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Molecular Insights: The Gut Microbiota's Impact on colorectal Cancer

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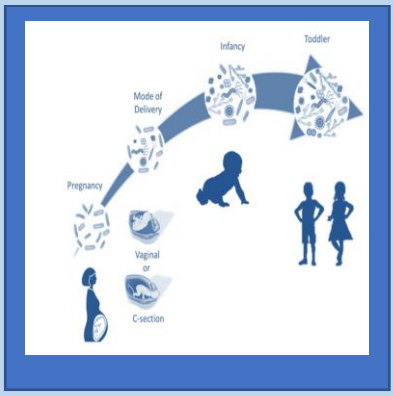
Abstract: Globally, colorectal cancer (CRC) ranks second in terms of cancer-related fatalities. Several lines of evidence point to the gut microbiota's role in increasing inflammation and tumour growth, despite the fact that CRC is believed to be the result of a genetic-environmental interaction. A person's gut microbiota is made up of around 40 trillion bacteria. Next-generation sequencing technologies and metagenomics have advanced the understanding of the ecology of gut microbes and have contributed to the connection between gut microbiota and colorectal cancer. Numerous investigations conducted on both human and animal models have highlighted the part that specific gut bacterialike Fusobacterium nucleatum, Bacteroides fragilis with enterotoxigenic properties, and Escherichia coli play critical role in the development and progression of colorectal cancer (CRC). New directions for the use of gut microbiota in CRC diagnosis, prevention, and therapy have been made possible by metagenomic research. The contribution of gut microbiota to colorectal cancer development and its possible therapeutic uses are outlined in this review paper.



Keywords: Molecular Insights, Gut Microbiota, Colorectal Cancer, Biomarkers.

Introduction

Colorectal cancer (CRC) is the second most prevalent cause of cancer related deaths globally and the third most common kind of cancer, accounting for almost 2 million new cases annually [1]. In men, it ranks as the third most prevalent type of cancer, whereas in women, it ranks second. 2020 had 1.9 million new cases and 0.9 million deaths globally due to colorectal cancer (CRC), an increase in incidence from previous years [1-2]. By the year 2030, it is estimated that there will be approximately 2.2 million incidents and 1.1 million fatalities due to colorectal cancer annually [3].

Only 10 to 15% of instances of colorectal cancer (CRC) are inherited, highlighting the significant influence of environmental factors in shaping the genetic makeup of CRC cases [4]. The gut microbiome, which has long been understudied and has only lately gotten enough attention, has also been linked in recent years to a growing role in the development of colorectal cancer (CRC) [5].

Human Microbiota

The gastrointestinal tract consists of a rich and varied assembly of microorganisms, including fungi, viruses, and bacteria, collectively known as the "gut microbiome." The human body hosts approximately 100 trillion symbiotic microbial cells from more than several hundreds of different species, which genomes can entail, globally, over three million genes, which make a huge gene pool [6]. An organism's microbiome is comprised of its genetic repertoire, which includes the bacterial gene pool (bacteriome), viral gene pool (virome), and fungal gene pool (mycobiome) [7]. The majority of our microbiota consists of bacterial cells (~99%). The gut alone has as many bacteria, It can surpass the overall count of cells in the human body. [8-9].

This intricate ecosystem should not be viewed merely as a passive colonizer of our gut; instead, it engages in mutualistic interactions with the host across various dimensions. The microbiota represents a metabolically dynamic ecosystem, comprised of diverse bacterial populations. These populations maintain a mutualistic relationship and reside within the epithelial barriers of various host organs, playing a crucial role in human health. [9].

Our microbiota coexists with the body in a state of symbiosis and has a huge influence on how our body functions, ranging from the development and regulation of the immune system to the growth of our nervous and cognitive systems [5]. Since gut microbiota is a significant environmental component for our body, it has a significant impact on human health and disease. In many ways, it functions like an additional organ in the body [10-11].

Balancing Act: The Microbiota's Role in Host Immunity

At various times throughout their lives, humans come into contact with various microbes. A newborn's mother is the first source of its microbiota. In this sense, the infant's birth method (vaginal versus C-section) also affects how its microbiota

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develops, as the method of delivery influences the newborn's first exposure and microbiota's composition (Fig.1) [12-14].

Through complex interactions, the microbiota and the newborn's immune system shape each other during the course of life. Leading to homeostasis, this delicate process, called microbiota eubiosis [15-16].

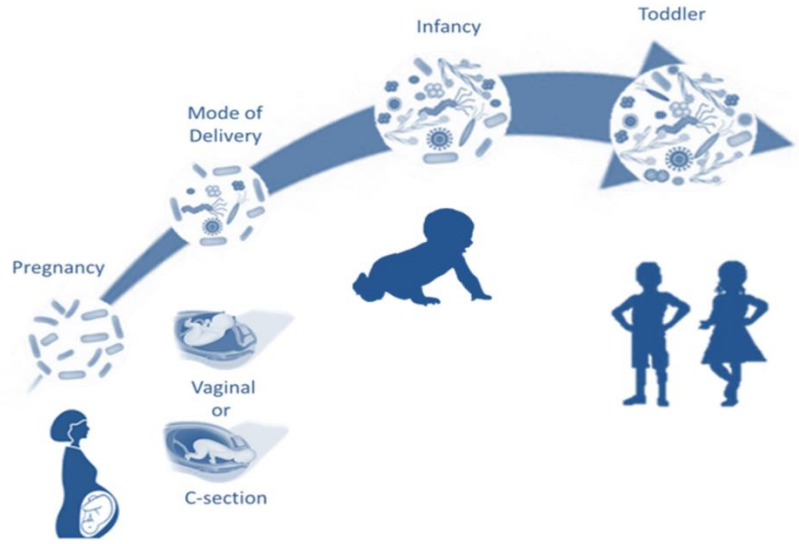

Figure (1): Microbiota Composition Across Different Life Stages. This figure illustrates the dynamic changes in microbiota composition across various life stages.

Eubiotic Microbiota Dynamics: Insights and Consequences

Microbiota eubiosis, characterized as the harmony between microbiota and the immune system, defines a state in which the emerging microbiota is termed eubiotic microbiota. This microbiota confers significant advantages for both physical and mental health as well as for an individual's development [17].

The host's genetics, environment, lifestyle, and food preferences all have a major influence on the dynamics of the microbiota during life [17-19]. Any one of these factors changing can have a significant impact on the gut microbiota [20]. Microbiota dysbiosis is the term used to describe a negative change in the gut microbiota that enhances the host's susceptibility to illnesses [21]. The delicate balance that existed between the immune system and eubiotic commensals is disrupted by this dysbiosed microbiota. These occurrences have the potential to cause long-term effects like inflammatory bowel disease (IBD) or accelerated tumorigenesis in cancer patients by directing cells and tissue toward inflammation [22-23]. Microbiota dysbiosis can arise from several significant contributors, including the consumption of a high-fat, high-protein, low-fiber diet commonly referred to as the "Westernized diet." Additionally, factors such as early life exposure to antibiotics or the overuse of broad-spectrum antibiotics can disrupt the balance of the microbiota. Unhealthy lifestyle habits like alcoholism and smoking also play a role in contributing to dysbiosis. Furthermore, genetic mutations in regulatory genes, such as sirtuins, nucleotide-binding oligomerization-domain (NLRP) genes, mucin 2, and lipocalin 2 (LCN2), can impact the equilibrium of the microbiota and contribute to dysbiosis. (Fig.2) [24-25].

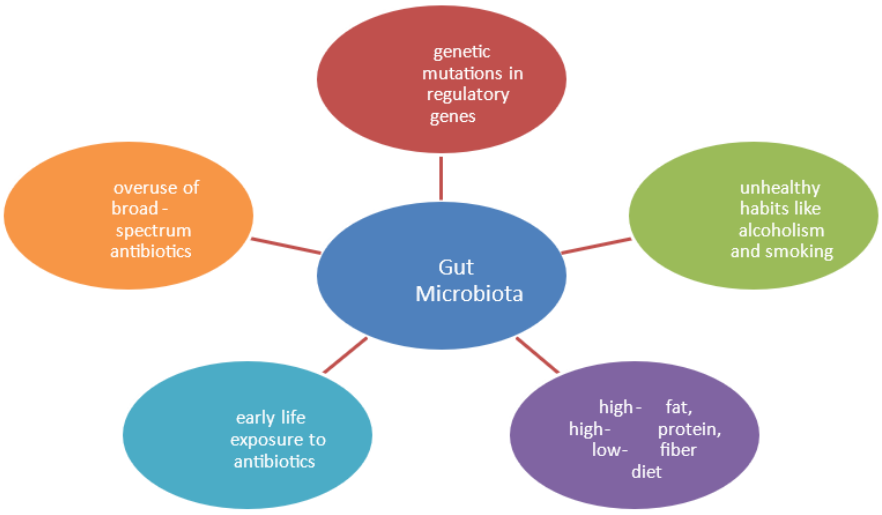

Figure (2): Factors that influence how the gut microbiota develops over time Most of these regulatory genes have a significant impact on controlling the barriers between the microbiota and host tissue in the human body.

The microbiota-host relationship is a dynamic and complex intricate process, and regulatory genes play a critical role in preserving the equilibrium, limiting detrimental interactions, and preserving host health [26]. The human body has a number of biochemical and physical barriers that keep the microbiota from host tissues. These consist of the mucous membranes, the skin, and the gut lining [18]. The integrity and operation of these barriers are controlled by these regulatory genes, thus preventing inflammation such as autoimmune diseases, The integrity and operation of these barriers are controlled by regulatory genes, thereby preventing inflammation observed in autoimmune diseases—where the body's immune system unintentionally targets its own tissues. Inflammatory bowel disease (IBD) serves as a distinct condition, not classified among autoimmune diseases. [27].

These regulatory genes are also important in determining the gut microbiota's makeup. While *NLRP3* gene is involved in the activation of proinflammatory responses. It has been reported that the *NLRP3* gain of function mutation positively contributes to the composition of the microbiota, resulting in a microbiota that induces an anti-inflammatory state and prevents colorectal cancer (CRC) [26]. The *LCN2* gene produces the lipocalin 2 protein, which is also referred to as lipocalin 2 or neutrophil gelatinase-associated lipocalin (*NGAL*) [25]. In addition to regulating inflammation, it is involved in the innate immune response and the movement of small hydrophobic molecules, among other physiological and pathological processes. The binding and transportation of small hydrophobic molecules, such as siderophores, which are involved in the transport of iron, is one of its crucial roles. When there is a deficiency of *LCN2*, the intestines produce more iron-bound siderophores, like enterobactin, which leads to an overabundance of *Alistipes* species, which need the siderophore-bound iron to grow. Right-sided intestinal tumours have been demonstrated to be caused by the well-known carcinogenic bacteria *Alistipes* spp [26].

Dysbiosed Microbiota and its Impact on Cancer

Regarding cancer, microbial dysbiosis refers to changes over time in the total microbial population, the proportional distribution of various microbial taxa, and the operational taxonomic units at a specific site. [28]. As a result of all these findings, numerous microbiota-related biomarkers for the early identification of cancers have been proposed [29]. Because the microbiota in a tumour microenvironment changes over time as the tumour progresses through different stages, it can be challenging to identify and treat a dysbiotic microbiota, which has the propensity to drive the process of tumorigenesis [30]. The question of whether the current microbiota's bacterial population is to blame for the development or progression of cancer or if it is merely connected to the site because it prefers the tumour niche for survival becomes challenging to answer. Anaerobic bacterial populations, for example, might be favored by a hypoxic tumour niche. Since the location of cancer and the culprit microbiota are typically different in nonintestinal cancer cases—dysbiosed gut microbiota can promote tumorigenesis in the liver or breast, drawing links between microbiota and cancer becomes even more difficult [31].

Recently, the involvement of microorganisms in the development of cancer has received a lot of attention. According to research, 20% of malignancies, including CRC, are influenced by microorganisms [32]. A study published in 2017 showed that intestinal carcinogenesis was facilitated by the gavage of faecal materials from CRC patients to conventional, germ-free mice. Research conducted on CRC-modeling germ-free mice and rats has shown a lower tumour burden than in animals raised conventionally. In addition to the tumour location, adjacent healthy tissues that have the same microbiota makeup as the tumour tissue also exhibit changes in the microbiome. There is a correlation between changes in gut microbiota and several malignancies, such as pancreatic, breast, and hepatocellular carcinomas [33].

Human studies have demonstrated that the gut microbiota of CRC patients is different from that of healthy individuals, exhibiting a greater concentration of procarcinogenic bacteria and a lower quantity of commensal bacteria [34]. Research has also revealed variations in the mucosal and faecal microbiota of CRC patients. Touchefeu et al. found that CRC patients had considerably lower levels of *F. prausnitzii, Eubacterium siraeum, Bacteroides eggerthii, Alistipes finegoldii,* and *Barnesiella intestinihominis* than controls. In faecal and mucosal samples, a number of research have attempted to delve deeply into the microbiome makeup linked to colorectal cancer. These investigations have found a change in the CRC microbiota's worldwide makeup. Research carried out on many populations has demonstrated the connection between specific bacterial species and colorectal cancer (Table 1) [35].

Table (1): Various pathogenic bacteria, their mechanisms of operation, and the types of cancer they induce. [5]

Microbe	Mode of Action	Cancer Type
Fusobacterium nucleatum	Alters the β-catenin/E-cadherin pathway and inhibits the immune response to tumours	Colorectal, Stomach, Oral, and Lung.
Enetrotoxigenic Bacteroides fragilis	The production of reactive oxygen species (ROS) results in DNA damage, disruption of the colon epithelial barrier, and the depletion of mucous membranes, ultimately leading to heightened inflammation.	Colorectal, Stomach and Lung
E. coli	Leads to the occurrence of breaks in both strands of the DNA double helix.	Stomach and lung.
Streptococcus bovis/gallolyticus	Elevates the expression of β-catenin, fostering inflammation and cellular proliferation.	colorectal cancer.
Enterococcus faecalis	Causes DNA damage through the generation of reactive oxygen species (ROS) and extracellular superoxide.	colorectal cancer.
Helicobacter pylori	Activates β-catenin/MAPK signaling pathway	colorectal and Stomach
Peptostreptococcus anaerobius	Interacts with TLR-2 and TLR-4 on colon cells to induce ROS formation	colorectal

Many studies have revealed that CRC has a decreased bacterial diversity and an increase in certain pro-tumorigenic bacteria. *Bacteroides fragilis, Fusobacterium nucleatum, E. coli, Streptococcus bovis/gallolyticus, Clostridium septicum, Enterococcus faecalis,* and *Peptostreptococcus anaerobius* are among the bacterial species that are frequently linked to colorectal carcinogenesis. Seven bacterial species were found to be enriched in CRC in a core sample of 536 faecal shotgun metagenomes that underwent metaanalysis. *Fusobacterium nucleatum, Bacteroides fragilis, Parvimonas micra, Prevotella intermedia, Porphyromonas asaccharolytica, Alistipes finegoldii,* and *Thermanaerovibrio acidaminovorans* were among them (3638). 29 core species were found to be enriched in CRC in eight distinct geographic locations according to a 2019 study [39]. In the gut microbiota of CRC patients, many studies have indicated that the activation of Th17 cell response and induction of DNA damage by certain bacteria, such as *E. Coli*, *Bacteroides fragilis,* and *Peptostreptococcus anaerobius*, may be linked to colorectal carcinogenesis [40].

In human CRC samples, many viruses, including the cytomegalovirus and human papillomavirus, have been found in addition to bacteria. Changes in the intestinal virome profiles were linked to the survival outcomes of individuals with colorectal cancer, as per a 2018 research [41]. On the other side, the mycobiome of CRC samples has been seen to alter in a few investigations. An increase in *Trichosporon* and *Malassezia* proportions was found in one study that examined the Ascomycota/Basidiomycota ratio; on the other hand, an increase in *Malasseziomycetes* and a decrease in *Saccharomycetes* and *Pneumocystidomycetes* were found in the study that examined the Basidiomycota/Ascomycota ratio [42]. In patients with colorectal cancer,

Wang et al. found a statistically significant rise in *Candida albicans* [43]. *Aspergillus rambellii, Cordyceps sp. RAO-2017, Erysiphe pulchra, Moniliophthora perniciosa, Sphaerulina musiva,* and *Phytophthora capsici* have been found to be more abundant in CRC patients' mycobiota, according to a recent study. Significant enrichment of *Lachancea waltii*, *Aspergillus rambellii,* and *Phanerochaete chrysosporium* was identified in CRC by another recent investigation [44]. According to Coker et al., individuals with colorectal cancer (CRC) have altered archaeomes, showing a decrease of methanogens and enrichment of halophiles [45].

The "bacterial driver-passenger" model explains the role of microorganisms in the development of colorectal cancer (CRC). This hypothesis suggests that "driver" bacteria, which are pathogenic gut microbes, produce genotoxins that damage DNA, leading to genomic instability and the initiation of CRC. Opportunistic bacteria, known as "passenger bacteria," then thrive in the CRC environment. These bacteria have a competitive advantage, allowing them to proliferate and display carcinogenic properties, outgrowing other microbial populations [46].

Fungi also contribute to CRC by inducing inflammation and disrupting gut homeostasis. Specific fungal species can create a pro-carcinogenic environment through the production of mycotoxins and other metabolites that cause DNA damage and promote tumor development [40].

Viruses are another significant factor in CRC. Oncogenic viruses can integrate into the host genome, leading to mutations and genomic instability. Human papillomavirus (HPV) and Epstein-Barr virus (EBV) are examples of viruses associated with CRC, transforming normal cells into cancerous ones by inhibiting tumor suppressor genes and activating oncogenes [40].

Despite these insights, it remains unclear whether dysbiosis is a cause or an effect of CRC, as the relationship between microbial communities and cancer development is complex and bidirectional. [46 - 47].

Microbiological Mechanisms Associated with Colorectal Carcinogenesis

Through both in vivo and in vitro research, common mechanisms employed by an aberrant microbiota in contributing to the pathogenicity of various cancer types in its host have been identified. A bacterial clade can utilize multiple mechanisms simultaneously to expedite the tumorigenesis process, especially when encountering a neoplastic environment conducive to its survival. Some bacterial clades are even implicated in cancer metastasis and its recurrence post-chemotherapy. The dysregulated microbiota primarily utilizes key mechanisms to induce colon cancer, including genotoxins and virulence factors, gut microbial metabolites, pathways of inflammation, oxidative stress, and modulation of anti-oxidative defense.

Genotoxins and virulence factors

As a result of acquiring different virulence factors during biological evolution, gut bacteria became pathogenic by being able to pass through intestinal epithelial cells and the gut mucosal barrier, which acts as a barrier between human tissues and microbiota [48]. Inflammation arises from a breach in this barrier [49]. The causes of pro-carcinogenic and diseasepromoting effects are virulence factors. Because of their Afa and Eae adhesins, CRC-associated E. coli strains are able to attach to intestinal epithelial cells, invade them, and start inflammatory processes [50]. By binding to E-cadherin through its FadA virulence factor, F. nucleatum promotes colon carcinogenesis by activating the β-catenin signalling pathway [51].

Toxins produced by numerous pathogenic bacteria are linked to the onset of colorectal cancer (CRC). The NF-κB and Wnt/βcatenin pathways are activated by ETBF's production of *B. fragilis* toxin (BFT), which increases cell proliferation, damages DNA, and releases pro-inflammatory mediators [52]. Moreover, BFT is capable of hydrolyzing E-cadherin's extracellular domain [53]. Numerous bacteria in the gut create genotoxins, which harm DNA. Cyclomodulins are genotoxins that cause damage to DNA and disrupt the cell cycle. Examples of these include colibactin, cytolethal distending toxins (CDT), cycle inhibiting factors, and cytotoxic necrotizing factors (CNFs). Since double-stranded DNA breaks are caused by CDTs and colibactin, they are regarded as true genotoxins that directly mediate DNA damage. The majority of Gram-negative bacteria linked to colorectal cancer (CRC), including Escherichia and Campylobacter, produce well-studied toxins called CDTs. Interactions with host cells are permitted by the CdtA and CdtC subunits, while the CdtB subunit has the ability to translocate to the nucleus and harm DNA in host cells [54]. Moreover, IL-6, TNF-α, NFκB, and cyclooxygenase 2 are all stimulated by CDTs. Colibactin causes cell cycle arrest, ROS production, and DNA damage. In a mouse model, tumours have been demonstrated to decrease when colibactin production is targeted. Despite making up a small percentage of the gut microbiota, an

examination of CRC tissue samples indicates that the toxins produced by these bacteria are highly expressed [55]. As a result, treating CRC with these toxins may have therapeutic benefits.

Gut Microbial Metabolites and Products

Colon cancer has been linked to specific metabolites produced by microbes in the gut. The microbial byproducts influencing the development of colon cancer include secondary bile acids, acetaldehyde, trimethylamine-N-oxide (TMAO), and glucuronidase. [56].

Secondary Bile Acids: Within bile are steroid acids, one kind of which is called bile acid. The process of emulsification, absorption, and elimination of fats and cholesterol is attributed to them. Primary bile acids produced in the liver are called chenodeoxycholic acid (CDCA) and cannabinoid acid (CA). Anaerobic bacteria in the colon utilise primary bile acids as a source of energy to produce secondary bile acids like deoxycholic acid (DCA) and lithocholic acid (LCA). CRC has been linked to high-fat diets. This is likely due to the fact that high-fat diets increase the secretion of primary bile acids, which are then transformed into secondary bile acids by gut microbes. CRC is induced and inflammation is increased when mice fed secondary bile acids [57].

Gene expression and intracellular signalling can both be regulated by DCA.

Additionally, it has the ability to downregulate the expression of miR-199a5p and induce the expression of the orphan nuclear receptor Nur77 [58]. By upregulating angiogenic VEGF (vascular endothelial growth factor) and anti-apoptotic BRE (brain and reproductive organ-expressed protein), Nur77 encourages the growth of tumours. As a result of its ability to target and degrade CAC1 (CDK2-associated cullin domain 1), a novel cell cycle regulator that is extensively expressed in colorectal cancer, miR-199a-5p has the ability to suppress tumour growth [59]. Tumour formation and oxidative DNA damage can be directly induced by DCA. Intestinal carcinogenesis is caused by DCA's induction of the epithelial-mesenchymal transition and activation of vascular endothelial growth factor receptor 2. The expression of urokinase-type plasminogen activator receptor (uPAR) is known to be induced by LCA. This may activate the MAPK signalling pathway and aid in the progression and metastasis of cancer [60].

Epithelial cell proliferation can be induced by secondary bile acids through their activation of G-protein-coupled bile acid receptor 1 (GPBAR1) [61]. Secondary bile acids can cause damage to DNA (by generating ROS and reactive nitrogen species (RNS), control gene expression and membrane permeability, and initiate the protein kinase C pathway and the epidermal growth factor receptor (EGFR) pathway signaling [62]. Furthermore, bile acids exhibit potent antimicrobial activities and modify the gut microbiome through microbe selection and elimination. The population of Gamma-proteobacteria and Bacteroidetes linked to CRC increases as a result [63].

Ruminococcus gnavus produces ursodeoxycholic acid (UDCA), a bile acid that inhibits the growth of colon cancer. It acts on colon cancer cells by modulating EGFR/Raf-1/ERK signalling, which prevents COX-2 expression and DCA-induced apoptosis [64].

Acetaldehyde : Bacteria in the gut that are facultative anaerobic and aerobic convert ethanol to acetaldehyde. Overindulgence in ethanol is thought to increase the risk of developing several cancers, including colorectal cancer. Acetaldehyde is a highly carcinogenic and toxic substance. It may lead to colorectal carcinogenesis by causing damage to DNA and hindering the repair of DNA excision [65].

Trimethylamine-N-oxide (TMAO): The interaction between flavin monooxygenase and trimethylamine (TMA), a microbial metabolite originating from red meat and fats, leads to the formation of trimethylamine-N-oxide (TMAO). Because gut microbes convert L-carnitine (a TMA) into TMAO, a diet high in fats and red meat produces more TMAO. TMAO has been associated with a higher risk of developing CRC and cardiovascular illnesses. It's likely that TMAO induces oxidative stress, inflammation, protein misfolding, and DNA damage in order to cause CRC [66].

Glucuronidase: Patients with CRC have elevated faecal glucuronidase activity. By conjugating glucuronic acid with certain carcinogens, the liver renders them inactive, and these substances are then eliminated via the digestive system. It is possible for bacterial glucuronidase to reactivate carcinogens in the colon by reversing this process. Bacterial glucuronidase has been shown to be responsible for the progression of colorectal cancer (CRC) by reducing the number of tumours in a mouse model when inhibiting the enzyme. Additionally, the activity of some anti-tumor medications is impacted by bacterial glucuronidase, which may have an impact on the course of treatment [67].

Inflammation and Host Immunity

The host immune system's adaptive response is inflammation. Healthy microbiota do not cause significant inflammation because the host immune system is designed to identify normal gut microorganisms. Gut microbes and host immune cells interact constantly, favouring and tolerating beneficial bacteria while eradicating harmful ones [68]. Changes in the gut microbiota contribute significantly to inflammation, which encourages the development of CRC. Dendritic cells (DCs), neutrophils, macrophages, and ROSproducing red blood cells (RNNS) damage DNA, and cyclooxygenase 2 is produced [69]. These inflammatory events are linked to the development of colorectal cancer (CRC). Furthermore, pathogen invasion of the intestinal mucosa results in immune cell activation as well as the release of growth factors and cytokines, which propel the inflammatory process. Cancer is caused by persistent inflammation, which also induces angiogenesis, the proliferation of epithelial cells, and the inhibition of apoptosis. Th17 cell differentiation is triggered by pro-inflammatory cytokines like TNF and IL-6 that are secreted by T cells and macrophages. Poor survival in colorectal cancer is linked to the protracted presence of Th17 cells and increased levels of related cytokines, including IL-17 and IL-22. Reducing Th17 cells has been shown in studies to lower the risk of carcinogenesis. Angiogenesis and STAT3 activation depend on the significant cytokine IL-6. Serum levels of TNF and IL-6 have been used as prognostic indicators for low CRC patient survival. Tumour suppressor genes can be inactivated or oncogenes can be activated by inflammation-associated factors [70].

Inflammatory bowel diseases (IBD), like ulcerative colitis and Crohn's disease, are linked to inflammation. Since 20% of patients with ulcerative colitis go on to develop CRC, CRC is

known as colitis-associated cancer, and it is linked to an increased risk of developing IBDs [71]. Patients with limited colitis are less likely to develop colorectal cancer (CRC) than those with pancolitis. According to meta-analyses, patients with Crohn's disease have an 8.3% chance of developing colorectal cancer and those with ulcerative colitis have an 18.4% risk [72]. Colitis promotes the growth of genotoxic bacteria, which in turn promotes the development of cancer. Patients with inflammatory bowel disease (IBD) have higher proteobacteria in their gut microbiota, especially Enterobacteriaceae like Escherichia coli. In a study using IL10-deficient mice given the genotoxic drug azoxymethane, the function of colibactin-producing E. coli in causing inflammation and intestinal tumours was clarified. Compared to mice receiving similar treatment but lacking pks+ E. coli, fewer intestinal tumours occurred in this type of mouse model. By triggering NF-κB and STAT3 signalling in colonic epithelial cells, ETBF stimulates inflammation. The genes that encode TNF and COX-2, which are typically overexpressed in

IBDs and CRCs, are regulated by the NF-κB pathway. Additionally, B. fragilis causes colonocytes to express spermine oxidase, which results in the generation of ROS and DNA damage [73].

IBDs can also be caused by other bacteria, such as *Mycobacterium* and *Citrobacter rodentium*, which also encourage inflammation. Because IBDs alter the homeostasis of the gut microbiota, patients are more susceptible to colorectal cancer. In addition, compared to healthy subjects, IBD patients have lower levels of *Firmicutes* and *Bacteriodetes* in their dysbiosis. Given that long-term NSAID use lowers the risk of CRC, there may be a connection between inflammation and the development of CRC [74].

Toll-like receptors (TLRs) and nucleotidebinding oligomerization (NOD)like receptors (NLRs) are examples of pattern-recognition receptors (PRRs) that are involved in identifying the unique molecular patterns known as microorganismassociated molecular patterns (MAMPs) that are present in pathogenic microorganisms [75]. PRRs are able to identify molecules on the surface of microorganisms, including flagellin, lipoproteins, lipoteichoic acid, lipopolysaccharides, and peptidoglycan. Lipoteichoic acid induces the secretion of proinflammatory factors by binding specifically to TLR-2 or CD14 [76]. One important class of PPR expressed by dendritic cells and macrophages is TLRs. In the event that the mucosal barrier is compromised, they identify microbes and trigger an immune response. TLR signalling improves barrier function, preventing microbial invasion, and starts immune defence by generating pro-inflammatory cytokines. The two primary Tolllike receptor (TLR) pathways are Myeloid Differentiation Factor 88 (MyD88) adaptor-protein-dependent and TRIF-dependent. In the MyD88dependent pathway, NF-κB and MAPK serve as downstream activators. Studies have suggested the participation of MyD88 in the onset of colorectal cancer (CRC). The deactivation of MyD88 led to a reduction in tumor count in APCmin/+ mice treated with AOM. [77].

In order to maintain the composition of the gut microbiota and reduce inflammation, TLR-2 expression is crucial. TLR2 may be crucial for preserving gut homeostasis because it has been observed that tumour numbers are higher in TLR2-deficient mice than in control mice. A rise in

TLR4 triggers NF-κB, resulting in the expression of COX-2 and a higher risk of colorectal cancer. In addition to decreasing survival, elevated levels of TLR4 and MyD88 in CRC patients raise the risk of liver metastasis. TLR4 expression inhibition guards against colorectal cancer. Additionally, a study discovered that persistent TLR9 activation may lead to hyperproliferation and the development of CRC [78].

The cytoplasm of both immune and non-immune cells contains NLRs. Proinflammatory cytokine production and autophagy are triggered by NLR activation [79]. According to a study, patients with colorectal cancer (CRC) had significantly different NLR signalling in their tumour and non-tumor tissues. Mice treated with AOM/DSS and lacking either NOD1 or NOD2 exhibit a marked increase in the number of CRCs. Moreover, NOD2 mutations are linked to a higher risk of colorectal cancer and Crohn's disease [80].

Oxidative Stress

An imbalance between oxidative molecules, like ROS and RNS, and antioxidative defences results in oxidative stress. Biomolecules are impacted by oxidative stress, which also weakens cell membranes and causes mutations and breaks in DNA. Oxidative stress activates NF-κB and increases the expression of anti-apoptotic signalling and pro-inflammatory cytokines. There is a direct correlation between the induction of CRC and ROS production [81]. The host immune system, including neutrophils and macrophages, as well as the gut microbiota, produce reactive oxygen species (ROS) in reaction to inflammation brought on by pathogenic bacteria or other environmental stimuli. Certain bacterial species, including *Bifidobacterium* and *Lactobacillus*, produce RNS. Certain species, like *E. faecalis*, can produce hydroxyl radicals, which cause chromosomal breaks and point mutations that aid in the development of colorectal cancer. Additionally, *H. pylori* causes oxidative stress, which leads to gastric carcinogenesis [82].

Oxidative stress is balanced by a number of anti-oxidative defence mechanisms, including DNA repair. In the colon cells, the base-excision repair system is the only one that performs more than 10,000 repairs every day. It is discovered that CRC has changed antioxidative defence mechanisms. Research has revealed that certain enteropathogenic *E. coli* strains and a CRC mouse model induced by colitis downregulate the MMR system [83]. Belcheva et al. found that gut microbes could cause colorectal cancer (CRC) in MMR-deficient epithelial cells using APCmin/+ MMRdeficient mice [84].

The Role of Gut Microbiota in CRC Therapy

Research has demonstrated that the gut microbiota can affect the effectiveness, toxicity, and responsiveness to immunotherapy, radiation, and chemotherapy, hence influencing the therapeutic benefits of cancer treatments. It has been demonstrated that dietary interventions using probiotics and prebiotics can affect how well most cancer treatments work [85].

Probiotics

When taken in sufficient quantities, living bacteria known as probiotics offer a variety of health advantages [86]. By substituting helpful microorganisms for pathogenic ones, probiotics alter the makeup of the gut microflora [87]. Numerous health advantages of probiotics include immune system control, decreased blood cholesterol and colitis, suppression of harmful bacteria, and prevention of colorectal cancer. Probiotics have been shown in studies to help prevent intestinal infections by preventing the colonisation of harmful bacteria through

competition for resources, adhesion to epithelial cells, or mucus. Furthermore, several metabolites that probiotics create can prevent the development of pathogens. Probiotics may avert the development of colorectal cancer by reducing the risk of inflammation and intestinal infections [88].

One of the novel strategies for treating IBD and CRC is the restoration of normal gut microflora with probiotics, as CRC is associated with dysbiosis of the gut microbiota. Through the production of short-chain fatty acids, antioxidants, and anti-cancer compounds, the downregulation of inflammatory pathways, the reduction of cyclooxygenase-2 expression and cell proliferation, the induction of cancer cell apoptosis, and the stimulation of tumour suppressor gene expression, probiotics inhibit colorectal cancer (CRC) in a number of ways [88-89]. Probiotics have been shown to be beneficial in the treatment of IBDs, CRCs, and other malignancies in several trials. *Lactobacillus* and *Bifidobacterium* are the two main probiotics [90]. The gut microbial composition is impacted by the administration of

Bifidobacterium lactis Bl-04 and *Lactobacillus acidophilus* NCFM.

Probiotics have been demonstrated to reduce CRC-associated bacteria like

Fusobacterium and enhance butyrate-producing bacteria like *Faecalibacterium*. Inflammation and apoptosis suppression were described by Kuugbee et al. When *Lactobacillus rhamnosus* and *Bifidobacterium lactis* Bb12 are combined with inulin, cell proliferation is decreased and barrier performance is enhanced [91]. According to Benito et al., quercetin and Bifidobacterium bifidum together prevented the development of colorectal cancer in Apcmin/+ mice. *Clostridium butyricum* has the ability to reduce the occurrence of tumours in mice by increasing Th2 and Th17 cell counts and lowering the release of inflammatory factors including NF-κB and IL-22. It is also known that *C. butyricum* alters the microbial composition, reduces the incidence and extent of CRC, and induces the formation of CRC in Apcmin/+ mice fed a high-fat diet. Other than these genera, *Streptococcus thermophilus* has been shown to decrease tumour growth in mice by producing galactose that is dependent on β-galactosidase and increases phosphorylation while decreasing the activity of the Hippo pathway kinase [92].

Bifidobacterium

The human intestine contains the Gram-positive, non-motile, anaerobic bacteria which is *Bifidobacterium*. An indication of the gut microbiota has been the ratio of Bifidobacterium to *E. coli* [93]. In CRC, there has been a noted reduction in Bifidobacterium and a rise in E. Coli. It has been discovered that Bifidobacterium alone, administered orally, can affect the immune response against colorectal cancer. Bifidobacterium may potentially increase the effectiveness of chemotherapy by lowering the activity of glucuronidase. B. breve used orally considerably reduces ulcerative colitis. In mice carrying MC38 colon cancer, B. breve inhibits the development of tumours and increases the effectiveness of cancer treatments. The B. infantis and B. breve strains can activate Foxp3+ regulatory T cells, IL-10-producing Tr1 cells, intestinal dendritic cells, and toll-like receptors (TLRs) [94].

Lactobacillus

Most probiotics contain the facultative anaerobic, Gram-positive bacteria Lactobacillus. By causing apoptosis, regulating cytokine-producing dendritic cells, and lowering the expression of β-catenin and NF-κB, lactobacillus can lower the risk of colorectal cancer [95]. Additionally, it improves the function of the intestinal epithelial barrier and controls the expression of toll-like receptors. The inhibition of STAT3 and NF-κB signalling by *L. rhamnosus* GG and L. acidophilus results in the downregulation of Th17 cell expression. In a CRC-affected animal model, *L. rhamnosus* GG reduces tumour burden by boosting colonic CD8 T-cell responses [96].

Oral administration of *L. casei* strain has been shown in studies to considerably treat ulcerative colitis and reduce the incidence of colorectal cancer in high-risk individuals. *Ferrichrome*, a chemical produced *by L. casei*, can cause tumour cells to undergo apoptosis by activating the JNK pathway [97]. In rat models induced by 1, 2-dimethylhydrazine, the administration of *L. salivarius Ren* and *L. paracasei* may inhibit the development of colorectal cancer. 5-fluorouracil (5-FU) and *L. paracasei* subsp. *paracasei* NTU 101 together were effective in lowering CRC cell viability [98]. It has been found that L. acidophilus NCFM inhibits the formation of tumours in mice by downregulating MHC class I in tumour cells and lowering the expression of CXCR4 [99]. It has been demonstrated that *L. rhamnosus* and *L. plantarum* increase the synthesis of mucin. It has been discovered that *H. pylori's* adhesion to GES-1 cells is inhibited by *L. acidophilus* and *L. bulgaricus*. It was discovered that *L. bulgaricus*, in particular, inhibits the TLR4/IκBα/NF-κB pathway, hence preventing GES-1 cells from producing IL-8. By reducing the levels of IL-6, TNF-α, IL-17, IL23, and IL-1β, *L. bulgaricus* reduces intestinal inflammation and may thus have a chemopreventive impact on colon cancer linked to colitis. Enteropathogenic *E. coli* was reported to be inhibited by *L. reuteri* strains ATCC PTA 6475 and ATCC 53608. *L. reuteri* raises reactive oxygen species within tumours while inhibiting the development of colon tumours. In both mouse and human CRC, *L. reuteri* and its metabolite, reuterin, are downregulated. In a rat model of colorectal cancer, *L. plantarum* and *L. salivarius* were shown to increase IL-18 production. According to a recent study, giving *L. gallinarum* to Apcmin/+ and AOM/DSS-treated mice might prevent the development of colorectal tumours. According to a different recent study, *L. coryniformis* MXJ32 reduced intestinal inflammation and increased the expression of tight junction proteins by downregulating the production of inflammatory cytokines. This resulted in a decrease in the average tumour width and the number of tumours [100-101].

Prebiotics

Food ingredients known as prebiotics help to maintain a healthy gut flora, which has positive health effects. Numerous food ingredients function as prebiotics. According to clinical investigations, probiotic microorganisms including *Ruminococcus*, *Faecalibacterium*, *Rosebura*, and *Akkermansia* are more prevalent when prebiotics are administered [101]. By interacting with bacterial receptors and blocking pathogens from adhering to epithelial cells, prebiotic oligosaccharides can hinder the colonisation of pathogens. According to a study, polydextrose has positive impacts on preserving a balanced gut microbiome. The concentration of butyrate in faeces is raised by fructans and galactooligosaccharides, which also boost the number of good bacteria like *Lactobacillus* and *Bifidobacterium*. Polysaccharide inulin, which is present in artichokes, bananas, asparagus, and wheat, inhibits the action of glucuronidase and lowers the pH and concentration of phenol, pcresol, and indole in the colon, so reducing the formation of precancerous lesions. It has also been

demonstrated that consuming inulin increases Bifidobacterium abundance. Agro-oligosaccharides modify the synthesis of SCFAs and secondary bile acids, which regulate the progression of CRC. *Lachnum sp*. polysaccharides change the gut microbiota and lower tumour incidence and inflammation [102].

Oats contain avenanthramide-C, which is converted by gut bacteria into bioactive substances with anti-tumor properties. By regulating inflammation and gut bacteria, nutmeg can prevent colon cancer. One kind of omega-3 fatty acid that reduces inflammation and CRC linked to colitis is eicosapentaenoic acid (EPA) [103]. Studies on the impact of polydextrose, resistant dextrin, fructo-oligosaccharides, and xylo-oligosaccharides on the gut microbiota of perioperative CRC patients revealed a decrease in Bacteroides and a rise in Bifidobacterium and Enterococcus abundance. According to recent research, berberine, which is present in berberis, influences CRC cell invasion, migration, and proliferation as well as causing apoptosis. It also lowers inflammatory modulators and NF-κB expression while raising the amounts of butyrate, acetate, and propionate in the faeces. In a CRC linked with colitis generated by AOM/DSS, ginsenoside compound K, derived from ginseng saponins, enhances the abundance of A. muciniphila and reduces tumour development. Model of Balb/C mouse [104-105].

Faecal Microbiota Transplantation (FMT)

A developing biotherapeutic technique called faecal microbiota transplantation (FMT) seeks to improve a variety of gastrointestinal illnesses, including IBDs, by restoring the natural gut microbial ecology. Transplanting a microbial population from a donor to a recipient is the process of FMT. Some research has been conducted in this area, and the potential benefits of employing FMT to improve CRC treatment remain mostly undiscovered. Faecal microbiota transplants from wild mice to laboratory mice have been shown to provide resistance against DSS/AOM-induced colorectal carcinogenesis [106]. However, FMT's poor effectiveness, safety concerns, and logistical challenges have prevented its broader application. After receiving FMT, a few individuals had side symptoms as constipation, diarrhoea, and distension in the abdomen. Furthermore, a major concern associated with FMT is the spread of germs resistant to drugs, which might result in potentially fatal illnesses such bacteremia caused by *E. coli*. Tight donor screening procedures can stop these illnesses from happening. The transfer of chronic illnesses linked to the microbiome, including autoimmune, cardiometabolic, and gastrointestinal problems, is another concern that comes with FMT. According to a study, transplanting human faeces from fat people into germ-free mice led to obesity. Similar outcomes were observed in a human study where a lady who had an FMT from an overweight donor went on to become obese [107].

Conclusion

The gut microbiota may have a significant role in the onset and course of colorectal cancer, according to an increasing amount of data. To establish a connection between the gut microbiota and colorectal cancer, it is imperative to investigate alterations in the gut microbiome as the disease progresses. A patient's reaction to chemotherapy and immunotherapy, as well as their chance of getting colorectal cancer (CRC), may be predicted by the presence of certain bacterial species. Intestinal microflora profiles can be used in combination with other variables, such as age, body mass index, family history, food, and geography, to assess the risk of colorectal cancer (CRC). Antibiotic overuse should cease indiscriminately in order to avoid disrupting the intestinal microecology, which has been connected to colorectal cancer and inflammatory bowel disease. Nutritional therapies have the potential to modify the gut microbiota and aid in both CRC prevention and treatment. Diagnostic diagnostics based on gut microbiota can reliably and accurately identify the risk factors for colorectal cancer (CRC). This can aid in the creation of probiotic therapies tailored to each patient for the treatment of colorectal cancer.

More of these causes from our gut and oral flora must now be found by future study. Furthermore, to develop targeted medication delivery strategies, it is essential to focus on mitigating the impact of microbiota on various aspects of cancer, including its development, acceleration, metastasis, and resistance to chemotherapy. Potential targets for such an approach may encompass β-catenin molecules within tumor cells, IL-6 and associated Toll-like receptors (TLRs) in regions of pronounced inflammation and microbiota dysbiosis, overexpressed molecules on tumor cells like E-cadherin and Gal–GalNac, which act as binding sites for bacteria or their effector molecules, and microRNAs implicated in tumor cell autophagy and heightened proliferation. Additionally, there is significant ongoing research to establish microbiota-based biomarkers for the early detection of cancer.

Author's contribution

Jarrar S. and Adwan G. contributed equally to the conceptualization, literature search, data collection, and synthesis of findings from the selected studies of this study. Both authors contributed to the writing and revision of the manuscript.

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Conflicts of interest

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