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Review Article

An Insight on Gut Flora, Colorectal Cancer Mechanism, and Treatment Strategies

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The microbiota in the stomach functions like an actual organ. To maintain gut homeostasis, the digestive tract's symbiotic relationships with the local microorganisms are crucial. This symbiotic connection may be upset, and illnesses like inflammatory bowel disorders and cancer can be promoted. Infections, dietary changes, and lifestyle modifications are a few examples of environmental factors that might alter the microbiome. It is becoming increasingly clear that the microbiota plays a part in the development of colorectal cancer. The complex interplay of tumour cells, nonneoplastic cells, and a large variety of microbes results in colorectal cancer. About 10% of new cancer cases globally are colorectal cancer instances (CRC). The gut microflora, which is situated adjacent to the colorectal epithelium, is made up of a sizable population of bacteria that interact with host cells to control a variety of physiological functions, including energy production, metabolism, and immune response. Sequencing research has revealed microbial compositional and ecological changes in CRC patients, while functional research in animal models has identified several bacteria, including *Fusobacterium nucleatum*, specific strains of *Escherichia coli*, and *Bacteroides fragilis*, as key players in the development of colorectal cancer. In this review, we focus on dysbiosis and the potentially carcinogenic characteristics of bacteria to evaluate the possible connections between the bacterial microbiota and colorectal carcinogenesis. We also discuss pertinent mechanisms in microbiota-related carcinogenesis, the potential for using the microbiota as CRC biomarkers, and the possibility of manipulating the microbiota for CRC prevention or treatment.

1. Introduction

There is broad consensus on the significant impact of the gastrointestinal microflora on both humans' and animals' health [1]. Numerous bacterial species have developed and adjusted to survive and proliferate in the human gut. A person's intestinal environment comprises between 300 and 500 distinct kinds of bacteria, and the number of microbial cells in the gut lumen is approximately 10 times more than the number of eukaryotic cells in the human body [2]. Natural selection has shaped the structure and makeup of the gut flora at both the microbial and host levels, fostering cooperation and maintaining the functionality of this intricate ecosystem [3]. We still do not fully understand the processes that determine the gut flora's makeup and how it is built up. However, it is evident that facultative aerobes, such as *streptococci* and *Escherichia coli*, colonise humans when they are born. However, at the crucial time of weaning, there is a major change in the flora, with obligate anaerobes, notably *Bacteroides* species, becoming dominant [4]. Up to 10^5 CFU/ml are present in the upper bowel's (stomach, duodenum, and jejunum) scanty microbiota. 10^5 organisms/g of stomach contents can be observed immediately after a meal, but the number reduces along with the drop in pH and falls below 103/g after an hour. In the ileum, the concentrations progressively rise to 10^{11}–10^{12} CFU/g in the colon [5]. In order to do a traditional bacteriological examination of the intestinal flora, it is necessary to cultivate bacteria with great care on a variety of growth media and identify isolates using a variety of techniques. Anaerobic bacteria are 100–1000 times more numerous than aerobic bacteria, according to the findings of some studies. Aerobes (facultative anaerobes) including *Escherichia*, *Enterobacter*, *enterococcus*, *klebsiella*, *lactobacillus*, and *proteus* are among the subdominant genera. The most common

genera in humans include *Bacteroides*, *Bifidobacterium*, *eubacterium*, *clostridium*, *Peptococcus*, *Peptostreptococcus*, and *ruminococcus* [2] as shown in Figure 1. Metchnikoff made the initial theory on the significance of *lactobacilli* for human health and lifespan at the beginning of this century. He claimed that the majority of gut microorganisms were harmful rather than helpful and that yoghurt bacteria could only replace them to provide the desired results. The pharmacokinetics of various probiotics in humans have been the subject of several research, and our understanding of these effects, particularly the intricate mechanism behind them, is continuously expanding [1]. Therefore, consuming live microorganisms in the form of concentrated preparations like powders, pills, or capsules, or through meals like yoghurt and other fermented foods, can have a probiotic effect on the gut microbiota. They may contain a single species of microorganism or multiple [6]. Due to its intricacy, the ecosystem's inaccessibility in some areas, and the temporal and geographical variety, methods for identifying the gut flora are restricted [7]. New identification technologies have allowed us a fresh look at the gut microbiota. Genome analysis is being used to enhance standard microbiological techniques like as culture and microscopy. Techniques for detecting and semiquantifying both culturable and nonculturable gut bacteria are now accessible, sparking increasing interest in the study of the intestinal flora [7]. There are no single bacteria in the faecal flora that has been linked to the aetiology of inflammatory bowel illness. Several investigations employing various approaches, however, have frequently demonstrated that the stool microbiota differs between people having inflammatory bowel disease and healthy controls. In terms of mucosal adherent flora, several investigations have consistently demonstrated that as compared to controls, individuals with inflammatory bowel illness have a larger concentration of bacteria in the mucosa. This appears to be true both in the mucus layer and at the epithelial surface, where bacteria are significantly less prevalent physiologically. The bacterial makeup of the mucus layer has been demonstrated to stay stable throughout the colon in both Crohn's disease patients and healthy persons [8]. The distribution of gut flora is confounded by changes not just along the gastrointestinal tract, but also inside the intestinal lumen. The metabolism of pharmaceuticals and other xenobiotics by gut flora has led to the conclusion that metabolic alterations in the gut flora are considerably more widespread than in any other area of the body. The toxicological relevance of gut flora metabolism was initially emphasised. Hydrolysis, dihydroxylation, decarboxylation, dealkylation, dehalogenation, deamination, heterocyclic ring fission, reduction, aromatization, and oxidation have all been recognised as metabolic processes done by gut flora. The majority of drug metabolic alterations by gut flora are studied in terms of enzymatic changes, although chemical reactions including the formation of H_2S and methanethiol in the gut contents should also be considered [9]. Probiotics may have an important role in the treatment of inflammatory bowel disease, according to mounting data from both human and animal research. Ingestion of a significant number of live bacteria necessitates a guarantee of safety. If bacteria obtained from natural flora are employed, their natural existence attests to their safety [7].

2. Colorectal Cancer

Nearly 2 million new instances of colorectal cancer are diagnosed each year, making it the second most prevalent cause of cancer-related deaths globally [10]. CRC reduced somewhat among individuals aged $p > 50$ years, in contrast to a rise in incidence of around 20% among adults aged 50 years, with a 10% increase in death [11]. Numerous epidemiological studies have shown that consuming an excessive amount of animal protein and fat, particularly red meat, and processed meat, may induce colorectal carcinogenesis [12]. Climate conditions, socioeconomic circumstances, education, and stress are examples of general external influences. Infections, radiation, alcohol, smoking, nutrition, physical activity, antibiotics, and pharmaceuticals are examples of external environmental influences. Internal environmental factors include metabolic parameters, the gut microbiota, oxidative stress, inflammation, and hormones [13]. The gut microbiota is one of the most significant internal environmental influences. Under some conditions, exposure to an external environmental element, such as stress or antibiotics, or an internal one, such as inflammation, causes dysbiosis in the gut microbiota (shown in Figure 2) and, as a result, CRC. Certain bacteria, for example, mediate the effects of a certain diet on CRC risk by producing butyrate, folate, and biotin, all of which play important roles in the control of epithelial proliferation. The microbiota associated with CRC also contributes to carcinogenic epigenetic signatures [14]. The bidirectional contact of tumour cells with their milieu has been observed in chick and zebrafish models, where the embryonic microenvironment restored the cancer phenotype of transplanted tumour cells [15]. In 1975, researchers found that germ-free rats had fewer chemically induced colorectal tumours than conventional rats, which led to the first finding associating gut microbiota with CRC [16]. These findings have been replicated in CRC-prone mice [17].

3. Diet and the Gut Microbiota's Composition

Transit time, pH, oxygen exposure, nutrition availability, host secretions (including bile and digestive enzymes), mucosal surfaces, and immune system interactions are all variables that affect microbial colonisation in the various regions of the digestive tract [18]. In healthy people, the large intestine possesses the densest and most metabolically active microbial community, which is dominated by anaerobic bacteria from two phyla (*Firmicutes* and *Bacteroidetes*), as well as *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [19]. Many of the taxa that respond to changes in carbohydrate consumption appear to be nutritionally adapted *Firmicutes* and *Actinobacteria* [20]. *Ruminococcaceae* increase in response to resistant starch-enriched diets, but *Lachnospiraceae* increase in response to wheat bran-enriched diets [21]. Although the association between dietary fibre intake and the risk of cancer has been disputed, recent meta-analysis studies demonstrate that a high intake of dietary fibre, especially from cereals and whole grains, is linked to a lower risk of CRC [22], and patients with advanced colorectal adenomas—which are CRC precursor lesions—have lower dietary fibre intakes than healthy controls [23]. On the

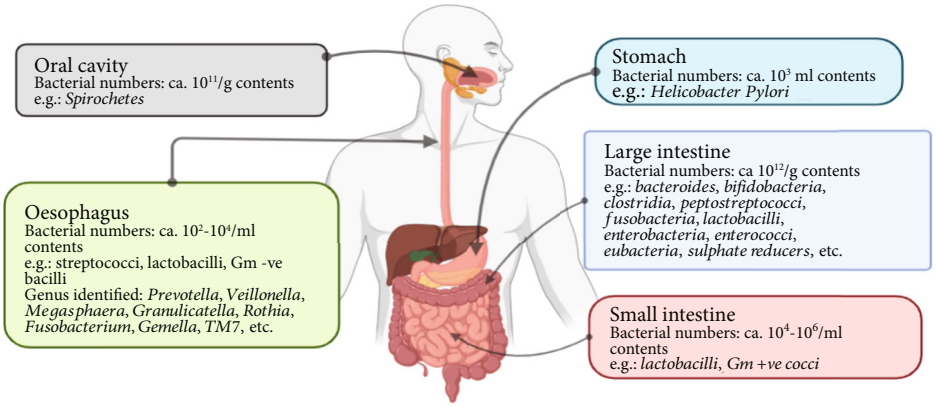

Figure 1: Microbiota present in the human gastrointestinal tract.

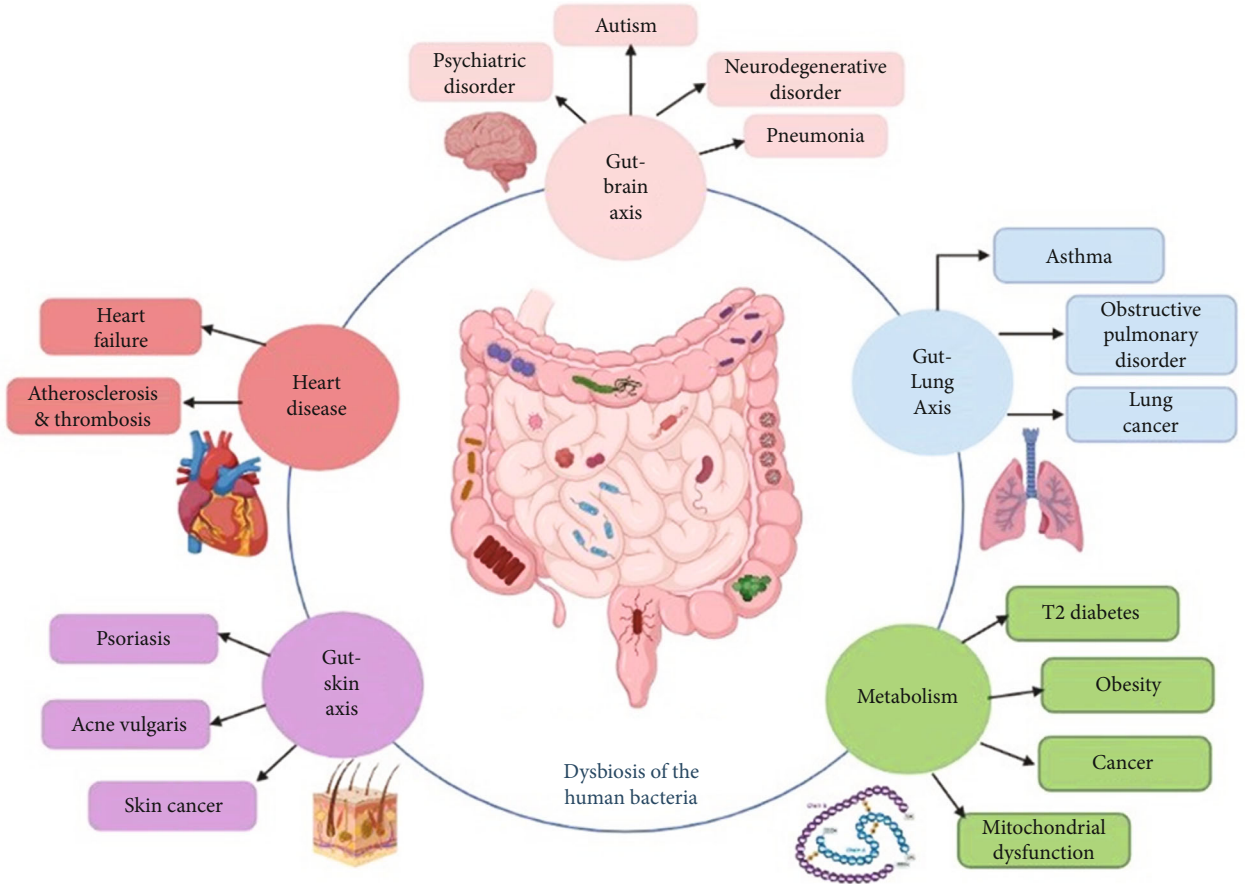

Figure 2: The gut microbiota dysbiosis in human disease.

other hand, a higher risk of CRC has been associated with diets heavy in red and processed meat, fat, and alcohol [24].

4. Inflammation and Gut Flora

Gut microbiota has a substantial impact on immune responses, and chronic inflammation is well-known to increase the risk of colorectal cancer. T cells, B cells, tissue-associated macrophages, and other innate immune cells engage in direct interactions with one another and other cells in the tumour microenvironment or send signals via cytokines and chemokines to regulate tumour development [25]. T cells are the immune cells that are most common in the cancer microenvironment, followed by TAMs. T lymphocytes may both encourage and inhibit the development of tumours. Increased numbers of CD4+ T helper 1 (TH1) and CD8+ cytotoxic T cells are linked to the direct lysis of cancer cells and the generation of cytotoxic cytokines, which slow the development of colorectal cancer [26]. Notably, the absence of gut microbiota or microbial products prevents inflammation from causing CRC [27]. Through a variety of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) that regulate the inflammatory response to molecular

patterns associated with microorganisms (MAMPs), including lipopolysaccharide (LPS), flagellin, and nucleic acids, the microbiota can recognise the host [28]. In the colonic environment, PRRs are essential for preserving mucosal homeostasis and limiting inflammation. Changes in TLR4 signalling, which is the main LPS receptor, have been specifically associated to the development of CRC [29].

5. Gut Microbiota in Colorectal Adenoma

The majority of CRC are known to have colorectal adenomas as their precursor [30]. The high-risk advanced adenoma is a significant focus for CRC screening since the majority of CRC starts with the malignant transformation of benign polyps. New methods to identify, control, or stop the evolution of these CRC precursors may emerge as a result of better understanding the biology of colorectal adenomas [31, 32]. In a 2019 investigation, 616 people who had colonoscopies provided stool samples, and it was shown that microbiota changes were present in cases of multiple colorectal adenomas and intramucosal carcinomas [33]. Stage-specific investigations revealed that some bacterial species, such *F. nucleatum* and *Solobacterium moorei*, showed a gradual rise in abundance from early to late stages of carcinogenesis, while other species, like *Atopobium parvulum* and *Actinomyces odontolyticus*, were raised in abundance only at an early stage of carcinogenesis (in patients with multiple adenomas or intramucosal carcinoma). In a research that examined the mycobiome of people with colorectal adenomas of various sizes and stages, the variation in abundance of numerous fungal taxa, such as Basidiomycota, was also noticeable [34]. Due to existing stool-based occult blood tests' inability to detect early adenomatous lesions, these variations in abundance make them potentially useful as indicators for these lesions.

6. Mechanisms in Colorectal Carcinogenesis

The development of colorectal cancer is a complex process that is impacted by both hereditary and environmental variables and has a variety of etiological processes. Inflammation, immunological control, dietary component metabolism, and the formation of genotoxin are a few of these processes that have been well examined and are strongly related to the gut microbiota [35]. Here, we focus on a few microbiota-associated elements in CRC carcinogenesis that may be modified and applied in the clinic.

6.1. Inflammation and Immune Regulation. The gastrointestinal tract serves as a crucial point of contact for interactions between the host immune system and the gut bacteria [36]. Chronic inflammation is a hallmark [37, 38] and a recognised risk factor for CRC, as demonstrated by the higher risk of the cancer in people with inflammatory bowel disease compared to the general population [39]. Patients with Crohn's disease have a risk of 8.3% [40], whereas those with ulcerative colitis had a risk of 18.4% over the course of 30 years [41]. However, the reported levels of risk varied depending on the study population (for instance, high-risk cases versus population cohorts), hospital setting (for instance, tertiary versus general hospitals), and clinical practise (for instance, rate of proctocolectomy) [42]. The gut microbiota can have an impact on the inflammatory process in the gastrointestinal tract by intimately interacting with the host immune system. Since microorganisms in the gut can cause chemotactic factors (such as CXCL9 and CXCL10 for cytotoxic T lymphocytes and type 1 T helper (TH1) cells, and CCL17 and CCL20 for IL-17-producing TH cells) to recruit T cells into tumours [43], oral administration of stool from CRC patients to germ-free and carcinogen-fed mice increased histological inflammation and the expression of inflammatory gene markers [44]. Regarding specific bacterial species, it has been documented that *F. nucleatum* can activate the nuclear factor-B pathway and cause myeloid cell infiltration in tumours [45], creating an inflammatory environment that is favourable for the progression of colorectal neoplasia in *ApcMin* mice (a popular mouse model of CRC) [46]. Through its virulence component *B. fragilis* toxin, enterotoxigenic *B. fragilis*, a strain that is more prevalent in CRC patients [47], may cause an inflammatory cascade involving IL-17, signal transducer and activator of transcription 3, and nuclear factor-B signalling in colonic epithelial cells [48]. These signalling pathways then cause CXC chemokines to attract polymorphonuclear immature myeloid cells in *ApcMin* mice, resulting in the development of an inflammatory milieu, particularly at the distal colon [49]. Other bacteria, such as the CRC-enriched genotoxic polyketide synthase (pks)+ E. coli [50–52], E. faecalis [53], and *A. finegoldii* [54], also contribute to the development of cancer by causing inflammation. The human immune system and the gut bacteria are connected through pattern recognition receptors (PRRs). Through a downstream cascade of signalling molecules, PRRs that recognise microbial antigens trigger the intestinal immune system. Toll-like receptors (TLRs) [55], nucleotide-binding oligomerization-like receptors [56], RIG-I-like receptors [57], and missing in melanoma 2-like receptors [58] are a few of these PRRs that have been linked to colitis-associated carcinogenesis in animal models. Particularly, *F. nucleatum*, a CRC-enriched bacteria, may stimulate TLR4 signalling to increase tumour growth in mice [59, 60], whereas *Peptostreptococcus anaerobius*, another bacterium, can activate TLR2 and/or TLR4 to promote carcinogenesis in mice [61].

6.2. CRC Induced by Heme Iron from Red Meat. Red and processed meat consumption, poor dietary fibre, and other lifestyle variables such as alcohol, cigarettes, and inactivity are all causally related to CRC [62, 63]. According to epidemiological research, eating meat increases the risk of developing CRC [64, 65]. The International Agency for Research on Cancer rated red meat as probably carcinogenic in humans in 2015 and processed meat consumption as carcinogenic. In comparison to white meat, like chicken, red meat—such as beef and lamb—has a higher myoglobin content and contains more heme iron [66, 67]. Interestingly, eating heme considerably raises the risk for CRC, according to a meta-analysis of epidemiological research [68].

Heme is a collective term for several proteins, including the oxygen-transporting haemoglobin and myoglobin [69].

Haemoglobin is made up of two and two-chain subunits that can bind up to four oxygen molecules in the lung and carry them to the tissue through the bloodstream. The monomeric myoglobin in tissue with a single chain receives oxygen thanks to the Bohr effect [70]. It is well-known that (heme-) iron catalyses the Fenton's reaction, which produces ROS [71]. Ferrous iron (Fe2+) is oxidised to ferric iron (Fe3+) in this redox reaction, while H2O2 is reduced to a hydroxyl anion and a hydroxyl radical. The latter directly interacts with macromolecules at its place of origin and has a relatively short half-life. By abstracting hydrogen atoms from the C-H bonds of the 2-deoxyribose sugar moiety and adding to the double bonds of DNA bases, the hydroxyl radical damages DNA and causes oxidative DNA lesions like 8-oxoguanine (8-OxoG) and thymine glycol, single-strand breaks, and abasic sites [72]. Specific DNA glycosylases, such as 8-oxoguanine glycosylase-1 (OGG1), which removes the damaged base as the initial stage of base excision repair (BER), can detect oxidative base damage [73]. Surprisingly, there are surprisingly few studies available that address the oxidative DNA damage caused by heme or haemoglobin in cultured cells. According to an FPG-modified alkaline comet assay, haemoglobin at high dosages was found to marginally exacerbate oxidative DNA damage [74]. Dietary heme disrupts the intestinal barrier and results in a microbial dysbiosis, which exposes the epithelium to enterobacteria and, consequently, bacterial lipopolysaccharides (LPS). Although one study found no indication of innate immune reaction and inflammation pathways induced by Toll-like receptor 4 (TLR4) in heme-fed mice, it is plausible that bacteria reaching the epithelium will provoke an immune response and maybe worsen intestinal inflammation [75]. Inflammatory mechanisms are known to promote colorectal carcinogenesis [76, 77]. Supporting evidence comes from two recent investigations that found eating heme makes chemically induced colitis in animals worse [78, 79]. Aside from these indirect effects, heme directly promotes or inhibits the activity of several populations of blood cells. The primary cell type that absorbs heme from ageing erythrocytes and provides heme iron for erythropoiesis is macrophages. Heme's toxicity towards healthy tissue is reduced by internalisation into macrophages, and pathogens are prevented from accessing heme iron, which is necessary for their proliferation [80].

6.3. Intestinal Tumorigenesis, Heme Iron, and Red Meat. Consuming red meat and heme iron increases the likelihood of developing CRC, according to epidemiological studies. The colon cancer incidence was higher in the group with haemoglobin when the alkylating chemical methylnitrosurea was given intrarectally to rats over a period of two weeks, followed by a high-fat diet with or without 3% haemoglobin for 36 weeks [81]. A later study established a direct connection between dietary heme iron and colon cancer for the first time [82]. Azoxymethane (AOM), a colonotropic tumour inducer, was administered to rats in this study before heme iron or haemoglobin was administered for 100 days. When isolated colon tissue was stained with methylene blue, it revealed an increase in the size and number of dysplastic aberrant crypt foci (ACF), which are thought to be the precursor lesions of colon cancer [83]. Additionally, dietary supplementation with high calcium or antioxidants decreased the number as well as the size of ACF, and heme iron was found to be a more potent inducer of ACF than haemoglobin [82]. To confirm these conclusions, meals containing beef or black pudding considerably induced the development of ACF and mucin-depleted foci (MDF) in AOM-initiated rats, but eating chicken only slightly increased the ACF and MDF number [84]. Importantly, animals lacking the tumour initiator AOM did not develop intestinal neoplasia or ACFs when fed a diet containing 2.5% haemoglobin (1.5 mol heme iron/g food) [85], indicating a significant role for heme iron in the production of tumours. An experiment feeding rats processed meat made from gammon further supported this. Following a ham-based diet (0.25 mol heme iron/g diet) for 100 days, the mice received an injection of the alkylating chemical dimethylhydrazine as a tumour initiator, which accelerated the production of ACF and MDF [86].

7. Use Microflora as a Biomarker

A biomarker is a biological sign of a disease's presence or severity. A reliable and noninvasive screening test might significantly lessen the burden of CRC on global health, given the strong evidence that identifying average-risk persons for screening can lower CRC incidence and mortality [87]. The possibility for employing these indicators for treatment prediction and prognostication has also increased because of the connections between bacterial markers and treatment efficacies or clinical outcomes that have been documented in a number of studies. Potential biomarkers can be found in abundance by studying the gut microbiome. We go over possible applications for CRC screening and prognostication of microbiota-related indicators in this section.

8. Screening Biomarkers

8.1. CRC Detection by Faecal Markers. In particular, early CRC, which may be treated with outstanding clinical results, requires accurate biomarkers for screening. In contrast to 14% in distant metastatic cancers, localised CRC, for instance, has a 5-year survival rate of 90% [88]. The current faecal immunochemical test (FIT) only has a 79% sensitivity rate to detect CRC [89] and a 25–27% rate to detect advanced colorectal adenomas [90]. Despite having a higher sensitivity than FIT (92.3%), the multitarget stool DNA test is nonetheless constrained by a worse sensitivity for detecting advanced adenoma (42.4%) [91]. Therefore, it would be ideal to have a test that is sensitive enough to detect advanced adenomas as well as CRC and is also accurate, inexpensive, and noninvasive. An abundant source for creating faecal microbial markers for illness detection is the growing quantity of metagenomic datasets in the CRC. Many studies have used the prevalence of different bacterial species to distinguish between CRC patients and healthy people, including two case-control studies that achieved areas under the receiver operating characteristic (ROC) curves (AUC) of 0.84 to 0.85 using 22 and 34 microbial markers, respectively [92, 93]. A group of 20 microbial genes were linked to disease presence in a prior metagenomic investigation that

compared CRC ($n = 74$) and healthy persons ($n = 54$) in Hong Kong [94]. Butyryl-CoA dehydrogenase from *F. nucleatum* and RNA polymerase subunit (rpoB) from P. micra, two useful biomarkers that can be quantified by PCR, might be used to reduce this group of gene markers and reach an AUC of 0.84. In one study using a Bayesian methodology, a logistic regression model combining data on the abundance of six different bacterial species in faeces could distinguish between patients with CRC and healthy people with an AUC of 0.80. This AUC increased to 0.92 when age, race, and BMI were factored into the model [95]. Additionally, given the newly discovered metagenomic landscapes of the virome and mycobiome in CRC, microbial signatures derived from these communities may be used as screening biomarkers for CRC [96, 97]. When measured either by itself [98, 99] or in combination with other bacteria [93, 100], particularly *Clostridium* symbiosum [101], *C. hathewayi*, and bacteria that produce colibactin (clbA+) [102], *F. nucleatum* stood out as a major marker among a number of bacterial contenders. In comparison to utilising FIT alone, the faecal abundance of *F. nucleatum* can enhance the effectiveness of FIT and provide higher sensitivity and specificity for the detection of CRC [92, 103]. For example, it has been demonstrated that increasing the FIT with faecal *F. nucleatum* abundance causes the AUC to rise from 0.86 to 0.95. While the best panel will likely have the variety of markers balanced between accuracy, logistic feasibility, and simplicity of the analysis, this method demonstrates the benefits of a multitarget test in which separate components can complement one another to decrease the cases of missed malignancy. Only 16 species could accomplish cross-validation AUC > 0.8 for the bulk of the datasets in a large-scale meta-analysis research that tested a random forest classifier and was published in 2019 [104]. This discovery supports the development of a precise stool-based diagnostic test that uses markers that focus on a small subset of bacteria species or genes. Lastly, meta-analyses using metagenomes from varied geographic regions reveal that polymicrobial classifiers are resilient against regional and technological variations and are relevant globally [104–106].

8.2. Faecal Indicators for Adenoma Detection. It has been determined that the progression of CRC from a healthy mucosa to a precursor lesion and then a malignant tumour occurs over time. The main lesion that precedes CRC is an adenoma, which can be surgically removed to stop the progression of cancer after being discovered [107]. To prevent and lower CRC, it is therefore important to find adenomatous polyps, especially advanced neoplasms. Colorectal adenomas are difficult to detect using the current noninvasive stool-based screening methods, such as FIT and the multitarget stool test. After integrating abundance data from five bacterial species with clinical characteristics, faecal microbial indicators could discriminate patients with colorectal adenoma from healthy controls with an AUC of 0.90, resulting in a 4.5-fold increase in posttest chance of identifying an adenoma [95]. These findings imply that microbial composition data might be used to diagnose colorectal adenomas [27, 92, 95]. Nonetheless, in identifying individuals with adenomas from healthy controls, a meta-analysis study of faeces metagenomes revealed a lower AUC value (highest AUC 0.58) [104]. According to several studies [87, 99, 108–110], *F. nucleatum* was shown to be a particular individual bacterium that was more prevalent in colorectal adenomas; however, the magnitude of the abundance differences was lower than that between patients with CRC and healthy persons. With the help of other microbial markers [93, 95] or in combination with FIT [98], it has been demonstrated that the quantification of *F. nucleatum* in faecal samples can distinguish patients with colorectal adenomas from healthy individuals as controls. However, differences between adenoma cases and controls were less pronounced [111, 112].

9. Cancer Biomarkers for Prognosis

In addition to their potential as diagnostic tools for CRC, connections between bacterial biomarkers and the clinical outcomes of CRC have increased the likelihood of their use as prognostic indicators. Tumoural *F. nucleatum* levels in tissue, as determined by quantitative PCR, were found to be negatively correlated with CRC survival in many molecular epidemiology investigations [87, 113, 114]. The hazard ratio for CRC-specific death in *F. nucleatum*-low and *F. nucleatum*-high patients, respectively, was 1.25 and 1.58, respectively, as compared to *F. nucleatum*-negative individuals [113]. This discovery emphasises the potential for measuring *F. nucleatum* in tumour tissue as a prognostic marker and, more crucially, offers encouragement that the bacterium's elimination may enhance prognosis and disease survival. Nevertheless, some research indicates that *F. nucleatum* may be related to the CRC genetic subtype (high lesions with the CpG island methylator phenotype) and tumour site (proximal malignancies) [92, 115, 116]. These variables may confuse the prognostication; hence, additional validation studies are essential before using these biomarkers in a clinical setting.

10. Modifying the Microbiome to Prevent CRCs

A tempting approach to lessening the burden of CRC is prevention. Numerous risk factors for CRC have been found by extensive epidemiological research, including eating patterns, obesity, and other lifestyle variables that may be easily modifiable [117]. According to two studies conducted in the United States, changing one's lifestyle might prevent more than half of CRC incidents [118, 119]. Additionally, a number of probiotic microorganisms have been researched in relation to CRC prevention. Here, we examine how these elements could affect the gut microbiome to lessen the risk of CRC.

10.1. Manage Obesity. Obesity has been identified as a risk factor for CRC. Accumulation of fat and the risk of CRC have a strong dose-response positive association, with individuals with a BMI over 27 kg/m2 having a nonlinearly greater risk [117]. The modulation of microorganism-derived proinflammatory molecules and metabolites by the gut microbiota has emerged as a key player in the relationship between

obesity and CRC among several mechanisms involving insulin or insulin-like growth factor 1 signalling, adipokines, sex hormone, and systemic inflammation. Obesity is linked to decreased microbial diversity [120] and a change in the makeup of the gut microbiota [121, 122]. In animals, diet-induced obesity can, in a way reliant on the gut microbiota, result in extensive histone methylation and acetylation along with a transcriptome pattern that, downstream, resembles the development of cancer [123, 124]. The gut flora can alter significantly when weight is controlled in obese people because of these interactions [125, 126]. Few clinical research have examined the impact of preventing weight gain or generating weight reduction on cancer risk generally, despite the fact that obesity and CRC have favourable relationships. Although the connection is not clear for women, observational studies point to a decreased risk of CRC in males with lesser weight increase over adulthood [127]. Although this study lacked the capacity to examine the risk of a specific cancer type, it was noted that people who were obese and had maintained weight reduction following bariatric surgery had reduced risks of obesity-related malignancies than matched obese controls who did not undergo surgery [128]. Observational data with conflicting findings were published, with the risk of CRC following bariatric surgery either rising [129] or remaining unchanged [130]. On the other hand, compared to 66,427 matched nonsurgical persons, 22,198 people who underwent bariatric surgery had a decreased incidence of obesity-related malignancies, including colon (but not rectal) cancer [131]. An article written in 2018 highlighted the varying impact of bariatric surgery on the risk of CRC [132].

10.2. Diet/Nutritional Interventions. The gut microbiota is largely determined by diet [133, 134]. Populations with diverse diets have significantly variable gut microbial assemblages, which are therefore associated with varying CRC risks. Researchers found that African Americans had a larger abundance of *Bacteroides* than the rural African group in one research comparing their diets, and that this difference was connected with higher intakes of animal protein, fat, and fibre in African Americans [135]. There has been a lot of interest in using dietary treatments to affect CRC incidence and development through the gut microbiota since dietary changes have the potential to significantly alter our microbiome [136]. One research found that converting African Americans to a high-fibre, low-fat diet for two weeks might alter the gut microbiota and lessen indicators of inflammation and cell growth in colon tissue [137]. On the other hand, moving rural Africans to a high-fat, low-fibre diet had striking reciprocal effects on mucosal cancer risk indicators. Dietary fibre, which may be obtained from natural food sources or added as a prebiotic preparation, has been identified by studies on individual dietary components as a significant influence regulating gut microbial composition and diversity [138, 139]. Prebiotics are described as a substrate that host bacteria use only when they want to provide a health advantage [140]. Through microbial fermentation, dietary fibre interventions such as fructans and galactooligosaccharides changed the gut microbiota's composition to raise the number of *Bifidobacterium* and *Lactobacillus* spp., and they elevated the content of butyrate in human faeces [141]. Importantly, a gnotobiotic mouse research has shown that dietary fibre suppresses tumour growth in the setting of colorectal neoplasia in a way that is reliant on both the microbiota and butyrate [142]. Clinical trials examining the effects of fibre supplementation in people with a history of colorectal adenomas, however, found little evidence that it might stop recurrent adenomas [143, 144]. The discrepancy in these results may be due to the diverse effects of SCFAs at the cellular level [145]. Strong epidemiological research indicate that consumption of processed and red meat is linked to a higher risk of CRC [146, 147]. Red and processed meat diet rises linearly with the risk of CRC up to a threshold of 140 g per day, according to a comprehensive assessment of data from 13 prospective studies [148]. As a result, processed meat has been labelled as a carcinogen (class 1) by the International Agency for Research on Cancer, whereas red meat has been categorised as a possible carcinogen (class 2A) [149], and consumers are advised to limit their intake of processed meat. A follow-up of vegetarians for 7.3 years demonstrated a 20% decreased incidence of CRC compared to nonvegetarians, lending validity to this advice [150]. The makeup of the gut microbiota can be significantly influenced by dietary fat consumption, as demonstrated in animal models [151, 152]. The enterohepatic circulation of bile acids, including deoxycholic acid, which accelerated the development of intestinal tumours in an *ApcMin* mice model, can be increased by dietary fat, which can also stimulate the hepatic production of bile acids to assist fat emulsification [153, 154]. However, association studies in people have shown contradictory findings on the link between dietary fat intake and CRC [155, 156]. The incidence of colorectal neoplasia has not been shown to be reduced by low-fat diets, either on their own [157, 158] or in combination with a high-fibre component [159, 160].

10.3. Probiotic Administration. Probiotics are living microorganisms that, when consumed in sufficient quantities, might provide health benefits [161]. The notion of probiotics has been around for over a century, and these microbes have been researched for their anticancer properties, with many putative immunological pathways hypothesised [162]. In preclinical studies for CRC, several bacteria, including *Bifidobacterium* and *Lactobacillus* spp., demonstrated anticancer properties through various mechanisms [163], including inhibiting cell proliferation [164, 165], inducing cancer cell apoptosis [166, 167], modulating host immunity [168, 169], inactivating carcinogenic toxins [170, 171], and producing anticarcinogenic compounds [172]. Probiotics' effectiveness for CRC in people has not been well studied in clinical studies. When *Lactobacillus* casei was given orally to patients who had undergone resection, it decreased the frequency of moderate- or high-grade dysplastic tumours but not the overall number of tumours [173]. Additionally, a synbiotic intervention using the prebiotic inulin along with the probiotics *Lactobacillus rhamnosus GG* and *Bifidobacterium* lactis Bb12 caused changes in the faecal microbiota (increased *Lactobacillus* and *Bifidobacterium*, decreased *Clostridium* perfringens), reduced cell proliferation, and improved epithelial barrier function in

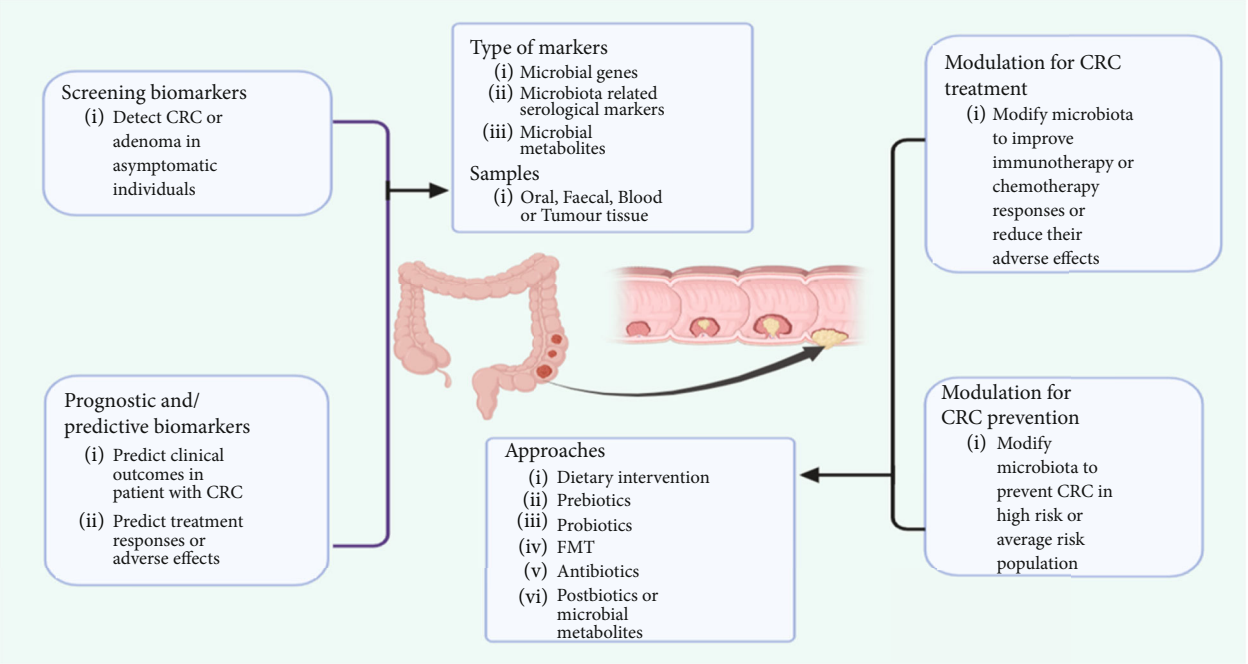

Figure 3: Possibilities for clinical use of microbiota in CRC.

patients with a history of colonic polyps [174] Nevertheless, despite changes in the faecal microbiota with a higher percentage of patients having *Lachnospiraceae* spp., a subsequent symbiotic intervention involving resistant starch and B. lactis in 20 human volunteers failed to replicate the changes in cell proliferation or other physiological markers [175]. However, there is only preliminary direct evidence that using probiotics can prevent CRC, despite in vitro and in vivo experimental results to the contrary. Probiotics' therapeutic usefulness for CRC prevention will be defined by more clinical research.

11. Utilising Gut Bacteria in CRC Treatment

There is growing evidence that the gut microbiota modulates the effectiveness and toxicity of chemotherapy and immunotherapy in addition to its functions in carcinogenesis and tumour growth [176]. This makes it possible to manipulate the gut microbiota for better cancer therapy and patient outcomes while also using it as a biomarker to identify treatment response or bad effects. Possibilities for clinical use of microbiota in CRC have been depicted in Figure 3.

11.1. Immunotherapy's Therapeutic Repercussions. Numerous tumours respond well to immunotherapy, which has grown to be a cornerstone of cancer care. Immune checkpoint drugs block inhibitory signals that prevent T cells from becoming activated, allowing tumour-reactive T cells to generate a potent antitumour response [177]. Immune checkpoint inhibitors that target the programmed cell death 1- (PD-1-) programmed cell death 1 ligand 1 (PD-L1) axis [178, 179] and the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) axis [180] can be affected by the gut microbiota, which is necessary for an efficient immune response in immunotherapy [181]. *Akkermansia muciniphila* [181], *B. fragilis* [180], *Bifidobacterium* spp. [179], *Eubacterium limosum* [182], *Faecalibacterium* spp. [183], and *Alistipes shahii* [184] are only a few of the bacteria that have been favourably connected with the success of immunotherapeutic treatment. Importantly, oral administration of *A. shahii* restored the immunotherapeutic defence against colon tumours in mice receiving antibiotic treatment [184]. Despite discrepancies in the main research, elevation of *A. muciniphila* and *Ruminococcus* champanellensis was seen in immunotherapy responders in a study that pooled shotgun metagenome data from three investigations on anti-PD-1 antibody response [185]. This discovery has opened up the idea of using these core microorganisms as biomarkers that can predict how well an immunotherapy would work. While a lack of genes in the pathways for polyamine transport and vitamin B production was linked to greater susceptibility to colitis, larger presence of the *Bacteroidetes* phylum was tied with resistant to immune checkpoint inhibitor-associated colitis [186]. Faecal microbiota transplantation (FMT) has also been shown to be a successful treatment for refractory immunotherapy-associated colitis in two reports of human cases, with gut microbial changes correlating with full resolution of colitis up to 53 days after one dose and up to 78 days after 2 doses of faecal microbiota transplantation, respectively [187]. Anti-PD-1 [188] and anti-CTLA-4 checkpoint inhibitors may offer a long-lasting response for metastatic CRC with high levels of microsatellite instability or a deficiency in DNA mismatch repair [189, 190], even though immunotherapy may not be as effective as a treatment for all CRC subtypes [183, 191] as it is for other cancers. This subgroup of CRC has characteristics that make it amenable to PD-1 inhibition, including high mutational load, cancer neoepitopes, lymphocytes that have infiltrated the tumour, and activation of immunological checkpoints [192, 193]. Given the growing usage of these

medications in some CRC patients, the gut microflora may be able to improve the effectiveness of treatment and decrease the side effects of immunotherapy [188].

11.2. Chemotherapy's Therapeutic Repercussions. An increasing body of research indicates that the gut microflora may mediate the anticancer effects of some chemotherapy drugs, such as 5-fluorouracil [194], cyclophosphamide [195], gemcitabine [196], and oxaliplatin [184], through a variety of mechanisms, including microbial translocation, immunomodulation, metabolism, and decreased ecological diversity [197]. Myeloid-derived tumour-infiltrating cells in mice treated with antibiotics or raised in germ-free environments did not respond well to treatment, leading to inadequate generation of reactive oxygen species and cytotoxicity following chemotherapy [184]. As demonstrated by experimental evidence, *F. nucleatum* may activate autophagy to impart resistance to oxaliplatin and 5-fluorouracil [198], the gut microbiota's function in chemotherapy resistance has also been examined. A new approach to prognosticating outcomes and treating CRC patients could involve measuring and focusing on *F. nucleatum*. Irinotecan (CPT-11), a prodrug of SN-38 and a topoisomerase inhibitor often used to treat CRC, is also metabolised differently and has more side effects because of the gut bacteria. Pharmacologically, host liver enzymes convert SN-38 into a conjugate that is inactive (SN-38G). The glucuronidase enzymes produced by gut bacteria hydrolyze SN-38G back to SN-38 when it enters the stomach by biliary excretion, leading to intestinal ulceration and chronic diarrhea [199]. A method of changing microbial activity to lessen the negative effects of chemotherapy treatments is shown by the administration of a specific inhibitor, which might prevent the reactivation of SN-38 in the gut and its associated toxicity in mice [200, 201].

12. Other Treatment Strategies Using the Microbiome

Tumours may be prevented or reduced as a result of cancer treatment using microbial agents or their products [162]. Using antibiotics to prevent microorganisms linked to cancer (e.g., metronidazole may decrease tumour size in *Fusobacterium*-positive xenografts in mice) [202], commensals (e.g., *Bifidobacteria* spp. [178], *A. muciniphila* [181], and *Bacteroides* spp. [180] may enhance antitumour T cell responses [162]), and small (e.g., targeting bacterial ClbP enzyme to reduce colibactin [203]) are some strategies for combating cancer-associated bacteria. Despite not having been studied in CRC patients, FMT is currently being evaluated in combination with chemotherapy or cancer immunotherapy to see how it affects the disease [204, 205]. The chemical method also provides a possible treatment option since the host-microbial interactions in CRC are partially influenced by microbial metabolites [206]. These include dietary substances known as prebiotics [207] or postbiotics [145, 208] that are metabolites produced by the microbiota. Using bacteriocins, bacteriophages, or genetically modified probiotics to alter the gut microbiota are some further innovative strategies. Bioengineering the gut microbiota is another.

13. Conclusions

It is complicated how the host and the gut-resident bacteria interact. Since birth, each person has carried a unique gut microbiota signature, and as they age, alter their food, and are exposed to a variety of environments throughout their lives, their intestinal microbiota changes and evolves. This equilibrium is indeed extremely fragile and undergoes several alterations throughout the course of a lifetime. During gut dysbiosis, certain bacterial subpopulations can grow, which in turn can cause the environment to become inflammatory and cancer-promoting. On the other hand, many probiotics originating from the gut have the ability to protect the host and restore the circumstances of a good gut microbiome in patients who are dysbiotic, including those who are suffering from cancer. The relevance of the gut microbiota in CRC has been the subject of exponential knowledge growth over the last few years. As meta-analyses have combined data from many populations to show the CRC microbiome landscape on a global basis, association studies have grown in frequency and sample size. Additionally, these investigations have advanced functional studies to investigate the part that certain microorganisms play in the development of cancer. Together, these findings have presented a once-in-a-lifetime chance to advance microbiome research toward therapeutic applications. The discoveries about the microbiota will probably usher in a new era of oncology in the coming years as additional advances are made in the fields of CRC genomics, metabolomics, and immunology.

Conflicts of Interest

The authors have declared that no competing interests exist.

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