitors can restore the expression of tumor suppressor genes and reverse drug resistance [12].

Third, dysregulated non-coding RNAs. Low expression of miR-143 and miR-145 results in high EGFR expression; high expression of lncRNA H19 activates the PI3K-AKT pathway; and circRNA CDR1as sponges miR-7 to regulate EGFR expression, all contribute to resistance. Next-generation sequencing allows comprehensive profiling to identify abnormal non-coding RNA expression.

4. Countermeasures for drug resistance of targeted therapy for colorectal cancer guided by NGS

Based on the drug resistance mechanisms analyzed by NGS and combined with clinical practice, targeted countermeasures can be formulated. The core is to accurately identify drug resistance targets through NGS to achieve individualized therapy and reverse or delay drug resistance.

4.1. Individualized medication adjustment: Replacing or combining targeted drugs based on NGS results

Personalized medication adjustment is a core strategy relying on NGS to clarify resistance targets, on the basis of which targeted drugs are combined or switched for precise treatment.

First, timely medication adjustment according to target abnormalities. For example, patients with EGFR inhibitor resistance and MET amplification can switch to MET inhibitors or receive combination therapy;

those with BRAF inhibitor resistance and secondary mutations may switch to combined MEK inhibitors or next-generation BRAF inhibitors[13].

Second, timely medication adjustment based on signaling pathway abnormalities. For example, for abnormalities in the RAS-RAF-MEK-ERK pathway, medications can be adjusted to MEK inhibitors or combined with EGFR inhibitors; for dysregulation of the PI3K-AKT-mTOR pathway, combination therapy with EGFR inhibitors plus PI3K/mTOR inhibitors is recommended.

Third, timely adjustment of personalized medication through dynamic monitoring. For instance, regular ctDNA detection via NGS enables early identification of new resistance targets, facilitating prompt adjustments to medication or treatment regimens. Liquid biopsy can monitor the dynamics of the resistance genome and screen patients eligible for anti-EGFR therapy rechallenge, which has been proven effective in clinical trials.

4.2. Combined therapy strategy: Reversing drug resistance through synergy

Combination therapy is an effective approach in targeted therapy for colorectal cancer. Specifically, it can delay, inhibit or reverse tumors through synergistic effects, while NGS ensures the precise implementation of combination regimens.

First, targeted drugs combined with chemotherapy to effectively enhance the sensitivity of targeted drugs and reduce tumor burden. For example, EGFR inhibitors combined with fluoropyrimidines, oxaliplatin, etc [14]. NGS can detect genes related to chemotherapy sensitivity, thus further improving the efficacy and safety of treatment.

Second, targeted drugs combined with immunotherapy. The combination of targeted drugs and PD-1/PD-L1 inhibitors produces a synergistic effect. For example, KRAS G12C inhibitors combined with PD-1 antibodies can significantly improve long-term survival rate in mice. NGS can detect biomarkers such as MSI and TMB to screen patients suitable for immunotherapy.

Third, combination of multi-target targeted drugs. For example, the combination of EGFR inhibitors and MET inhibitors, MEK inhibitors and PI3K inhibitors, etc., has been confirmed in clinical trials to effectively promote the improvement of therapeutic efficacy.

4.3. Target repair and epigenetic regulation: Reversing drug resistance phenotype

In response to target abnormalities and epigenetic abnormalities, target repair and epigenetic regulation can be used to reverse the drug resistance phenotype and restore drug sensitivity.

First, target repair. CRISPR-Cas9 technology is used to repair mutated target genes, such as editing EGFR T790M mutation sites, silencing mutated RAS genes, etc [15].

Second, epigenetic regulation. For example, DNA methyltransferase inhibitors can reduce the methylation level of genes such as MLH1, and HDAC inhibitors can regulate histone acetylation, thereby reversing drug resistance. NGS can accurately guide the selection of epigenetic modulators.

4.4. Tumor microenvironment regulation: Improving the tumor microenvironment and enhancing drug sensitivity

Tumor microenvironment regulation refers to regulating the tumor microenvironment based on NGS test results to improve drug sensitivity and reverse drug resistance.

First, inhibit the activation of cancer-associated fibroblasts (CAFs) by using FAP inhibitors, TGF-β inhibitors, etc., thereby reducing cytokine secretion and enhancing drug sensitivity. In this process, NGS can be used to screen suitable patients.

Second, regulate immune cell infiltration. CTLA-4 inhibitors and CD47 inhibitors can activate T cell function, promote macrophage phagocytosis of tumor cells, and increase $CD8^+$ T cell infiltration. NGS can detect immune cell infiltration and related gene expression to guide the selection of immunomodulators.

Third, regulate cytokines and extracellular matrix. IL-6 and IL-8 inhibitors can block drug resistance pathways, and matrix metalloproteinase inhibitors can enhance drug penetration, increase drug concentration and reverse drug resistance.

5. Conclusion

Drug resistance in targeted therapy of colorectal cancer is a major clinical bottleneck, involving target abnormalities, abnormal signaling pathways and other aspects. NGS can comprehensively and accurately analyze tumor molecular characteristics, providing support for the study of drug resistance mechanisms and the formulation of individualized strategies. In the future, with the continuous development of this technology, the goal of precise treatment of colorectal cancer will be achieved, and the quality of life and prognosis of patients will be greatly improved.

Disclosure statement

The author declares no conflict of interest.

References

[1] Wang B, Yang Y, Wei Y, et al., 2025, Research Progress on Biomarkers Related to Targeted Therapy of Colorectal Cancer. Chinese Journal of Gerontology, 45(11): 2792–2798.

[2] Zhang H, Bu J, 2024, Drug Resistance Mechanisms and Potential Solutions of Targeted Therapy for Colorectal Cancer. Chinese Journal of Practical Surgery, 44(07): 774–780.

[3] Wang Y., Jiang R, Liu H., et al., 2018., Feasibility Analysis of Targeted Next-generation Sequencing for Colorectal Cancer Colonoscopy Biopsy Specimens. Chinese Journal of Pathology, 47(7), 6.

[4] Li R, Han Z, 2023, Research Progress of Immunotherapy and Targeted Therapy for Colorectal Cancer. Journal of Qiqihar Medical University, 44(10): 979–983.

[5] Shi L, Ji W, 2023, Research Progress on the Role of EMT in CRC and Targeted Therapy. Chinese Bulletin of Life Sciences, 35(04): 463–471.

[6] Guo H, Zhou H, Zhu X, et al., 2022, Research Progress of Molecular Targeted Therapy for Colorectal Cancer. Oncology Progress, 20(19): 1950–1953.

[7] Yuan J, 2021, Exploring DNA Methylation for Noninvasive Early Diagnosis of Colorectal Cancer Based on Second-generation Sequencing, doctoral thesis, Southern Medical University.

[8] Tang P, Du Q, Wang M, et al., 2022, Research Progress of Targeted Therapy for Metastatic Colorectal Cancer with HER2 Gene Amplification or Overexpression. China Practical Rational Drug Use, 19(09): 29–34.

[9] Zhang C, Xue Y, Chen D, et al., 2022, Research Progress of Chemotherapy and Targeted Therapy for Colorectal

Cancer. Practical Pharmacy and Clinical Remedies, 25(05): 453–458.

[10] Zhou L, 2022, Research Progress of Targeted Drugs for Colorectal Cancer. Journal of Yan'an University (Medical Sciences), 20(01): 1–9.

[11] Li J, Mao Y, Zhang X, et al., 2021, Research on the Correlation Between Circulating Tumor DNA and Targeted Therapy and Drug Resistance Mechanisms in Metastatic Colorectal Cancer. Practical Preventive Medicine, 28(12): 1450–1454.

[12] Li C, Huang X, Wang P, 2021, Research Progress of Related Molecular Targets and Targeted Therapy for Colorectal Cancer. Shandong Medical Journal, 61(17): 108–112.

[13] Pan S, Fang J, 2019, Current Status and Prospects of Targeted Therapy for Colorectal Cancer. Chinese Journal of Gastroenterology, 24(11): 678–682.

[14] Xue Y, Li Q, 2017, Targeted Therapy and Drug Resistance Mechanisms of Colorectal Cancer. World Clinical Drugs, 38(11): 721–726.

[15] Xu K, Chen G, Peng H, 2017, Research Progress on Drug Resistance Mechanisms of Targeted Therapy for Colorectal Cancer. International Journal of Pharmaceutical Research, 44(05): 402–408.