



Review Article

An Overview of Advancements in Screening Methods and Point-of-care Diagnostics for Colorectal Cancer



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Abstract

Colorectal cancer (CRC) is a type of cancer that originates in the colon or rectum from precancerous polyps, which can evolve into cancerous growths over time. This review aimed to provide a comprehensive analysis of CRC, its subtypes, clinical manifestations, point-of-care diagnostic approaches, and management strategies. The clinical presentation of CRC often includes symptoms such as blood in stool, changes in bowel habits, abdominal discomfort, weight loss, fatigue, a feeling of incomplete bowel emptying, and anemia. The identification of these signs prompts healthcare professionals to initiate diagnostic measures without delay. Point-of-care diagnosis plays a pivotal role in the early detection of CRC, employing screening tests such as stool tests and colonoscopies. These diagnostic modalities enable healthcare professionals to identify precancerous polyps or early-stage tumors, facilitating timely intervention and significantly improving treatment outcomes. Adherence to screening guidelines is crucial for the prevention and early detection of CRC. Despite advancements in screening and treatment options, there remains a crucial need for more specific, minimally invasive screening methods with minimal side effects. By improving current detection methods, a better screening approach for CRC can be developed. Recent advancements, including single-cell sequencing, spatial transcriptomics, and artificial intelligence integration, hold great promise for enhancing early diagnosis and advancing personalized treatment strategies. Moreover, a healthy lifestyle, including a balanced diet, regular exercise, no tobacco use, and limited alcohol consumption, can significantly lower the risk of CRC. By emphasizing the importance of lifestyle modifications, early screening, and timely intervention, healthcare professionals can significantly reduce the burden of CRC and improve patient outcomes.

Introduction

Colorectal cancer (CRC) is a gradually progressing yet complex gastrointestinal malignancy affecting the colon or rectum, and it stands as a significant global health concern.¹ Rather than being a single, uniform disease, individual cases of CRC differ in terms of location, degree of malignancy, and the genomic and epigenomic alterations of the tumor.² CRC arises from the malignant transformation of epithelial cells, typically progressing from benign adenomatous polyps through a series of genetic and epigenetic alterations.³ Clinically, CRC often presents with symptoms such as altered bowel habits, rectal bleeding, abdominal pain, and unin-

tentional weight loss.² However, the asymptomatic nature of early-stage CRC emphasizes the critical role of screening and early detection in improving patient outcomes.⁴ Numerous risk factors contribute to CRC development, including age, family history, lifestyle factors, inflammatory bowel diseases (IBD), and hereditary syndromes. Emerging evidence also implicates the gut microbiota, chronic inflammation, and environmental exposures in CRC pathogenesis.⁵

The European Society for Medical Oncology Guidelines have improved CRC diagnosis and staging.⁶ Advances in point-of-care diagnostics offer non-invasive screening options that make CRC detection faster and easier. Innovative technologies, such as the fecal occult blood test (FOBT), stool DNA testing, and blood-based biomarker assays, enhance early detection and improve patient outcomes. However, current screening methods have limitations, and adherence to guidelines remains inadequate, particularly in underserved populations with limited access to healthcare services.^{2,7–9} Although early detection has improved outcomes for localized CRC, metastatic CRC remains a significant treatment challenge.¹⁰

Given the substantial healthcare burden posed by CRC, there

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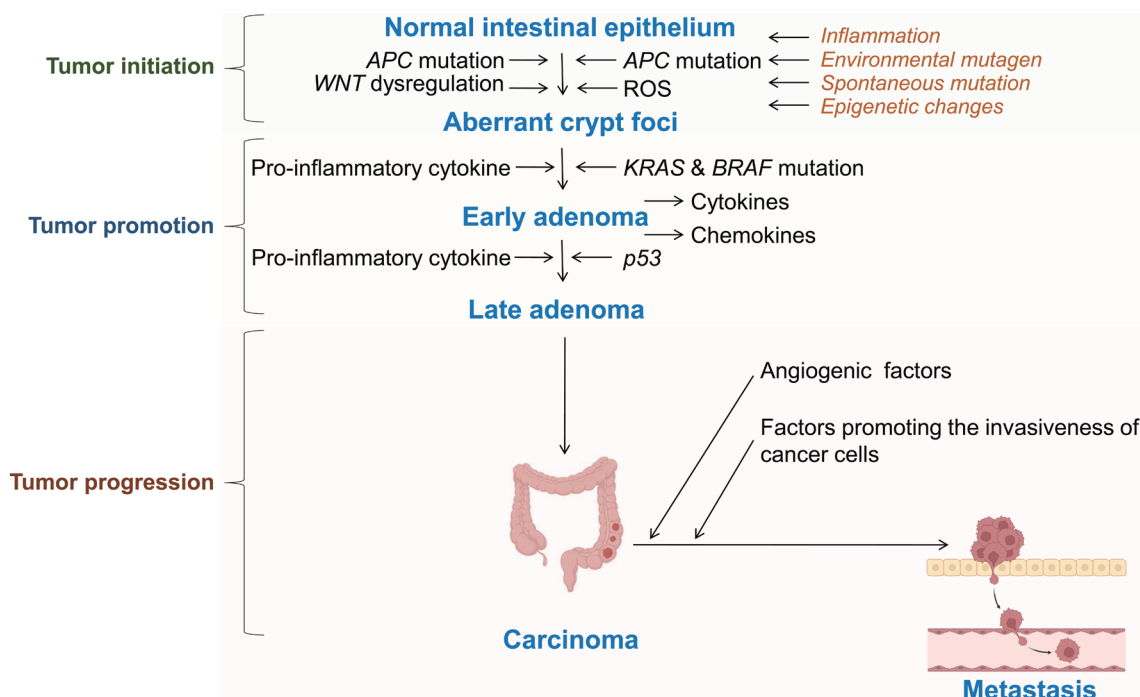


Fig. 1. CRC progression via initiation, promotion, progression, and metastasis. APC, adenomatous polyposis coli; BRAF, B-rapidly accelerated fibrosarcoma; CRC, colorectal cancer; KRAS, Kirsten rat sarcoma viral oncogene homolog; p53, tumor protein 53; ROS, reactive oxygen species; WNT, wingless/integration.

is an urgent need for more effective screening options to address this challenge. By exploring the complex interplay between genetic, environmental, and lifestyle factors in CRC development and progression, this review aimed to provide a comprehensive understanding of the disease landscape, as well as potential strategies for prevention, early detection, and personalized management approaches.

Etiology of CRC

CRC typically originates as abnormal tissue growth, or polyps, within the colon or rectum. These polyps undergo a gradual, multistep process involving various histological, morphological, and genetic changes over time, leading to the eventual transformation of normal epithelial cells into cancerous cells.^{11–13} Based on histological characteristics, polyps can be adenomatous or serrated, each with distinct features, and both can further transform into cancerous forms.¹⁴ Generally, adenomatous polyps are associated with a higher risk of transforming into cancerous types than serrated polyps. The transformation from polyp to cancer involves a sequence of genetic and epigenetic alterations leading to cytologic and histologic dysplasia.¹⁵

These genetic and epigenetic alterations in CRC progress through four stages: initiation (irreversible genetic damage), promotion (abnormal cell growth), progression (genetic and epigenetic changes leading to malignancy), and metastasis (spread to other tissues). Although it is challenging to precisely estimate the duration of each stage, the entire process spans decades in CRC.¹⁶ Different key genes and signaling pathways are involved at various stages of CRC progression.¹⁷ The adenoma-carcinoma sequence, or classical pathway, describes the stepwise progression from benign adenomatous polyps to carcinoma. Mutation in specific genes, such as the adenomatous polyposis coli (APC) gene, plays a crucial role in the initia-

tion of this pathway.^{18,19} Genetic instability, such as microsatellite instability (MSI) due to mutations in mismatch repair genes (mutL homolog 1, mutS homolog 1, mutS homolog 6, and postmeiotic segregation increased 2), and chromosomal instability (CIN) caused by mutations in APC and Kirsten rat sarcoma viral oncogene homolog (KRAS) leading to tumor protein 53 (p53) loss, also contribute to CRC development.²⁰ In addition, dysregulation of the wingless/integration (WNT) signaling pathway (due to APC gene mutation) is involved in CRC carcinogenesis (Fig. 1).²¹ Increasing DNA mutations or damage, whether acquired through environmental exposures or inherited over time, can result in high-grade dysplasia, which poses a high risk of progression to invasive carcinoma.²² Somatic evolution, through diverse mechanisms, leads to accumulating molecular changes that disrupt key regulatory networks. Over time, the integrity of WNT, mitogen-activated protein kinase, transforming growth factor-beta, phosphoinositide 3-kinase/AK strain transforming, and p53 signaling pathways is compromised, contributing to tumor development.²³ Early identification and removal of precancerous polyps are vital to disrupting the adenoma-carcinoma sequence, preventing CRC development and spread.²² Once these malignant cells establish themselves, they can infiltrate the colorectal wall and potentially spread through blood or lymph vessels, leading to metastases in distant organs and tissues such as the liver, lungs, or peritoneum (Fig. 2).^{8,11,24}

Based on anatomical locations (proximal colon, distal colon, and rectum) and molecular characteristics, CRC subtypes exhibit distinct molecular profiles. Hypermutated CRC shows unique genetic alterations, whereas non-hypermutated CRCs share common genomic features across colon and rectal cancers.²⁵ Molecular classification of CRC is primarily based on three key abnormalities: MSI, CIN, and cytosine-phosphate-guanine island methylator phenotype.^{2,25–27} Additionally, consensus molecular subtypes (CMS) further classify CRC into four distinct subtypes: CMS1 (MSI-immune), CMS2 (ca-

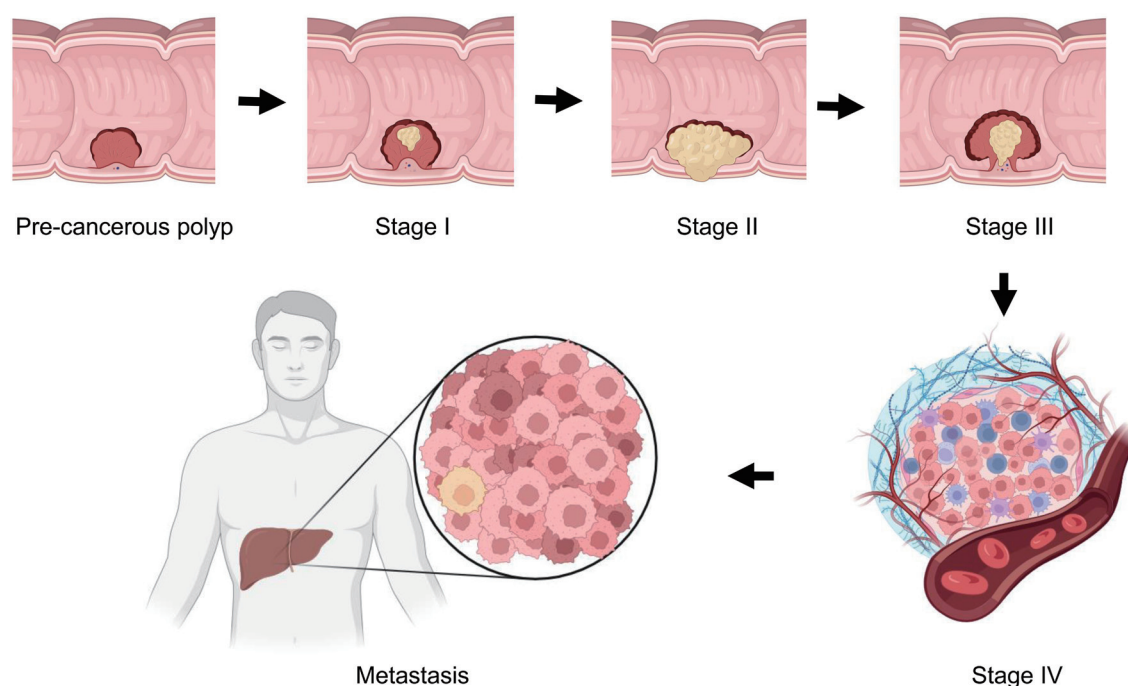


Fig. 2. Progression of CRC, from pre-cancerous polyp through various stages. CRC, colorectal cancer.

nonical), CMS3 (metabolic), and CMS4 (mesenchymal). Each CMS subtype exhibits unique genetic, epigenetic, and immune profiles, contributing to differences in tumor behavior, prognosis, and therapeutic response.²⁸ These subtypes influence CRC pathogenesis, progression, and response to treatment.

Risk factors associated with CRC

The risk factors for CRC include modifiable factors such as sedentary lifestyles, tobacco/alcohol use, obesity, and processed/red meat consumption, as well as non-modifiable factors like aging, IBD, prior abdominal radiation exposure, genetic alterations, and a family history of CRC or high-risk polyps.^{29,30} While the majority of CRC cases (90%) occur in individuals over 50, young individuals often present with a more aggressive form of the disease. Major risk factors include male sex, black or Asian race, and conditions such as abdominal obesity, polyps or precancerous conditions, elevated body mass index, environment, gender, ethnicity, and tall stature (Fig. 3).^{2,11,24,31}

Global survival disparities in both incidence and mortality across racial and ethnic groups are primarily linked to variations in access to diagnostic and treatment services.³² These risk factors are discussed in more detail below:

Genetic predisposition

A significant non-modifiable risk factor for CRC is a family history of the disease. Individuals with a first-degree relative diagnosed with CRC have a two- to four-fold higher risk.³⁰ The risk is even higher when at least two relatives are affected, especially if they were diagnosed with CRC before the age of 50.²⁵ Additionally, a family history of advanced colorectal polyps is a significant and often underestimated factor, associated with a 50% increased risk of CRC.³⁰ A family history of CRC significantly elevates the risk of developing the disease due to a combination of inherited genetic predisposition and shared lifestyle factors.^{2,7} Excluding rare

hereditary cancer syndromes, most inherited CRC mutations have low penetrance. Familial CRC cases often result from acquired genomic abnormalities, highlighting the role of environmental factors. Hereditary cancer syndromes elevate CRC risk through germline mutations in high-penetrance genes. Notably, CRC can occur in the absence of a family history, possibly due to parental germ cell mutations or post-fertilization mutations. In hereditary non-polyposis colorectal cancer (HNPCC), postmeiotic segregation increased 2 mutations have lower CRC penetrance than other causative genes, which may result in the absence of a family history despite inheritance.²⁵ On the other hand, the majority of CRC cases are sporadic, meaning family history is not involved in their development (they arise from point mutations, appear during life, and are not inherited).^{13,31}

Various types of genomic instability play a vital role in the development of both sporadic and inherited CRC. The molecular development of CRC is diverse, and mutations affect various oncogenes and tumor suppressor genes, initially including the *APC* genes. Following the *APC* mutation, mutations occur in *KRAS*, *p53*, and eventually deleted in colorectal carcinoma genes. Approximately 70% of CRC cases exhibit a specific sequence of mutations that results in a defined morphological progression, beginning with the emergence of adenomatous polyps and culminating in carcinoma within a 10-year time frame.³¹ Approximately 70% of CRC cases arise sporadically from somatic mutations, and dysfunction in the WNT pathway leads to the accumulation of β -catenin, promoting carcinogenesis.¹¹ However, approximately 5% of CRC cases are linked to specific hereditary syndromes (both polyposis and non-polyposis), caused by gene mutations. Inherited syndromes such as Lynch syndrome (non-polyposis, associated with DNA repair gene mutations) and familial adenomatous polyposis (FAP) (polyposis, involving multiple colon polyps) account for 2–4% and 25% of all CRC cases, respectively. In rare instances, CRC is also linked to Peutz-Jeghers syndrome, juvenile polyposis syndrome, and mutY homolog-associated polyposis.

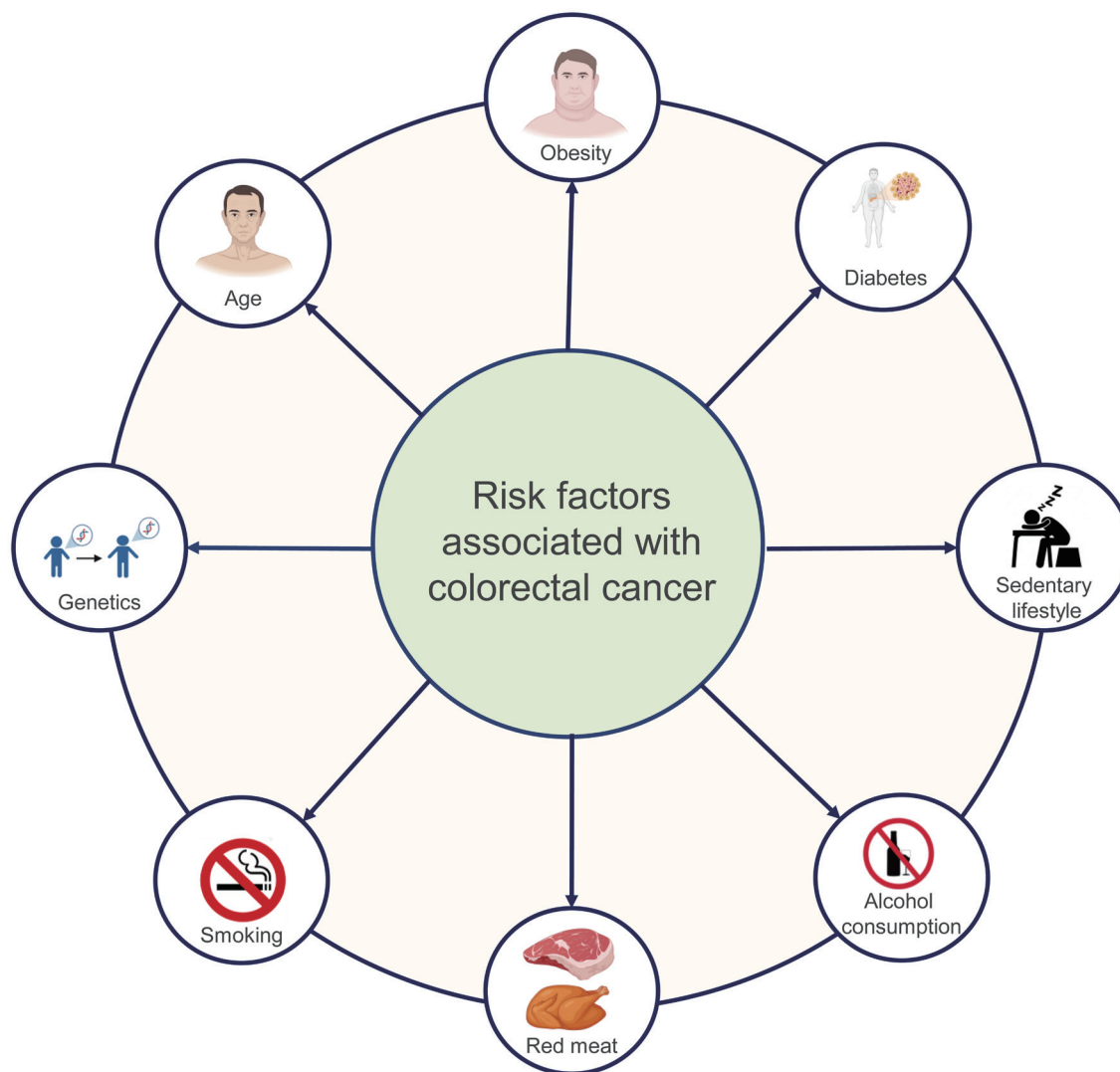


Fig. 3. Risk factors associated with the development of CRC. CRC, colorectal cancer.

sis.^{9,11,27,31} FAP involves hundreds to thousands of polyps, some of which progress to cancer, while Lynch syndrome sees most polyps progress to cancer.⁷ In terms of hereditary cancer syndromes, Lynch syndrome or HNPCC-associated CRC is predominantly found in the proximal colon, whereas FAP-associated CRC tends to occur in the distal colon.²⁵ Individuals with a history of Mendelian cancer syndromes like Lynch syndrome or polyposis syndromes such as FAP or mutY homolog-associated polyposis face a particularly elevated risk of CRC. These Mendelian CRC syndromes, contributing to about 5% of CRC cases, are more prevalent in individuals with early-onset CRC. Carriers of the genetic mutations associated with these syndromes may develop cancer at a much younger age compared to other high-risk groups, necessitating early screening, often starting in their first decade of life, depending on the specific syndrome and underlying mutation.³⁰

Lifestyle

Various lifestyle factors play a vital role in the development of CRC. Implementing a healthier lifestyle, particularly in terms of diet (high in fat, low in fiber), physical inactivity, sedentary hab-

its (smoking and alcohol consumption), and obesity, can mitigate certain driving risk factors for early CRC. In addition, diets high in red or processed meat, saturated fats, cholesterol, and spicy foods elevate the risk.^{11,12,31,33} Physical activity reduces colon cancer risk by 25%, while obesity increases the risk of both colon and rectal cancer. Obesity is responsible for nearly one-third of CRC cases and reduces survival rates. Notably, dietary intake and elevated levels of visceral adipose tissue (a hormonally active component of total body fat) are linked as risk factors for the development of CRC.^{11,34} Various lifestyle risk factors are discussed in more detail in the section below:

- **Diet:** CRC incidence is significantly affected by diet, which impacts the microbiome in the large intestine, immune response, inflammation, and tumor growth.¹¹ According to the International Agency for Research on Cancer Group, red and processed meats are considered probable carcinogens.² Red meat, when consumed, releases heme groups in the intestine, leading to the formation of carcinogenic *N*-nitroso compounds and cytotoxic aldehydes due to lipoperoxidation. After digestion, meat cooked at high temperatures generates heterocyclic amines and poly-

cyclic hydrocarbons. These can increase oxidative stress, leading to DNA damage, mutations, and epithelial cell changes that may progress to cancer.^{2,31} Certain high-temperature cooking methods for meat, like frying and broiling, are associated with CRC due to the formation of mutagenic substances. These substances, including heterocyclic amines and polycyclic aromatic hydrocarbons, can produce N-nitroso compounds, a recognized human colon carcinogen.³⁵

- **Smoking:** Tobacco smoking is linked to carcinogenesis due to its high carcinogen content (including nicotine, *N*-nitrosamines, polycyclic aromatic hydrocarbons, aromatic amines, aldehydes, and metals). Cigarette carcinogens and their byproducts, transported by the gastrointestinal tract and circulatory system, increase the risk of inflammation, mutagenesis, and carcinogenesis in the colorectal mucosa. These carcinogens lead to the formation, development, and growth of polyps by damaging DNA and inducing mutations in colorectal cells. Smoking can raise the risk of CRC by up to 10.8%, increasing the risk two to three times in smokers compared to non-smokers. Cigarette smoking is linked to up to 12% of CRC deaths. This risk is particularly significant for long-term smokers, regardless of their current smoking status.^{2,11,31,35}
- **Alcohol:** Alcohol, particularly ethanol in alcoholic beverages, is a recognized risk factor for CRC development.^{25,31} Compared to non/occasional drinkers, consuming two to three drinks daily is associated with a 20% higher CRC risk, while consuming more than three drinks raises the risk by approximately 40%. Those who regularly consume four or more drinks daily increase their CRC risk by up to 52%.^{2,35} Alcohol impacts folate synthesis. As alcohol circulates through the bloodstream to colon cells, microbes convert it into acetaldehyde (labeled as a human carcinogen), which degrades folate. Folate deficiency, essential for DNA synthesis and repair, can lead to chromosome breakage, uracil misplacement, and other DNA precursor imbalances, thus causing damage and increased cell growth in the colon lining, contributing to carcinogenesis.^{2,25,35} Various other mechanisms have been proposed to explain alcohol-induced carcinogenesis, including the generation of reactive oxygen and nitrogen species during ethanol metabolism, the production of mutagenic acetaldehyde (as an initial ethanol metabolite), depletion of *S*-adenosylmethionine leading to epigenetic changes, inactivation of tumor suppressor genes, hormonal imbalances, reduced folate levels, and disruption of retinoic acid metabolism.² Tobacco-induced mutations are less effectively repaired when alcohol is consumed.¹¹

Gut microbiota

The gut microbiota, which consists of a diverse population of microorganisms (such as bacteria, viruses, fungi, and protozoa), plays a significant role in CRC development (through initiation, promotion, and progression).^{2,31} Several bacteria, such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, and enteropathogenic *Escherichia coli*, contribute to this multi-step process. Harmful metabolites produced by these microorganisms can induce DNA damage, disrupt cell cycles, provoke immune responses, and compromise the integrity of the intestinal barrier. This primarily works through a chronic inflammation mechanism when there is an imbalance in the intestinal microbiome, known as dysbiosis. Dysbiosis is linked to various lifestyle factors, including diet.^{2,8,31,36}

Geographical

CRC incidence varies globally, with around 55% of cases found

in more developed countries. Geographical differences are likely shaped by a combination of diverse dietary and environmental exposures, set against a backdrop of genetically determined susceptibility.³² CRC, often seen as prevalent in developed nations, is now on the rise in countries undergoing economic development and lifestyle changes.³⁷ Low socioeconomic status is also associated with an increased risk of CRC, likely due to higher rates of modifiable risk factors and lower levels of CRC screening. High CRC incidence is noted in Australia, New Zealand, Europe, and Northern America, while developing countries, especially in Western Asia (Kuwait and Israel) and Eastern Europe (Czech Republic, Slovakia, and Slovenia), are also witnessing an increase. This rise is associated with a higher prevalence of westernization-related risk factors such as unhealthy diets, obesity, and smoking. The global burden of CRC is expected to escalate due to population growth and aging.³² Migrants from countries with low CRC rates quickly adopt the higher rates of their new adoptive country.

Age

Age is a major risk factor in the development of CRC. After the age of 50 years, the chances of developing CRC markedly increase, with 90% of new cases and 94% of CRC-related deaths occurring in those aged 50 and above.^{22,31} Generally, apart from inherited history, CRC onset below the age of 50 is rare.³¹ In women, the median age at diagnosis is 72, whereas it is 68 in men.¹² However, CRC is also observed in younger individuals due to dietary habits and lifestyles in countries such as the United States, Europe, and China.^{2,38}

Gender and race

In CRC development, biological differences in carcinogenic risk and exposures may exist. Globally, CRC incidence rates are notably higher in men than women, with the disparity likely arising from complex interactions between sex-specific risk factors, protective effects of hormones, and gender-specific screening practices.³² According to the American Cancer Society, men have a 30% higher risk of CRC than women and a worse prognosis, with a 40% higher mortality rate.² Proximal colon cancer is more common in women (increasing with age and often detected at an advanced stage), older individuals, and both white and black populations. Distal colon cancer is more prevalent in men and younger individuals, while rectal cancer tends to occur in early-onset cases (diagnosed before age 50) and among Asians.^{2,25} Gender disparities may be linked to risk factors, diet, and hormones. CRC incidence also varies by race, with non-Hispanic blacks having a 50% higher rate than Asians/Pacific Islanders and 20% higher than non-Hispanic whites.² CRC rates are higher in black individuals compared to whites, likely due to complex interactions between screening practices, etiologic factors, and potential socioeconomic influences. Black individuals, compared to whites, tend to be diagnosed at a younger age, have CRCs in proximal or transverse regions, and present with lower tumor grades.³² In the USA, proximal colon cancer predominates among both white and black individuals, while rectal cancer is highest among Asians.²⁵ Ashkenazi Jews have a higher CRC risk (9–15%) than the 5–6% average in Western populations.⁷ African Americans show CRC at younger ages, with higher prevalence, carcinogenesis, and mortality rates, as well as lower five-year survival rates compared to other ethnicities. Asian Americans, despite lower CRC incidence, have it as their most diagnosed cancer. American Indian/Alaskan native populations have higher CRC incidence/mortality than whites, with no decline observed. CRC screening rates are lower in this population compared to African Americans and whites.^{30,35}

Other disease conditions

Various disease conditions, such as IBD, obesity, and diabetes, are potential risk factors associated with CRC development. Moreover, physical inactivity, obesity, and diabetes are interlinked and are discussed in detail below¹²:

- **Physical inactivity:** It is estimated that people who are physically inactive are 50% more likely to develop CRC compared to those who engage in regular physical activity.² Prolonged sitting, which impairs skeletal muscle function, contributes to insulin resistance and promotes colorectal carcinogenesis.²⁵
- **Obesity:** Obesity is one of the major factors contributing to CRC development and results from certain lifestyle habits.^{2,38} High-fat red and processed meat consumption can also contribute to obesity and further increase CRC risk.²

Specifically, abdominal obesity has a stronger association with CRC in both men and women, with a more significant impact on colon cancer than rectal cancer.³⁶ Recent epidemiological research indicates that the connection between obesity and CRC is more pronounced in men than in women.³³ Elevated CRC risk in men and susceptibility to visceral obesity compared to women may be due to sex hormones. Higher levels of natural estrogens in women offer CRC protection, while increased testosterone in men may lower the risk. Aging-related adiposity affects hormones differently; postmenopausal women's increased estrogen from adiposity might reduce obesity-associated CRC risk, while decreasing testosterone in men could amplify this relationship.²⁵ Visceral adipose tissue can contribute to the onset of CRC by releasing pro-inflammatory cytokines. This, in turn, triggers inflammation in the colon and rectum, promotes insulin resistance, and influences the activity of metabolic enzymes like adiponectin and leptin.³¹ The mechanism involves changes in adipose tissue hormone and cytokine release in obese individuals, including factors like leptin, resistin, tumor necrosis factor- α , and various interleukins (ILs) (IL-1, IL-6, IL-7, and IL-8). These substances can promote cell growth, inhibit cell death, induce oxidative stress, dampen the immune response, and reduce insulin-like growth factor 1 activity, all of which are associated with cancer development and progression.² Elevated body fat is linked to CRC risk through potential mechanisms such as increased insulin levels, insulin resistance, inflammation, altered immune response, oxidative stress, and disruptions in growth factors, adipokines, and sex steroids.³⁹ Additionally, investigations into the impact of cholesterol on CRC metastasis have revealed a positive correlation between low-density lipoprotein cholesterol levels and liver metastases. Elevated expression of the low-density lipoprotein receptor is linked to advanced stages (nodes and metastases - N and M stages) of CRC.³³

- **Diabetes:** Epidemiological evidence shows that diabetes is an independent risk factor for CRC, with individuals having type-2 diabetes being two to three times more likely to develop CRC. This increased risk is linked to elevated insulin levels, inflammation, and the role of insulin-like growth factor 1 in promoting cell growth.² High blood insulin levels increase CRC risk by promoting colon cell proliferation and reducing apoptosis through hyperinsulinemia.³⁵ Chronic inflammation associated with diabetes also contributes to carcinogenesis and tumor progression through pro-inflammatory cytokines like tumor necrosis factor- α and IL-6. Consumption of high-fat red and processed meat can also contribute to insulin resistance, further increasing CRC risk.²
- **IBD:** In terms of CRC risk, IBD ranks third after HNPCC and FAP.² A personal history of IBD substantially heightens the risk of developing CRC, with ulcerative colitis increasing the risk

by 3.7% and Crohn's disease by 2.5%. Chronic inflammation related to IBD can lead to dysplasia, increasing the chances of these abnormal cells progressing into tumors.^{12,31,35,40} Individuals with long-term IBD, especially ulcerative colitis, have a higher risk of CRC. The risk in ulcerative colitis is tied to disease duration and extent, with cumulative risks of 2%, 8%, and 18% at 10, 20, and 30 years, respectively.⁹ Both sporadic CRC and colitis-associated cancer may display tumor-induced inflammation, sharing genetic alterations but differing temporally in key genes like APC and p53. APC mutations are early events in sporadic CRC, while they occur later in colitis-associated cancer, with the opposite pattern for p53 mutations.⁴¹

- **Colon polyps:** Colon polyps or precancerous lesions are abnormal growths in the mucosal layer of the colon.² The malignancy risk of polyps depends on factors like size, histology, and epithelial atypia.⁷ These are categorized as neoplastic (adenomatous) and non-neoplastic (hamartomatous, hyperplastic, serrated, and inflammatory polyps),^{2,7} with adenomatous and serrated polyps being major precursors to most CRCs.²⁵ Adenomatous polyps are often well-defined, elevated, and may have a stalk, while sessile serrated lesions are flat, lack a stalk, and often have an unclear border due to a "mucus cap". Adenomatous polyps are particularly significant as they can evolve into CRC, accounting for about 95% of CRC cases. However, only around 5% of polyps progress to CRC, with the transition period taking five to fifteen years. The risk increases with polyp size, degree of dysplasia, and age. Larger polyps, high dysplasia, and older age are unfavorable factors.² Additionally, sessile serrated lesions share histological features with benign hyperplastic polyps.⁷ Individuals with large polyps (>1 cm), numerous polyps (>2), villous histology, and signs of dysplasia face a higher CRC risk.^{12,25} Hyperplastic polyposis syndrome, marked by multiple hyperplastic polyps, is a rare condition linked to increased CRC risk. Hyperplastic polyps lack nuclear hyperchromatism and atypia.⁷

Detection of CRC (point of care diagnosis: invasive and non-invasive)

CRC often grows slowly and is generally asymptomatic until it reaches a sizable stage.²² Detecting CRC at an early stage poses challenges, and it is often identified and diagnosed at advanced stages (when successful treatment is no longer possible).^{25,38} CRC development involves histological, morphological, and genetic changes, which are useful in early screening (precancerous form) and detection in individuals at high risk.²² Clinicians determine screening criteria by evaluating an individual's risk based on factors such as age, personal and family history of CRC, genetic syndromes, inflammatory bowel disease, and a history of childhood cancer with abdominal radiation therapy. Individuals without these risk factors are considered at normal risk, while the presence of any indicates an increased risk, necessitating different screening or surveillance strategies.⁹

Initiating early screening is crucial for reducing CRC incidence and mortality by removing precancerous lesions and detecting or treating CRC at an early stage.^{25,29} Additionally, removing precancerous growths can lower the risk of developing cancer.⁸ Effective screening requires tools and methods that are highly sensitive, specific, and safe.³⁸ There are several screening methods for CRC, which are essential for early detection.^{24,25} These methods include both non-invasive and invasive techniques. Non-invasive techniques include FOBT, guaiac-based FOBT (gFOBT), fecal immunochemical test (FIT), fecal DNA test, and computed tomography (CT) colonography.⁸ In contrast, invasive techniques involve

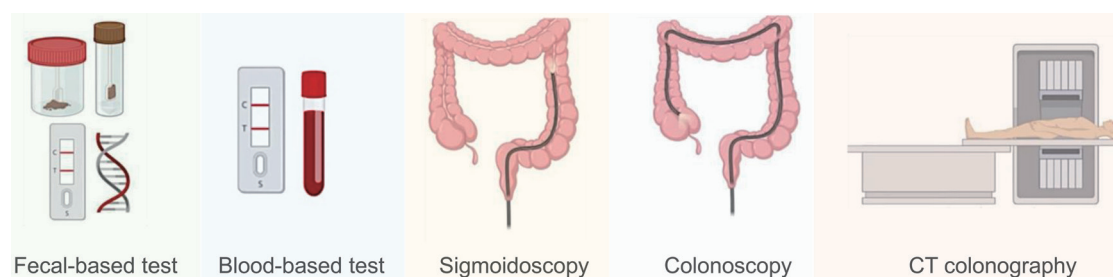


Fig. 4. Various screening methods to diagnose CRC. CRC, colorectal cancer; CT, computed tomography.

endoscopic procedures (such as colonoscopy, flexible sigmoidoscopy, capsule endoscopy, and rectoscopy, which provide direct visualization), along with blood tests like the Septin9 gene test, and barium enema (Fig. 4).^{2,7-9}

Some of the various methods for detecting CRC are discussed in detail below:

Fecal-based tests

Colonic polyps, adenomas, and cancers can release cells and cause bleeding into the colon, with markers potentially found in feces. Fecal-based tests often target the detection of small amounts of blood in feces, which may indicate the presence of polyps or CRC.⁷ Over the past few decades, various fecal-based tests have been proposed for CRC screening, each with differing levels of effectiveness. These include FOBT or gFOBT, FIT, and fecal DNA tests.⁹ These early detection tests identify microscopic amounts of blood linked to advanced adenomas and most cancers. Positive results often lead to endoscopy for further evaluation, considered a preventive measure.⁴² Fecal-based screening methods are in high demand for the early detection of CRC and precancerous polyps, aiming to boost participation while reducing the drawbacks and costs of screening.⁸ Fecal-based screening offers advantages such as being non-invasive, cost-effective, and convenient for at-home testing.^{2,8} Various stool-based CRC screening tests are discussed in detail in the section below:

FOBT

The FOBT is a home-based, non-invasive method that checks stool for subtle blood traces, indicating possible precancerous lesions or cancer.^{22,25,35} These tests are simple, inexpensive, and non-intrusive, recommended annually or biennially. Positive results prompt a colonoscopy to confirm or rule out tumors.^{2,9,38} FOBT is recommended for patients with low-risk bowel symptoms only and is not advised for all symptomatic patients.² Additionally, blood in the stool is not a reliable sign of CRC, as it can originate from both cancerous growths and polyps.³⁸

The gFOBT, a specific type of FOBT, is named after the *Guaia-cum* tree paper used in the device and detects microscopic amounts of hemoglobin in stool. The standard test card has two panels for a fecal smear, and the common approach is to use three cards, each with two panels, over three consecutive bowel movements. However, due to limitations in providing a definitive diagnosis (non-exclusive positive results and limited negative predictive value in symptomatic patients), imaging techniques have largely supplanted this practice.⁴² The widely used gFOBT detects fecal blood by leveraging the pseudo-peroxidase properties of hemoglobin. This involves liberating oxygen from 3–6% hydrogen peroxide in ethanol or methanol.⁷ The gFOBT offers cost-effectiveness, easy distribution, simplicity, and stability. The gFOBT's ability to assess multiple fe-

cal samples reduces sampling errors and helps manage colonoscopy demand by selecting patients based on positive results and setting a threshold. However, the gFOBT has drawbacks, including limited sensitivity for cancer and adenoma detection, dependence on manual analysis, and an inability to automate.⁴² Other limitations include the need for three samples on different days, the inability to determine the bleeding source (upper or lower GI tract), and the requirement for dietary restrictions before testing.⁹ The gFOBT lacks specificity for human hemoglobin and can be influenced by diet or medications, requiring restrictions before testing.² Dietary vitamin C can lead to false negatives by inhibiting peroxidase activity, while red meat ingestion, containing dietary hemoglobin, may result in false positives.²² False positives may also occur due to similarities between human and non-human hemoglobin in dietary meat. Vegetable peroxidases in the diet can also interfere. The gFOBT has drawbacks such as subjectivity, manual processing, and a fixed cutoff for hemoglobin concentration.²²

FIT

FIT, or immunochemical FOBT, is a non-invasive, home-based assay for the quantitative detection of human globin in minimal stool samples. It offers both qualitative and quantitative tests.^{22,25,42} This test is generally recommended for patients with low-risk CRC symptoms, unexplained bowel changes, or iron deficiency anemia (age > 60).^{2,8} FIT employs antibodies specific to human globin to identify hemoglobin, making it more targeted towards human blood.²² FIT accurately identifies the origin of lower gastrointestinal bleeding and has superior CRC detection rates, making it the preferred screening choice. Moreover, it requires fewer stool samples than FOBT.^{9,30} Its advantages include reduced invasiveness and lower cost, which appeals to patients who are averse to sedation or bowel preparation.³⁰ FIT, which does not require dietary restrictions before sampling, provides higher sensitivity and a lower incidence of interval CRC after a negative screening test. FIT or immunochemical FOBT is often preferred over the older gFOBT. FIT, without dietary restrictions before sampling, provides higher sensitivity and a lower incidence of interval CRC post a negative screening test.²⁵ Their advantages over gFOBT include human specificity, avoiding nonhuman blood cross-reactivity, and lower sensitivity to upper gastrointestinal bleeding. While faster globin degradation in the GIT makes FIT less sensitive to upper GIT bleeding, this may reduce sensitivity for proximal colon lesions. FITs provide heightened sensitivity for neoplasia, with a lower blood-detection threshold, and are performed on a single stool sample. To enhance performance, extending FIT testing over two days is recommended due to the assumption of intermittent bleeding from neoplastic lesions.⁴² However, it is less effective in detecting advanced adenomas and has limited sensitivity for sessile serrated polyps compared to colonoscopy.³⁰

Fecal DNA test

The fecal DNA test, also referred to as the FIT-DNA test or multi-target stool DNA test, is a non-invasive test approved by the U.S. Food and Drug Administration (FDA).^{8,22} It checks for altered DNA and the presence of blood. This test combines FIT, which detects altered or abnormal DNA (DNA markers such as bone morphogenetic protein 3, N-myc downstream regulated gene, mutant KRAS, β -actin) in stool, occult blood in stool (hemoglobin immunoassay), and molecular abnormalities associated with CRC (quantitative molecular assays for biomarkers like KRAS mutations and aberrant N-myc downstream regulated gene/bone morphogenetic protein 3 methylation).^{8,22,25} This test, which requires a patient-collected stool sample, doesn't involve bowel preparation, medication, or dietary changes. It is done once every three years. Despite being relatively new with limited evidence, the test boasts high sensitivity, prioritizing this crucial characteristic in screening evaluations.^{9,22} According to studies, when compared with FIT, it has higher sensitivity but lower specificity, leading to more false positives (which result in unnecessary colonoscopies).^{9,25} Additionally, it is expensive, requires a full stool sample, and has limited value as a screening tool.²⁵

Cologuard® is an FDA-approved, commercially available multi-target stool DNA test that identifies ten DNA-based markers and one hemoglobin biomarker, yielding a composite negative or positive result. A positive result prompts referral for colonoscopy and lesion removal, while a negative result indicates normal internal control DNA, prompting continued routine screening.²²

Blood tests

Blood tests are among the diagnostic tools that may be used for CRC detection. However, they are not typically used as the primary method for CRC diagnosis.⁴³ Various blood-based tests for CRC detection are discussed in detail below:

Septin9 assay

The FDA has granted approval for this test, which identifies methylated septin9 DNA. This test is currently available for screening individuals who decline alternative screening options.⁸ The septin9 assay is the only plasmatic test recommended for CRC screening every three years. However, its low sensitivity for adenoma detection has led to guidelines discouraging its use in CRC screening.⁹

Liquid biopsy

Liquid biopsy is a non-invasive technique used to analyze blood for circulating tumor DNA, circulating tumor cells, or other molecular markers associated with CRC.^{44,45} Liquid biopsy is employed in situations where an insufficient quantity of tissue sample is available.⁴⁶

The advantages of liquid biopsy include the ability to capture tumor heterogeneity and the potential for real-time monitoring of treatment response. However, it is important to note that liquid biopsy is not yet a standalone diagnostic tool for CRC. It is often used in conjunction with other diagnostic methods, such as imaging studies and traditional biopsies, to provide a more comprehensive understanding of the disease.^{44,45,47}

Tumor biomarker identification in blood & feces

In CRC, three significant alterations—MSI, CIN, and cytosine-phosphate-guanine island methylator phenotype—lead to changes in DNA, RNA, proteins, or metabolites. These changes can be detected in tumor samples, blood, or stool, making them useful

as biomarkers (Table 1).^{7,25,31,38,48-55} In various types of CRC, common genetic mutations (e.g., cellular myelocytomatosis or c-MYC, KRAS) and non-coding RNA alterations (e.g., long non-coding RNA, microRNA) impact critical pathways and serve as predictive markers. To enhance prognosis and treatment selection, gene and mRNA panels are evolving. MSI and KRAS mutations in tumor samples are the primary biomarkers for tumor classification, prognosis, and treatment decisions in CRC. Key biomarkers for tumor classification, prognosis, and treatment include MSI and KRAS mutations. Additionally, deficient mismatch repair/MSI-H is a well-established biomarker.³¹ Stool samples are extensively studied for DNA, RNA, and protein biomarkers as screening tools for CRC and its precursors.

Several commonly used tumor markers in clinical practice for CRC, such as carcinoembryonic antigen (CEA), carbohydrate antigen 19-9, tissue polypeptide-specific antigen, tumor-associated glycoprotein-72, and hematopoietic growth factors, can be found in various bodily materials like tumor tissue, lymph nodes, bone marrow, blood, urine, ascites, and stool.³⁸ These key biomarkers are important in understanding the biological heterogeneity of the disease. Moreover, identifying these biomarkers helps classify the disease into subtypes for predicting prognosis, treatment response, and recurrence risk, potentially leading to personalized therapies.^{27,38}

Increased levels of blood proteins such as CEA and pyruvate kinase muscle isoenzyme 2 (PK-M2) are observed in CRC. CEA, a glycoprotein involved in cell adhesion, is commonly used for post-treatment monitoring, aiding in staging and indicating suspicion of metastatic spread. PK-M2, expressed in tissues with elevated nucleic acid synthesis, can be measured in plasma and feces, with fecal PK-M2 being more indicative of CRC than other cancers. The effectiveness of PK-M2 and CEA in CRC screening remains uncertain.⁷

Transferrin, present at lower blood concentrations (2.0–3.6 g/L), has potential as a gastrointestinal bleeding marker, with resistance to degradation. Tests for transferrin in feces show usefulness in diagnosing CRC, but its role in screening average-risk individuals for CRC requires further research. Calprotectin, associated with inflammation, is found in neutrophils and linked to bowel conditions, but its sensitivity and specificity for CRC detection are reported to be less than gFOBT. Calprotectin is likely unsuitable for population CRC screening, and its potential merits for CRC tumor staging are unclear based on early research.⁷

Gene expression profiling-based studies compare normal and tumor tissue gene samples or tissue from different stages of the disease. These comparisons provide information about the prognosis of the disease and the most suitable treatment choice for each patient.³¹

Endoscopy

The National Comprehensive Cancer Network advises endoscopic assessment for concerning signs such as rectal bleeding, anemia, or altered bowel habits in younger individuals.⁸ Endoscopy is considered the most reliable method for CRC diagnosis as it enables real-time assessment, targeted biopsies, and histopathological analysis.³⁸ Visualization techniques, such as endoscopy, can be either invasive (colonoscopy, sigmoidoscopy, double contrast barium enema) or non-invasive (computed tomography colonography or CT-colonography).⁷ Although invasive and expensive endoscopy techniques such as sigmoidoscopy and colonoscopy not only enable early detection but also help prevent CRC by removing precursors.⁴² Flat (sessile) polyps, often found in the right colon (as-

Table 1. Various biomarkers associated with CRC, their clinical utility, and the methods used for their detection

Sr. no.	Biomarker type	Identification method	Clinical utility	Reference
1.	CEA	Blood test (immunoassay)	Detecting recurrence	38
2.	PK-M2	Blood test (immunoassay)	Detecting recurrence	38
3.	CA 19.9	Blood test (immunoassay)	Detecting recurrence	38
4.	TPS	Blood test (immunoassay)	Detecting recurrence	38
5.	TAG-72	Blood test (immunoassay)	Detecting recurrence	38
6.	HGF-s	Blood test (immunoassay)	Detecting recurrence	38
7.	KRAS mutations	Genetic testing (PCR, sequencing)	Prognostic marker	31
8.	BRAF mutations	Genetic testing (PCR, sequencing)	Prognostic marker	25
9.	MSI/dMMR	Genetic testing (PCR, sequencing)	Prognostic marker	31
10.	p53 mutations	Genetic testing (PCR, sequencing)	Prognostic marker	48
11.	APC mutations	Genetic testing (PCR, sequencing)	Early detection, prognostic marker	48
12.	WNT	Immunohistochemistry, PCR	Prognostic and tumor progression marker	49
13.	SMAD4 mutations	Genetic testing (PCR, sequencing)	Prognostic marker	50
14.	PIK3CA mutations	Immunohistochemistry	Prognostic marker	51
15.	MicroRNA	RT-qPCR	Prognostic marker	52
16.	ctDNA	RT-qPCR	Detecting minimal residual disease	52
17.	MSH-2	Genetic testing (PCR, sequencing)	Identifying Lynch syndrome	51
18.	MSH-6	Genetic testing (PCR, sequencing)	Identifying Lynch syndrome	51
19.	MLH-1	Genetic testing (PCR, sequencing)	Identifying Lynch syndrome	51
20.	PMS-2	Genetic testing (PCR, sequencing)	Identifying Lynch syndrome	51
21.	CTNNB1	Genetic testing (PCR, sequencing)	Activating WNT signaling pathway	49
22.	SMAD2	Genetic testing (PCR, sequencing)	CRC progression	53
23.	SMAD3	Genetic testing (PCR, sequencing)	CRC progression	53
24.	MYC	Immunohistochemistry	Prognostic marker	31
25.	TERT	Genetic testing (PCR, sequencing)	Tumor progression, Poor prognosis	54
26.	Septin9	qPCR	Prognostic and tumor progression marker	55
27.	Albumin	Blood test (immunoassay)	Prognostic marker of liver metastasis	7
28.	Calprotectin	Fecal test	Prognostic marker of inflammation associated CRC	7

APC, adenomatous polyposis coli; BRAF, v-Raf murine sarcoma viral oncogene homolog B; CA 19.9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA; CTNNB1, catenin beta-1; HGF-s, hepatocyte growth factor splice variant; KRAS, Kirsten rat sarcoma viral oncogene homolog; MLH-1, MutL homolog 1; MSH-2, MutS homolog 2; MSH-6, MutS homolog 6; MSI/dMMR, microsatellite instability/deficient mismatch repair; MYC, myelocytomatosis oncogene; p53 mutations, tumor protein p53 mutations; PCR, polymerase chain reaction; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PK-M2, pyruvate kinase M2; PMS-2, postmeiotic segregation increased 2; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SMAD2, SMAD family member 2; SMAD3, SMAD family member 3; SMAD4 mutations, SMAD family member 4 mutations; TAG-72, tumor-associated glycoprotein 72; TERT, telomerase reverse transcriptase; TPS, tissue polypeptide-specific antigen; WNT, wingless/integration.

ending colon and cecum), can be identified through endoscopy.³⁰ First-degree relatives of individuals with sessile serrated polyps should undergo endoscopic screening at age 40 or 10 years before the family's earliest cancer diagnosis.⁹ Various endoscopic approaches, such as colonoscopy and sigmoidoscopy, are discussed below:

Colonoscopy

Colonoscopy is the standard technique for CRC screening, with superior sensitivity and specificity. Colonoscopy uses a fiberoptic

endoscope inserted through the rectum to inspect the entire colon up to the cecum, often with minimal sedation.^{7,30} Colonoscopy typically involves administering a benzodiazepine, particularly midazolam, intravenously with or without an analgesic. Propofol anesthesia is a common alternative to sedation. While some countries, like France, often conduct colonoscopy under complete anesthesia, others, such as Norway, frequently perform the procedure without sedation.⁴² Abnormalities such as polyps or suspected cancers can be removed or biopsied during the procedure. Recent advancements, like high-definition scopes, enhance its diagnostic

and therapeutic capabilities.⁷ The need for follow-up colonoscopies depends on initial findings.² A positive stool test mandates a follow-up colonoscopy, and the timing of this follow-up after a positive stool-based screening has been investigated.⁸ Artificial intelligence is also being used to improve polyp detection during colonoscopy.² Colonoscopies can typically identify distinct characteristics of pre-cancerous polyps. High-risk individuals, particularly those with a family history of CRC, should undergo earlier CRC screening, preferably with colonoscopy.³⁰ In high-risk individuals, colonoscopy is advised every 10 years, examining the entire colon and allowing for polyp removal. Individuals with serrated polyposis syndrome should undergo annual colonoscopies.^{9,35} The risk of CRC in the future and surveillance intervals depend on the characteristics of adenomas detected during the initial colonoscopy, considering histology, size, number, and patient factors. Increased CRC risk prompts follow-up colonoscopies, with the repeat interval determined by identified risk factors. Surveillance intervals may be adjusted based on repeat colonoscopy findings, and individuals may return to routine CRC screening when the risk decreases.⁷ According to U.S. guidelines, patients with serrated adenomas < 10 mm and no dysplasia should have a repeat colonoscopy in five years, while those with serrated adenomas ≥ 10 mm and/or dysplasia should undergo a follow-up colonoscopy after three years. Guidelines from the European Society of Gastrointestinal Endoscopy consider serrated adenomas < 10 mm and no dysplasia as low risk, suggesting a return to the CRC screening program, while serrated polyps ≥ 10 mm or those with dysplasia should be followed by a repeat colonoscopy after three years.⁹ However, drawbacks include the need for bowel preparation, sedation, potential uncovered costs, and rare procedural risks such as mucosal injury and infection.³⁰ It is more challenging to detect sessile serrated lesions during colonoscopy compared to adenomatous polyps.⁸ Furthermore, limitations include invasiveness, time off work, and post-procedure assistance. Risks such as perforation and post-colonoscopy bleeding contribute to low compliance, and detecting challenging lesions in the proximal colon remains difficult.²²

Sigmoidoscopy

Sigmoidoscopy is a minimally invasive diagnostic method utilized in CRC screening to inspect the lower part of the large intestine, from the rectum to the distal colon. This examination can be performed using either a flexible endoscope, known as flexible sigmoidoscopy, or a rigid instrument, referred to as rigid sigmoidoscopy.^{42,56} Among endoscopic methods, flexible sigmoidoscopy is one of the most widely used and studied techniques. Global recommendations suggest a follow-up sigmoidoscopy between five to ten years after the initial examination, and no later than a decade.^{9,35} Flexible sigmoidoscopy is a safer, faster procedure that is well-tolerated and focuses on the distal colon and rectum, where CRCs often develop. Insufflation is used, and the less burdensome self-administered enema for bowel preparation may improve compliance compared to colonoscopy.⁷ Like colonoscopy, it allows for the removal of cancerous and precancerous polyps (limited to the distal colon) and can be performed without sedation if needed.^{22,30} Compared to colonoscopy, sigmoidoscopy offers benefits such as limited bowel preparation, quicker procedure time, lower complications, and reduced costs.⁹ With a 95% sensitivity for CRC detection and 70% sensitivity for advanced adenomas (≥10 mm), it requires an enema for bowel preparation. If larger polyps or distal colon lesions are found, a follow-up colonoscopy is needed to check for potential proximal lesions.^{22,42} However, sigmoidoscopy

shares some drawbacks with colonoscopy, including the need for bowel preparation and safety concerns. Sigmoidoscopy, without typical sedation, may cause discomfort compared to colonoscopy under anesthesia, leaving a substantial portion of the colon unscreened.²² Around 37% of CRC cases are localized in the rectum, while 31% are in the sigmoid colon. The majority of colon cancers (65%) are distal to the splenic angle, making them detectable through sigmoidoscopy. However, a smaller proportion (35%) is located proximal to the sigmoid colon and is not easily detectable using this method.^{24,57} Although effective for distal CRC screening, sigmoidoscopy is now largely replaced by colonoscopy due to limitations in detecting proximal colon cancer.³⁰

Capsule endoscopy

Capsule endoscopy, a non-invasive alternative to colonoscopy, allows for comfortable colon examination without the discomfort associated with the procedure. It eliminates complications and sedation risks, promoting increased adherence to screening.⁹ This technique involves a pill-sized capsule with a camera that transmits images for eight hours in the gastrointestinal tract, eliminating the need for complete bowel lavage. It offers a potential alternative to colonoscopy by avoiding discomfort and risks. However, colonoscopy may still be required for removing suspicious lesions, and the cost-effectiveness of population-based screening remains uncertain.⁷

Radiology

Radiology plays an important role in the screening and management of CRC. Various imaging modalities (CT, magnetic resonance imaging, colonography, etc.) are used to detect the stage and monitor CRC,⁵⁸ as discussed in the sections below:

Computed tomographic colonography (CTC)

CTC, or virtual colonoscopy, is a non-invasive or semi-invasive, quick radiographic technique increasingly used for colon examination. It offers low radiation exposure and avoids intubation and sedation risks. CTC is a valuable alternative for those unable to undergo or tolerate colonoscopy, assessing the colon through 3D images (using CT and specialized software) and detecting polyps ≥ 10 mm in 90% of cases and polyps of 6–9 mm in 70–80% of cases.^{2,9,42,59} CTC is recommended every five years. Despite its advantages, CTC has limitations, including the need for bowel preparation, inability to detect small polyps, flat adenomas, and high-risk serrated lesions. Additionally, if lesions are found, subsequent colonoscopy is required for direct removal and better utilization, and the technique tends to be more expensive compared to alternatives.^{9,30} Compared to stool tests, CTC is more invasive and costly.⁵⁹ Patients must prepare for it in the same way as for a colonoscopy, and the procedure itself can be uncomfortable due to the insufflation process (involving insufflating the colon with air or carbon dioxide, and the scan is done while the patient briefly holds their breath). The overall sensitivity of CTC is similar to colonoscopy, but it is notably less effective at detecting polyps smaller than 8 mm.^{2,7,22} Limitations include concerns over ionizing radiation, safety considerations, and the need for specific healthcare facilities.²²

Double-contrast barium enema

A double-contrast barium enema is a diagnostic imaging technique used to examine the colon and rectum. It serves as both a means of screening for CRC and diagnosing a range of colorectal conditions.^{58,60} During the procedure, barium sulfate, a contrast agent,

is used to highlight the inner lining of the colon and rectum on X-ray images. The patient is placed on an X-ray table, and a tube is inserted into the rectum to introduce barium sulfate into the colon, coating its walls and outlining its structure. Air is then introduced to improve contrast, aiding in the clear visualization of the colon lining. X-ray scans are taken from different angles to detect abnormalities such as polyps or tumors. After imaging, the patient expels the barium and air, which may cause temporary discomfort and bloating.⁶¹ Historically, the barium enema was the primary method for detecting CRC, but it is now seldom used. It is considered for patients with significant co-existing conditions, requires bowel preparation, and is less effective than CT colonography, with lower sensitivity for detecting polyps compared to colonoscopy.⁷

Integration of recent technologies in CRC diagnosis

The integration of advanced technologies, such as single-cell sequencing (SCS), spatial transcriptomics (ST), and artificial intelligence (AI), has significantly enhanced CRC screening, offering improved detection, characterization, and predictive capabilities.⁶²

Single-cell sequencing in CRC screening

Single-cell sequencing allows for the analysis of genetic and transcriptomic profiles at the individual cell level, providing insights into tumor heterogeneity and the tumor microenvironment.^{63,64} This has been instrumental in identifying distinct subpopulations of tumor cells with unique mutational profiles, including key driver mutations in genes such as *KRAS*, *BRAF*, *p53*, *APC*, and *PIK3CA*.^{65,66} By elucidating the interactions between cancer cells and their microenvironment, SCS contributes to a deeper understanding of tumor progression and potential therapeutic targets.⁶⁷

Spatial transcriptomics for understanding the tumor micro-environment

Spatial transcriptomics enables the mapping of gene expression within the spatial context of tissue architecture, preserving the relationships between tumor cells and surrounding stromal and immune components. This approach has revealed critical insights into tumor-immune interactions, epithelial-mesenchymal transition, and MSI in CRC. By integrating ST with other imaging techniques, researchers can stratify CRC patients based on molecular subtypes, facilitating more personalized screening and therapeutic strategies.^{68–70}

AI in CRC screening and diagnosis

AI has revolutionized CRC screening by enhancing the accuracy and efficiency of diagnostic procedures:

- *AI in colonoscopy*: Deep learning-based computer-aided detection systems have improved the identification of colorectal polyps and adenomas during colonoscopy.⁷¹ Studies have demonstrated that AI-assisted colonoscopy increases adenoma detection rates and polyp detection rates, particularly for small and flat lesions that are easily overlooked.^{72,73} This enhancement reduces the risk of missed diagnoses and interval cancers.
- *AI in histopathology*: Convolutional neural networks have been employed to analyze histological slides, automating the differentiation between normal, dysplastic, and malignant tissues. This application of AI not only increases diagnostic accuracy but also reduces the time required for pathological assessments.⁷⁴
- *AI in multi-omics data integration*: By integrating data from genomics, transcriptomics, proteomics, and metabolomics, AI models can predict CRC risk and treatment responses with



Fig. 5. Various lifestyle approaches to manage CRC. CRC, colorectal cancer.

greater precision.⁷⁵ Machine learning algorithms have been utilized to correlate circulating tumor DNA, microRNA, and epigenetic markers with disease progression, enabling early non-invasive detection.^{76,77}

Future perspectives and challenges

While these technological advancements have transformed CRC screening, challenges remain in their widespread clinical implementation. High costs and technical complexities limit the accessibility of SCS and ST.⁷⁸ AI-driven screening tools require extensive validation across diverse populations to ensure reliability and generalizability.^{79,80} Additionally, ethical considerations, including data privacy and potential biases in AI algorithms, must be addressed.^{81,82} Despite these challenges, the integration of SCS, ST, and AI holds immense potential to make CRC screening more precise, minimally invasive, and patient-centered, ultimately aiming to reduce CRC-related mortality.

Lifestyle management of CRC

Lifestyle plays an important role in the management of CRC.⁸³ A reduction in CRC incidence and mortality can be achieved through dietary adjustments, increased physical activity, and secondary prevention via screening (Fig. 5).²⁹

The risk of CRC can be influenced positively or negatively by the collective impact of components within a dietary pattern.²⁵ Furthermore, diet plays a role in daily challenges to DNA repair from various carcinogens and influences early colon carcinogenic events. Genetic polymorphisms affecting enzyme activity and DNA repair proteins contribute to cancer risk, making them crucial in the mutational process.³³ Elevated CRC risk is associated with modifiable factors like alcohol, obesity, smoking, and processed/red meat consumption.²⁹ A different chemo-preventive approach proposes that incorporating prebiotics into the diet can help prevent CRC by enhancing intestinal function and promoting a healthy microbial environment in the colon. Modulating gut microbiota with probiotics and prebiotics, either alone or together, is an effective preventive approach for inflammation and CRC. Combining conventional treatment with natural compounds, such as *Ganoderma lucidum*, polysaccharides, and triterpenes, enhances therapeutic effects and minimizes side effects. According to studies, fruit and vegetable intake does not appear to be associated with the risk of proximal colon cancer and rectal cancer, except for *Brassica* vegetables. The risk of distal colon cancer significantly decreases with

the consumption of carrots, pumpkins, and apples.³³ Conversely, protective factors for CRC include a higher intake of dietary fiber, green vegetables, folate, and calcium.²² Natural compounds, even in high-risk populations, can modify the risk of tumor development, as seen in Chinese women with a genetic predisposition to breast cancer, where regular green tea consumption is linked to a reduced risk.³³ According to studies, calcium protects against CRC by precipitating carcinogenic substances (such as bile acids, ionized fatty acids, and heme iron) in the colorectal lumen, thereby diminishing their carcinogenic effects. Furthermore, calcium binding with calcium-sensing receptors on intestinal epithelial cells activates intracellular signaling pathways, inhibits proliferation, and promotes differentiation and apoptosis in intestinal epithelial cells. Regular physical activity is also associated with a reduced CRC risk, influencing factors such as gut motility, immune response, inflammation, and metabolic hormones.²⁵ Reduced risk is linked to physical activity, postmenopausal hormone therapy, and non-steroidal anti-inflammatory drugs. However, evidence on the impact of fruit and vegetable intake on CRC risk has been inconsistent.²⁹

Limitations

While this review offers a broad overview of CRC diagnostics and management strategies, several limitations should be acknowledged. This narrative review lacks quantitative analysis, which limits the strength and generalizability of its conclusions. Variations in CRC screening guidelines and the limited effectiveness of early detection are noted but not deeply explored. Key aspects such as molecular subtyping, treatment resistance, socioeconomic disparities, and patient-centered outcomes are not comprehensively addressed, highlighting the need for further multidisciplinary research.

Future perspective

CRC is a multifactorial disease influenced by genetics, environment, and lifestyle. It often begins as precancerous polyps and may progress silently, making early detection critical. Symptoms range from subtle bowel changes to advanced signs like weight loss and fatigue. Non-invasive screening tools such as FOBT, stool DNA analysis, and blood-based biomarkers have improved early diagnosis and patient outcomes. However, challenges remain due to CRC's asymptomatic nature and rising incidence, especially in younger populations. There is an urgent need for uniform global screening guidelines targeting high-risk individuals, including those under 50. Emerging technologies like SCS, spatial transcriptomics, and AI integration promise breakthroughs in personalized diagnostics and treatment. Additionally, promoting healthy lifestyle changes can help reduce CRC risk factors.

Conclusions

While CRC detection and management have seen notable advancements, significant challenges remain, particularly due to its asymptomatic nature and increasing incidence, especially among younger populations. Current screening programs lack standardization and fail to adequately address high-risk individuals, underscoring the need for universal, age-inclusive, and targeted screening strategies. Innovative technologies such as SCS, spatial transcriptomics, and AI-driven tools offer exciting potential for improving early detection and personalizing treatment. Additionally, addressing modifiable risk factors through lifestyle interventions and advancing

chemoprevention research are crucial steps toward reducing CRC incidence. A coordinated global effort that integrates improved screening, technological innovation, and public health strategies is essential to effectively combat CRC and improve long-term patient outcomes.

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Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

Collection of the information, drafting of the manuscript (PK), writing-review (PK, ZBZ, RS, SK), data curation, conceptualization, planning, analysis, review, and editing (KC, SVP). All authors have approved the final version and publication of the manuscript.

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