MASTERARBEIT | MASTER'S THESIS

Titel | Title

Gut Microbiome Signature as a Prognostic Marker for Colorectal Cancer

verfasst von | submitted by

Lena Kurz BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Magistra pharmaciae (Mag. pharm.)

Wien | Vienna, 2026

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 605
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Pharmazie
Betreut von | Supervisor:	Dr. Mohammed Aufy Privatdoz.
Mitbetreut von | Co-Supervisor:	Ahmed Mohamed Mahmoud Hussein B.Sc. M.SC. PhD PhD

Abstract (Deutsch)

Das Darmmikrobiom ist in den vergangenen Jahren zunehmend in den Fokus der Forschung gerückt. Zahlreiche Studien haben Veränderungen des Darmmikrobioms beim kolorektalen Karzinom untersucht. Ob Mikrobiomsignaturen jedoch einen klinisch relevanten Informationsgewinn bieten, ist derzeit noch unklar. Ziel dieser Literaturarbeit war es, den aktuellen Wissensstand zu Veränderungen des Darmmikrobioms beim kolorektalen Karzinom zusammenzufassen und deren Potenzial als prognostischer Biomarker zu bewerten.

Diese Literaturarbeit basiert überwiegend auf Beobachtungsstudien am Menschen sowie auf einigen Tierstudien. Die bisherige Evidenz zeigt konsistente Veränderungen des Darmmikrobioms bei Patientinnen und Patienten mit kolorektalem Karzinom. Mehrere Studien fanden Zusammenhänge zwischen spezifischen Mikrobiomsignaturen und dem Krebsrisiko, dem Tumorstadium, dem Überleben sowie dem Ansprechen auf Therapien.

Insgesamt deutet die derzeitige Evidenz darauf hin, dass das Darmmikrobiom künftig als prognostischer Biomarker beim kolorektalen Karzinom von Bedeutung sein könnte. Für eine Beurteilung des klinischen Nutzens sind jedoch weitere gut konzipierte Studien erforderlich.

Abstract (English)

The importance of the gut microbiome has become increasingly recognized over the past years. Many studies have investigated changes in the gut microbiome in colorectal cancer. However, it is still unclear whether microbiome signatures provide clinically relevant additional information. The aim of this literature review was to summarize the current evidence on changes of the gut microbiome in colorectal cancer and to evaluate their potential as a prognostic biomarker.

This review is based mainly on observational studies in humans together with a small number of animal studies. The available evidence shows consistent changes in the gut microbiome of patients with colorectal cancer. Several studies found associations between specific microbial signatures and cancer risk, tumor stage, survival, and response to treatment.

Overall, the current literature suggests that the gut microbiome could become a useful source of prognostic information in colorectal cancer. However, further well designed studies are needed to determine whether microbiome signatures provide enough additional information to be useful in clinical practice.

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1. The Human Gut Microbiome: Composition and Function

1.1 Definition and Concept of the Gut Microbiome

The gut microbiome is defined as the collective genetic material of the microorganisms that inhabit the human gastrointestinal tract. It is a complex ecological system composed of bacteria, archaea, viruses, and fungi that coexist with the host and contribute to human physiology, metabolism, and immune function. [1]

1.2 Composition and Diversity of the Human Gut Microbiome

The human gut microbiome is a highly dense and diverse microbial ecosystem found predominantly in the distal gastrointestinal tract. This ecosystem contains trillions of microbial cells whose collective gene content is much larger than that of the human host. Culture-independent sequencing approaches have shown that the gut microbiome contains bacteria, archaea, viruses, and eukaryotic microorganisms, with bacteria being the dominant fraction. At the phylum level, the gut microbiota of healthy adults is largely dominated by Firmicutes and Bacteroidetes, with additional contributions from Actinobacteria, Proteobacteria, and Verrucomicrobia. Despite this broad taxonomic diversity, the majority of microbial biomass is accounted for by a limited number of dominant phyla, reflecting a relatively stable but variable microbial community. [2,3]

Although hundreds of bacterial genera can be detected across populations, only a small subset is consistently shared among most individuals. Large population-based studies integrating multiple European cohorts have shown the existence of a core gut microbiota at the genus level. This core includes a limited number of genera that are present in the vast majority of individuals, while a much larger pool of low-abundance and variably present genera contributes to interindividual diversity. [2,3]

Although taxonomic composition varies between individuals, a conserved functional core exists at the gene level across the human gut microbiome. Metagenomic studies show that distinct microbial communities can encode similar metabolic pathways, reflecting

functional redundancy. Consequently, gut microbiome composition is more accurately characterized by shared functional capacity than by taxonomic structure alone. [2]

1.3 Development of the Gut Microbiome

The human gut microbiome begins to develop immediately after birth and follows a predictable developmental pattern during early life. Longitudinal metagenomic analyses show that the infant gut microbiome changes markedly during the first year of life, characterized by increasing microbial diversity, decreasing interindividual variability, and gradual development toward an adult-like microbiome. [4,5]

Mode of delivery is a factor influencing early microbial acquisition. Infants born vaginally get a microbial signature resembling the maternal gut microbiota, whereas infants delivered through c-section have reduced transfer of maternal microbiota and increased colonization by skin- and environment-associated taxa. These differences are greatest immediately after birth and decrease over time, yet specific taxa characteristic of vaginal delivery remain underrepresented in infants delivered through c-section throughout the first year of life. [5,6]

Feeding patterns further influence the development of the gut microbiome. Breastfeeding maintains a microbiome dominated by *Bifidobacterium* and *Lactobacillus*, whereas formula feeding and the discontinuation of breastfeeding are associated with accelerated microbial maturation and enrichment of taxa and metabolic pathways characteristic of the adult gut. Microbiome maturation correlates more strongly with stopping breastfeeding than with introducing solid foods, showing the importance of breastfeeding in shaping the microbiome. [5]

1.4 Spatial Organization Along the Gastrointestinal Tract

The gastrointestinal tract is not a uniform environment for microorganisms. Different regions provide physical and chemical conditions that determine which microbes can colonize and persist. As a result, different microbial communities are found along the gastrointestinal tract. [7]

Microbial density and diversity increase progressively from the small intestine to the colon. The small intestine provides a harsh environment for microbial growth due to rapid transit, exposure to bile acids, and host-derived antimicrobial factors. In contrast, the colon provides conditions that allow large and diverse microbial communities to develop.[7]

In addition to differences along the length of the gut, distinct microbial communities are found in the intestinal lumen, within the mucus layers, and in close association with the epithelium, including specialized niches such as colonic crypts.[7]

This spatial organization is not passive but is actively maintained by the host. The host secretes mucus, produces antimicrobial peptides, and contains microbes through immune mechanisms, restricting bacterial access to epithelial surfaces and helping preserve stable microbial localization within defined regions of the gut. [7]

Microbial location affects access to nutrients, exposure to host-derived compounds, and interactions with host tissues. As a result, it influences microbial metabolism and host–microbe interactions.[7]

An important implication of this spatial heterogeneity is the limitation of stool-based microbiome analyses. Fecal samples primarily represent luminal communities from the distal gut and do not capture regional or mucosa-associated microbial differences. [7]

1.5 Microbial Metabolism in the Gut

Microbial metabolites are a central link between the gut microbiota and the host and are a highly diverse group of small molecules. It is estimated that up to 50,000 microbial metabolites occur in the human gut, reflecting the extensive metabolic capacity of the microbiome. These metabolites fall into three main groups. The first group includes dietary-derived metabolites, representing a large and heterogeneous category, including, but not limited to, vitamins (e.g., vitamin K and certain B vitamins), polyphenol-derived metabolites, as well as compounds derived from amino acids such as tryptophan or choline. The second group includes metabolites synthesized de novo by microorganisms, most prominently short-chain fatty acids such as butyrate, propionate, and acetate. The

third group includes host-derived metabolites that are secreted into the intestinal lumen and modified by the microbiota, such as bile acids. [8]

1.5.1 Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are the main end products of anaerobic microbial fermentation of non-digestible carbohydrates in the human colon. Chemically, they are fatty acids with fewer than six carbon atoms and are produced almost exclusively by gut bacteria. Due to their weak acidic properties and the near-neutral intestinal pH, approximately 90–99% of SCFAs are present in the gut in their ionized forms and not as free acids. In contrast to the protonated acid forms, ionized SCFAs are less volatile and therefore largely odorless. [9] [10]

SCFAs are formed from dietary carbohydrates that escape digestion in the small intestine. These substrates include non-starch polysaccharides, resistant starch, and oligosaccharides, which reach the colon intact and are fermented by gut bacteria.[6]

The quantitatively dominant SCFAs in humans are acetate, propionate, and butyrate, which together account for the vast majority of total SCFA production in the colon.[6]

SCFAs are produced predominantly in the colon, where microbial density is highest and transit time allows extensive fermentation. In the stomach and small intestine, SCFA formation is limited due to low bacterial abundance and rapid movement of intestinal contents.[11]

SCFAs are not produced by single bacterial species but arise from metabolic cooperation between microbial groups. [11]

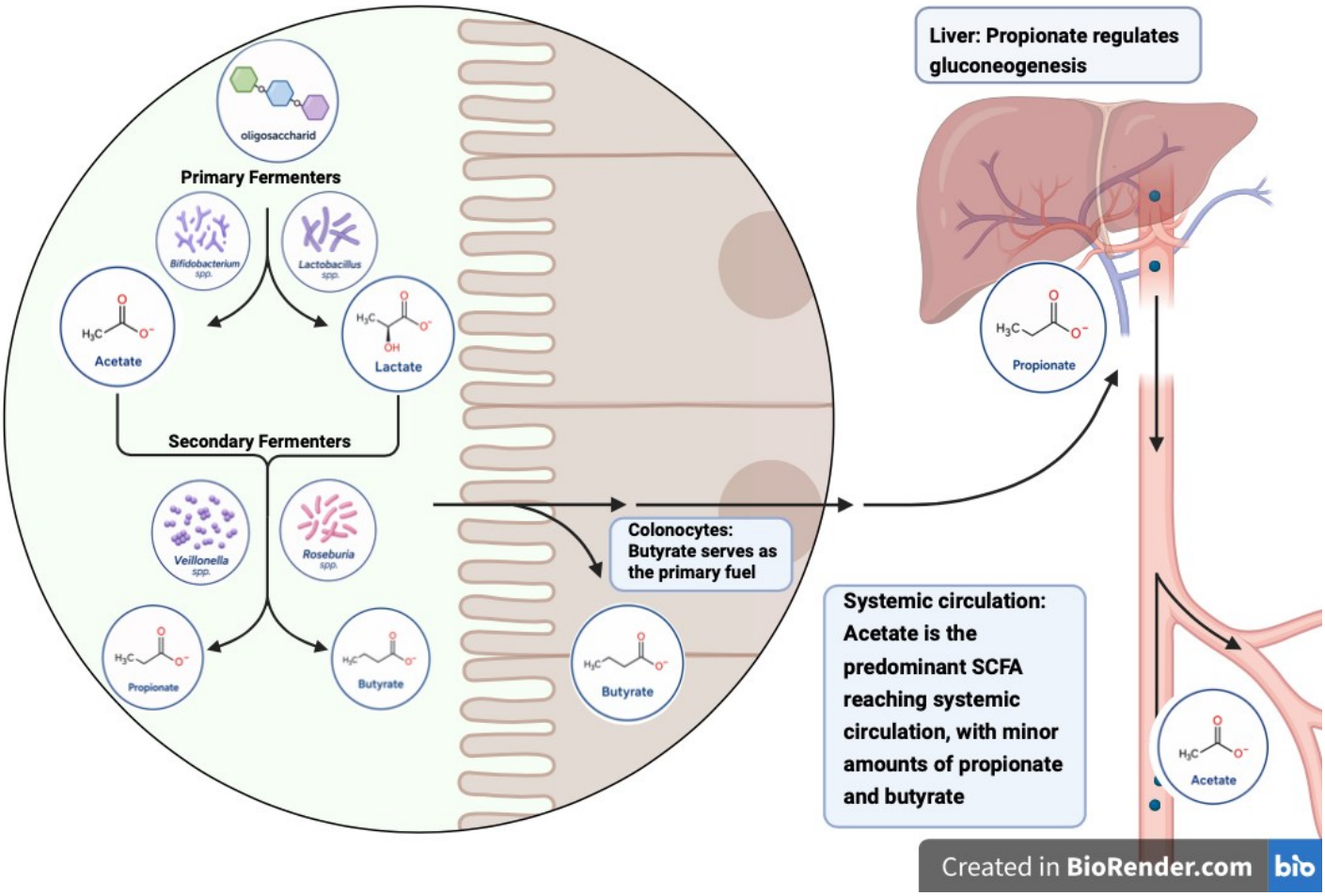

Figure 1: Overview of microbial cross-feeding involved in short-chain fatty acid (SCFA) production. Primary fermenters use oligosaccharides and monosaccharides to generate intermediate metabolites, including acetate and lactate, which are converted by secondary fermenters into butyrate and propionate. Butyrate is an energy source for colonocytes, whereas propionate is transported to the liver and acetate is the predominant SCFA reaching the systemic circulation. The bacterial taxa shown are examples and were selected for visualization purposes.

Polysaccharides that escape digestion are initially degraded by primary degraders into oligosaccharides and monosaccharides. These substrates are metabolized by primary fermenters, producing intermediate metabolites including fumarate, succinate, lactate, formate, and acetate. [12]

These metabolites can be used by secondary fermenters, contributing to SCFA production. Representative butyrate-producing bacteria include members of the Lachnospiraceae family (*Anaerostipes* spp., *Roseburia* spp., *Eubacterium* spp.,) and *Faecalibacterium prausnitzii*, whereas propionate producers include *Bacteroides* spp., *Negativicutes*, and some *Clostridium* spp. [12]

SCFAs are then absorbed by the host. Butyrate is the primary energy source for colonocytes, propionate is transported via the portal vein to the liver where it contributes to gluconeogenesis. Acetate reaches systemic circulation to a greater extent and is distributed to peripheral tissues. Bacterial species are not limited to a single metabolic role and can perform different functions depending on available substrates and environmental conditions.[12]

The proportions of acetate, propionate, and butyrate depend on the type of dietary fiber, microbial community composition, and colonic conditions such as transit time and available substrates.[11]

1.5.2 Microbial Protein Fermentation

Protein digestion and amino acid absorption in the small intestine are highly efficient, but not complete. A proportion of dietary protein reaches the colon, where it can be fermented by gut bacteria. In addition to dietary proteins, endogenous host-derived proteins such as glycoproteins and mucosal proteins also contribute to the nitrogen pool within the colon.[13,14]

Colonic bacteria preferentially ferment complex carbohydrates such as resistant starch. As these substrates become depleted from the proximal to the distal colon, bacterial metabolism increasingly shifts toward proteolytic fermentation, resulting in a specialization of the distal colonic microbiota in protein fermentation.[13,14]

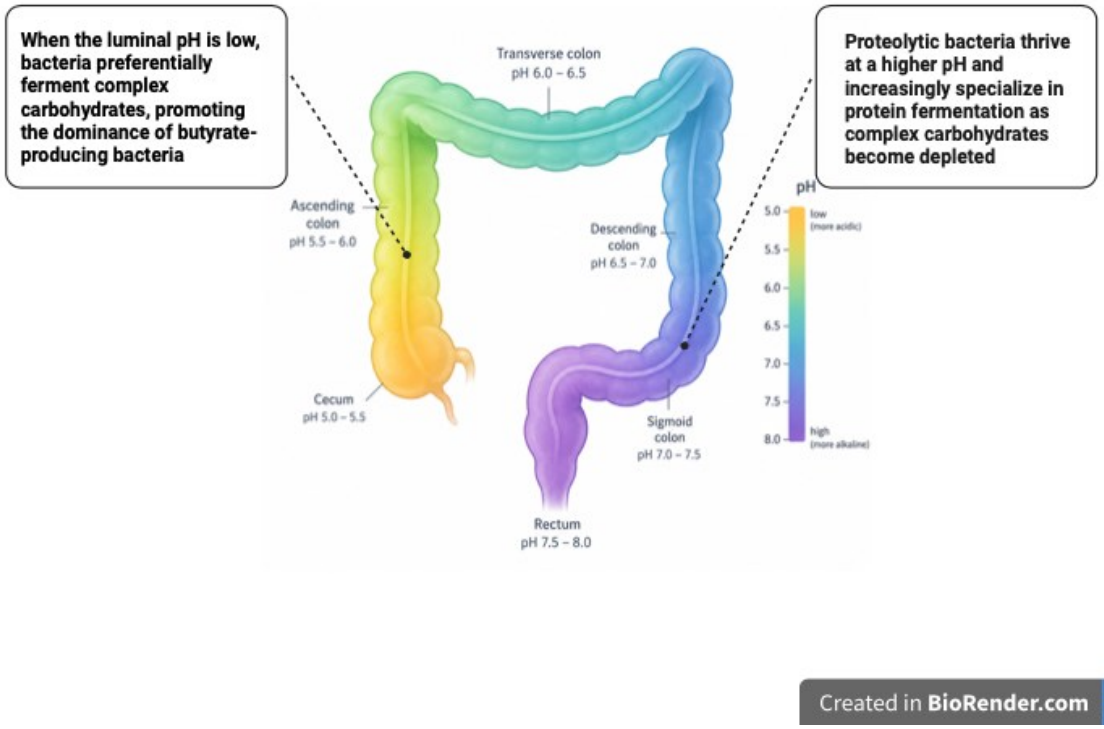

Figure 2: Representation of the pH gradient across the human colon and its influence on microbial metabolic activity. In the proximal colon (cecum and ascending colon), lower luminal pH values promote carbohydrate fermentation and favor saccharolytic, butyrate-producing bacteria. Along the distal colon, depletion of fermentable carbohydrates and increasing pH shift microbial metabolism toward proteolytic activity, resulting in increased protein fermentation.

This regional distribution of protein fermentation is further influenced by luminal pH. Since proteases exhibit higher activity at neutral pH, the less acidic environment of the distal colon (pH 6.5–7.0) may further promote proteolytic fermentation compared with the more acidic proximal/ascending colon (pH 5.5–6.5).[13,14]

The amount of protein reaching the colon depends on the total amount of protein in someone's diet as well as protein digestibility. Approximately 12–18 g of protein reach the large intestine daily. Protein digestibility is affected by several factors, including the protein source, food processing, and the surrounding food matrix. Plant proteins generally appear to be less digestible than animal proteins and may reach the colon in greater amounts. In addition, components of the food matrix such as lectins, phenolic compounds, and phytic acid can further impair protein digestibility. Food processing, particularly thermal processing, can additionally decrease protein digestibility and increase the amount of protein available for microbial fermentation in the colon.[13,14]

Many gut bacteria depend on external amino acids for growth, making amino acid availability an important determinant of microbial community composition. Amino acid fermentation in the colon is mainly associated with proteolytic taxa including Clostridia and Peptostreptococci, but also involves species belonging to *Fusobacterium*, Bacteroides, *Propionibacterium*, Actinomyces, *Enterobacteria*, *Eubacterium* spp., Lachnospiraceae and Ruminococcaceae.[15]

Microbial protein fermentation begins with the degradation of undigested proteins and peptides by bacterial proteases and peptidases, resulting in the release of free amino acids that can enter several microbial metabolic pathways. Depending on the available substrates and the composition of the gut microbiota, these amino acids are metabolized through reactions such as deamination, decarboxylation, and Stickland fermentation, generating a broad range of metabolites including ammonia, hydrogen sulfide, branched-chain fatty acids, amines, polyamines, as well as aromatic compounds such as phenols and indoles.[13,16]

1.5.2.1 Ammonia

Ammonia is primarily generated through bacterial deamination of amino acids during proteolytic fermentation in the colon. While many amino acids can contribute to ammonia formation, some, including aspartate, serine, lysine and glutamate, appear to be metabolized more extensively by the gut microbiota. Ammonia production is not dependent on a single amino acid species but rather reflects the overall availability of fermentable nitrogen substrates reaching the colon. These substrates originate from

undigested dietary proteins as well as endogenous nitrogen sources including mucus, host proteins and bacterial biomass turnover. Proteolytic fermentation is carried out by a diverse microbial community including species belonging to Clostridium, Enterococcus, Escherichia and Fusobacterium.[15]

1.5.2.2 Hydrogen Sulfide

Hydrogen sulfide (H_2S) is a gas involved in cellular signaling and redox reactions. In the colon, H_2S is generated through microbial sulfur metabolism from inorganic sulfur compounds such as sulfate and sulfite, sulfited food additives, sulfur-containing amino acids including cysteine and methionine, taurine, and through the catabolism of intestinal sulfomucins. Sulfur-metabolizing bacteria include Bilophila, Desulfovibrio, Desulfomicrobium, Fusobacterium and Clostridium-related species, which possess desulfhydrase enzymes such as cysteine desulfhydrase involved in cysteine degradation. Increased animal protein and meat intake are associated with enhanced sulfur metabolism and increased H_2S production within the gut. [17,18]

1.5.2.3 Branched-Chain-Fatty Acids (BCFA)

Branched-chain fatty acids (BCFA) are primarily saturated fatty acids containing a methyl branch, most commonly isobutyrate, isovalerate, and 2-methylbutyrate. In contrast to the short-chain fatty acids acetate, propionate, and butyrate, BCFA are generated during bacterial fermentation of branched-chain amino acids and are considered markers of proteolytic fermentation in the colon. BCFA are important structural components of bacterial membranes, where they contribute to membrane fluidity. [18–20]

Although BCFA can also be synthesized de novo in mammalian tissues, they are mainly derived from commensal bacteria and dietary sources, particularly animal fats and dairy products. In humans, BCFA are especially abundant in skin lipids, meibum, sebaceous glands, and vernix caseosa, where they are believed to exert protective and barrier-supporting functions. During late gestation, vernix particles suspended in amniotic fluid are swallowed by the fetus, making BCFA important constituents of the neonatal intestinal environment. BCFA are now increasingly recognized as bioactive metabolites

involved in host–microbiome interactions and modulation of intestinal microbial composition.[19,20]

BCFA are mainly formed from the bacterial metabolism of the branched-chain amino acids valine, leucine, and isoleucine. During this process, the amino acids first undergo transamination to branched-chain α-keto acids via branched-chain amino acid transferases, followed by oxidative decarboxylation through branched-chain keto acid dehydrogenase complexes. The resulting intermediates are substrates for BCFA synthesis. The main bacterial genera involved in this fermentation include Bacteroides and Clostridium. BCFA production depends on available substrates in the colon and increases when complex carbohydrates and dietary fiber become depleted in the distal colon. Accordingly, reduced fiber intake and high-protein, low-complex carbohydrate diets lead to elevated fecal BCFA concentrations.[19–21]

1.5.2.4 Phenolic Compounds

Phenolic compounds are end products of bacterial aromatic amino acid fermentation in the colon. They are primarily generated from tyrosine and phenylalanine that escape digestion in the small intestine and become available for microbial metabolism in the large intestine. Important phenolic metabolites include phenol, p-cresol, phenylacetate, phenylpropionate, hydroxyphenylacetate, hydroxyphenylpropionate, and 4-ethylphenol. Protein fermentation and aromatic amino acid catabolism increase particularly under conditions of carbohydrate depletion and increased proteolytic activity.[22,23]

Several intestinal bacteria are capable of producing phenolic metabolites from aromatic amino acids. *Clostridioides difficile* is one of the best characterized phenol-producing bacteria and converts tyrosine-derived p-hydroxyphenylacetate (p-HPA) into p-cresol through the p-HPA decarboxylase. P-Cresol is an antimicrobial metabolite and appears to provide a competitive advantage within the intestinal ecosystem by suppressing competing gut microorganisms, particularly Gram-negative bacteria. [24–26]

Besides *Clostridioides difficile*, additional intestinal bacteria associated with aromatic amino acid fermentation and phenol production include *Clostridium* spp., *Bacteroides*

spp., *Morganella morganii, Citrobacter freundii, Klebsiella pneumoniae, Peptostreptococcus* spp*., and Fusobacterium* spp.[22,27]

Phenolic compound production is strongly influenced by the colonic environment, particularly carbohydrate availability and luminal pH. Saccharolytic fermentation suppresses aromatic amino acid catabolism, whereas carbohydrate depletion favors proteolytic fermentation and phenol formation. In vitro studies showed that starch reduced the production of several phenolic metabolites, including p-cresol, while higher pH values promoted tyrosine and phenylalanine fermentation. In addition, naturally occurring dietary compounds may directly inhibit bacterial phenol-producing enzymes. Quercetin was recently identified as a competitive inhibitor of bacterial tyrosine phenol-lyase (TPL) and significantly reduced phenol production in bacterial cultures and mouse feces.[15,23,27]

1.5.2.5 Indolic Compounds

Tryptophan is an essential aromatic amino acid that is extensively metabolized by the gut microbiota into a broad range of indolic compounds. In bacteria, tryptophan is required for protein synthesis and is a precursor for several important metabolic processes including nicotinamide adenine dinucleotide (NAD) biosynthesis and maintenance of redox homeostasis. Intestinal microorganisms convert tryptophan through several distinct pathways including deamination to indole, decarboxylation to tryptamine, and transformation to indole-3-pyruvate followed by further conversion into downstream metabolites such as indole-3-acetate (IAA), indolelactic acid (ILA), indoleacrylic acid (IAcA), and indole-3-propionate (IPA). Besides functioning as metabolic end products, several of these compounds, particularly indole, also are signaling molecules involved in the regulation of bacterial physiology and microbial community interactions.[28–31]

Indolic compound production is highly species-specific and depends on the enzymatic capacities of individual gut microorganisms. Indole formation is primarily mediated by the enzyme tryptophanase (TnaA), which converts tryptophan into indole, pyruvate, and ammonia and is expressed by numerous intestinal bacteria including *Escherichia coli, Bacteroides* spp., and several Clostridium species. Other bacterial taxa use alternative pathways that generate metabolites such as indole-3-propionate (IPA), indolelactic acid

(ILA), indoleacrylic acid (IAcA), skatole, and tryptamine. Clostridium sporogenes and Peptostreptococcus species are among the best-characterized IPA producers, whereas *Lactobacillus* and *Bifidobacterium* species mainly generate ILA and related metabolites. Recent studies further showed that certain indolic compounds can also arise through cooperative interactions between bacterial species via cross-feeding mechanisms.[29,30]

The production of tryptophan-derived compounds is strongly influenced by environmental conditions within the colon, particularly luminal pH, dietary composition, and microbiota composition. Recent studies demonstrated that increased fecal pH is associated with elevated indole production and increased formation of indoxyl sulfate (IS), whereas lower pH conditions favor the reductive branch of tryptophan metabolism and promote metabolites such as indolelactic acid (ILA), indole-3-acetate (IAA), and indole-3-propionate (IPA). Mechanistically, acidic conditions inhibit bacterial tryptophanase activity and suppress the conversion of tryptophan into indole. Dietary factors additionally modulate these pathways, since starch suppresses aromatic amino acid fermentation, while fibers such as pectin were shown to enhance IPA production. Considerable interindividual variability further indicates that the composition and metabolic activity of the individual microbiome strongly determine the resulting indolic profile. [23,29,32]

1.5.2.6 Biogenic Amines

Biogenic amines are organic compounds generated through the decarboxylation of amino acids. They are commonly classified according to the number of amino groups into monoamines, diamines, and polyamines. Monoamines include compounds such as dopamine, norepinephrine, histamine, serotonin, and tryptamine, whereas putrescine and cadaverine belong to the diamines. Polyamines, such as spermidine and spermine, are an important subgroup of biogenic amines characterized by several amino groups.[33]

1.5.2.6.1 Polyamine

Polyamines (PAs) are molecules containing more than two amino groups and mainly include putrescine, spermidine, and spermine. In humans, polyamines originate from

three sources including dietary intake, biosynthesis by eukaryotic cells, and microbial synthesis in the intestinal lumen. Most dietary polyamines are absorbed in the small intestine, whereas gut microbiota contribute substantially to luminal polyamine concentrations in the colon. Polyamine biosynthesis by eukaryotic cells declines with age and is partially compensated by dietary intake and microbial biosynthesis in the large intestine. Polyamines originate primarily from the amino acids ornithine and methionine, while arginine and lysine are secondary precursor sources. Putrescine is synthesized through decarboxylation of ornithine catalyzed by ornithine decarboxylase (ODC), whereas spermidine and spermine are formed through S-adenosylmethionine decarboxylase-dependent pathways.[13,34,35]

In bacteria, polyamines are not only metabolic end products but are also involved in bacterial communication, signaling, and differentiation. Bacterial PA metabolism is heterogeneous: gut microbes can synthesize putrescine, spermidine, and spermine, but also other amines such as cadaverine, agmatine, homospermidine, norspermidine, and 1,3-diaminopropane. Their production depends on the bacterial strain, microbiota composition, precursor availability, and environmental conditions. Fermentable carbohydrates can increase bacterial PA formation because indigestible polysaccharides are fermented into SCFAs, lowering luminal pH and modifying microbial metabolism.[13,34,35]

1.5.3 Choline Metabolism

Choline is an essential nutrient mainly obtained from animal-derived foods such as eggs, red meat, fish, poultry, and dairy products. Choline is required for the synthesis of the neurotransmitter acetylcholine and is an important methyl-group donor essential for homocysteine degradation. Choline is essential for membrane integrity and hepatic lipid transport. In the liver, choline is required for the synthesis of phosphatidylcholine and very-low-density lipoproteins (VLDL), preventing hepatic lipid accumulation and fatty liver development. As a result, choline deficiency has been associated with hepatic steatosis, liver dysfunction, neurodegenerative diseases, and cognitive impairment.[36,37]

In addition to its physiological functions, there are gut bacteria that possess choline TMA-lyase activity (cutC/cutD) and metabolize dietary choline into trimethylamine (TMA), which is oxidized in the liver by flavin-containing monooxygenase 3 (FMO3) into trimethylamine-N-oxide (TMAO). Bacteria containing this enzymatic capacity belong mainly to the Firmicutes and Proteobacteria phyla and include species such as *Clostridium sporogenes, Clostridium hathewayi, Anaerococcus hydrogenalis, Escherichia fergusonii, Proteus penneri,* and *Edwardsiella tarda*. [36,37]

In a mouse study, pharmacological inhibition of microbial choline TMA-lyase reduced TMAO formation and significantly altered host cholesterol and bile acid metabolism, indicating that microbial choline metabolism directly affects host metabolic pathways.[38]

The MEATMARK intervention study showed that individuals with higher habitual meat consumption showed stronger TMAO responses following beef intake. In contrast, two weeks of dietary fiber supplementation significantly reduced microbial cutC gene abundance and attenuated TMAO formation after beef consumption, suggesting that microbial choline TMA-lyase activity is influenced by diet.[39]

1.5.4 N-Nitroso Compounds

Nitrate and nitrite originate from both endogenous and exogenous sources. Endogenously, nitrate and nitrite are generated through the oxidation of nitric oxide (NO), whereas exogenous exposure mainly occurs through dietary intake and drinking water. Dietary nitrate exposure is derived mostly from green leafy vegetables and other plant-based foods, while processed and cured meat products are an important source of dietary nitrite due to its use as a preservative. Following ingestion, nitrate enters the enterosalivary circulation and becomes concentrated in saliva, where it is mainly reduced to nitrite by commensal oral bacteria expressing nitrate reductase enzymes. These bacteria use nitrate as an alternative terminal electron acceptor during anaerobic respiration. Additional nitrate and nitrite reduction may also occur through bacterial species in the gastrointestinal tract and mammalian nitrate reductase enzymes present in tissues such as the liver, lung, kidney, and heart. [40,41]

Nitrite is a central intermediate within the nitrate–nitrite–NO cycle and a metabolic branching point. Under acidic conditions, nitrite is protonated to nitrous acid (HNO2), which can generate reactive nitrogen species such as dinitrogen trioxide (N2O3). These intermediates may either be reduced to nitric oxide or convert amines and amides to N-nitroso compounds (NOCs).[42,43]

Which pathway predominates depends on the local chemical and microbial environment, including pH, oxygen availability, redox conditions, antioxidant availability, heme iron, and the presence of nitrosatable substrates. Antioxidant-rich food matrices containing ascorbate, polyphenols, and tocopherols may inhibit nitrosation because these compounds react more rapidly with nitrosating intermediates such as N2O3 than amines do, shifting nitrite metabolism toward NO formation. In contrast, processed meat products provide nitrite, heme iron, and nitrosatable amine precursors that may promote endogenous NOC formation. Consequently, the biological effects of nitrate and nitrite appear to depend less on nitrate exposure alone than on the surrounding dietary and microbial context in which nitrite metabolism occurs.[44–46]

1.5.5 Purine Metabolism

Purine-derived nucleotides participate in nucleic acid synthesis, energy metabolism, and multiple regulatory processes. While many microorganisms are capable of synthesizing purines through de novo pathways, this process is metabolically costly and requires multiple enzymatic reactions. Preformed purines can additionally be recycled through salvage pathways, which is a more energetically favorable alternative. Not all microorganisms possess complete biosynthetic pathways, and some species rely on exogenous purine sources.[47]

Purines available within the intestinal environment originate from multiple sources, including dietary intake, host metabolism, cell turnover, and microbial activity. Approximately one-third of the total purine pool is estimated to derive from the diet and may be used by both the host and intestinal microorganisms. Following uptake and metabolism, purines can be converted into several metabolites, including inosine, hypoxanthine, xanthine, uric acid, and allantoin.[47]

Microbial purine metabolism does not merely affect local intestinal metabolite availability but also contributes to systemic purine homeostasis. Experimental studies comparing germ-free and conventionally raised mice showed altered purine profiles associated with microbial colonization. Colonized animals showed increased levels of guanine, guanosine, inosine, hypoxanthine, and xanthine, whereas germ-free mice had elevated levels of uric acid and allantoin, both considered waste products of purine metabolism. Such alterations may be clinically relevant, as both purine intake and serum uric acid levels show a U-shaped association with mortality. [47,48]

1.5.6 Bile Acids

Bile acids are cholesterol-derived molecules produced in the liver that are necessary for the absorption of fats. In humans, cholic acid and chenodeoxycholic acid are the primary bile acids. Before secretion into the intestinal tract, these bile acids are conjugated with glycine or taurine. After release into the duodenum in response to food intake, most bile acids are reabsorbed in the distal small intestine and transported back to the liver via enterohepatic circulation. Only a minor fraction avoids reabsorption and reaches the colon, where it is metabolized by the gut microbiota. [49]

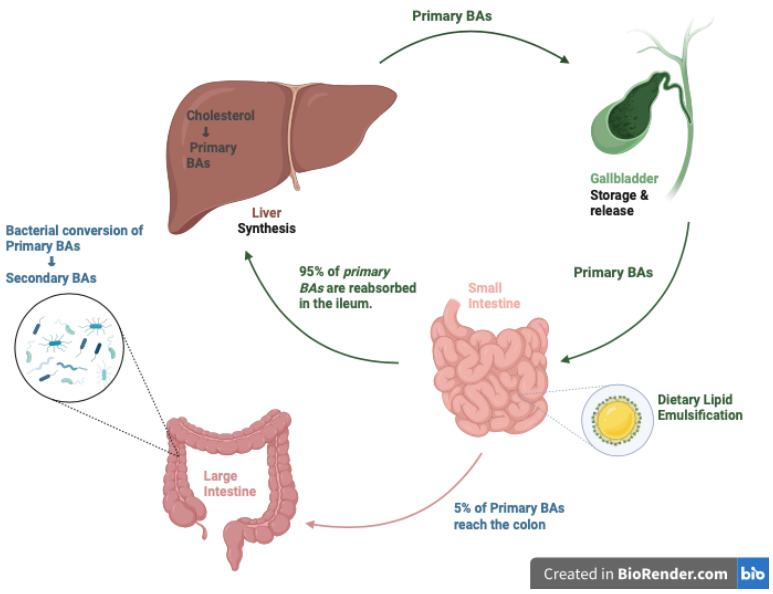

Figure 3: Schematic illustration of the enterohepatic circulation and microbial transformation of bile acids. The liver synthesizes primary bile acids from cholesterol. After storage in the gallbladder, they are released into the small intestine, where they emulsify dietary fats. Approximately 95% of primary bile acids are reabsorbed in the ileum and returned to the liver, whereas ~5% reach the colon, where gut microbiota convert them into secondary bile acids. This bidirectional interaction highlights the central role of gut microbiota in shaping bile acid composition and signaling.

Once bile acids enter the intestine, they are modified by gut bacteria. The first step of bacterial bile acid metabolism is the removal of glycine or taurine from conjugated bile acids. This reaction is catalyzed by bile salt hydrolase enzymes. Many gut bacteria express bile salt hydrolase and produce unconjugated bile acids. For bacteria, this reaction reduces bile acid toxicity and releases amino acids that can be used as nutrients.[49]

After deconjugation, a smaller group of intestinal bacteria can further modify bile acids by removing a hydroxyl group at position seven. This reaction converts the primary bile acids cholic acid and chenodeoxycholic acid into the secondary bile acids deoxycholic acid and lithocholic acid. The ability to perform seven-alpha-dehydroxylation is restricted to a limited number of anaerobic gut bacteria and is strongly dependent on microbial composition. As a result, secondary bile acid production varies between individuals and is sensitive to disturbances of the gut microbiome.[49,50]

1.5.7 Microbiota-derived Vitamins

The gut microbiome can synthesize several essential vitamins, including vitamin K as well as multiple B-group vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), nicotinic acid (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin (vitamin B7), folate (vitamin B9), and cobalamin (vitamin B12). [11,51]

The capacity to synthesize vitamins is unevenly distributed among bacterial taxa, with vitamin K production being often associated with members of the phylum Bacteroidetes (e.g. *Bacteroides* spp.), whereas the biosynthesis of several B-group vitamins is particularly linked to Actinobacteria, most notably *Bifidobacterium* spp. [11,51]

Microbiota-derived vitamins contribute to the host vitamin pool to varying extents, with vitamin K being almost entirely supplied by bacterial synthesis, whereas B-group vitamins contribute a substantial but incomplete fraction of host requirements, typically estimated at approximately 40–60%. [11,51]

A difference between dietary and microbially synthesized vitamins lies in their site of absorption. Dietary vitamins are absorbed in the small intestine, whereas microbiota-derived vitamins become available in the colon, where they can be used by other microbes.[11,51]

Microbial vitamin biosynthesis shows age-dependent differences. Early-life microbiomes have more pathways related to folate and vitamin B12 synthesis. [1]

1.6 Host–Microbiome Interactions

1.6.1 Immune Modulation

The immune system and the gut microbiota exist in a mutually beneficial relationship. Signals from the gut microbiota help the immune system maintain a balance between tolerance and defense. These signals promote immune tolerance toward commensal microorganisms while preserving the ability to respond to invading pathogens. In return, the immune system maintains conditions that allow commensal microorganisms to live within the intestine. [52,53]

Such signals can originate from microbial metabolites or components of bacteria, fungi, and viruses. Without these signals, immune development is impaired and susceptibility to immune-mediated diseases increases. [53]

1.6.1.1 Metabolites

1.6.1.1.1 Short-Chain Fatty Acids

Short-chain fatty acids (acetate, propionate, butyrate) are central immunomodulatory metabolites. They regulate immune responses through two main mechanisms: (i) inhibition of histone deacetylases (HDACs), leading to epigenetic reprogramming toward anti-inflammatory phenotypes, and (ii) activation of G protein–coupled receptors (e.g., GPR43, GPR41, GPR109A), which modulate immune cell signaling, inflammasome activation, and cytokine production. SCFAs strongly promote regulatory T cell differentiation and suppress pro-inflammatory pathways such as NF-κB signaling. [53]

1.6.1.1.2 Indolic Compounds (AHR ligands)

AHR ligands, especially microbiota-derived tryptophan metabolites (indole-3-acetate (IAA), indolelactic acid (ILA), indoleacrylic acid (IAcA), and indole-3-propionate (IPA)), regulate immune responses by inducing IL-22 production in Th17 cells and group 3 innate lymphoid cells. IL-22 is a effector cytokine that enhances antimicrobial immunity, including mechanisms that limit pathogen growth through the reduction of available metal ions. This pathway is critically involved in antifungal host defense, particularly against *Candida albicans*. [53]

1.6.1.1.3 Polyamine

Polyamines such as putrescine, spermidine, and spermine are derived both from dietary sources and from de novo synthesis by gut bacteria and human cells from arginine. Within this pathway, arginase and nitric oxide synthase compete for arginine, linking polyamine production to nitric oxide metabolism and immune regulation. Polyamines exert pronounced immunomodulatory effects, including a reduction in pro-inflammatory cytokines such as TNF-α and IL-6, alongside an increase in anti-inflammatory mediators such as TGF-β and IL-10, as well as enhanced IgA production in the small intestine. [53]

Through these mechanisms, they contribute to intestinal immune tolerance and barrier stability. Polyamine levels decline with age, which may be associated with impaired regenerative capacity and altered immune responses. In contrast, dysregulated and elevated polyamine levels are frequently observed in cancer, reflecting the high proliferative demands of tumor cells and their dependence on polyamine-mediated cell growth and metabolism.[53]

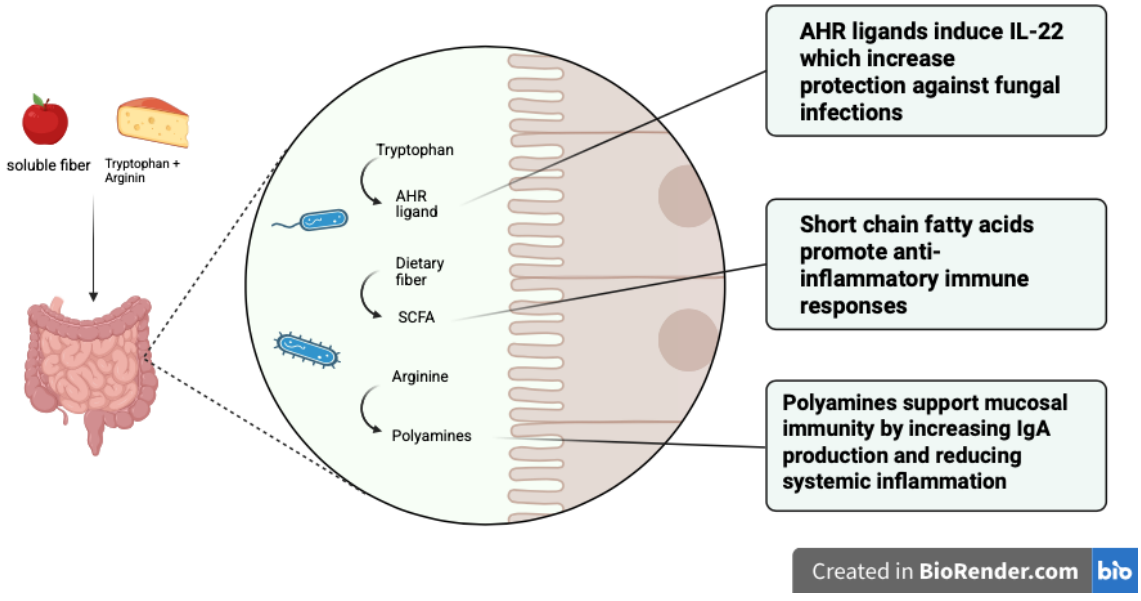

Figure 4: Overview of selected microbiota-derived metabolites and their immunomodulatory functions. Dietary components including soluble fiber, tryptophan, and arginine are metabolized by gut microorganisms into bioactive compounds such as short-chain fatty acids (SCFAs), aryl hydrocarbon receptor (AHR) ligands, and polyamines. These microbial metabolites regulate the immune system by promoting anti-inflammatory responses, enhancing IL-22–mediated mucosal protection against fungal infections, and supporting mucosal immunity through increased IgA production and reduced systemic inflammation.

1.6.1.1.4 Inosine

Inosine is a purine metabolite consisting of hypoxanthine linked to a ribose molecule and has two, context-dependent effects on the immune system. Both anti-inflammatory and antitumoral properties have been linked to inosine. [54,55]

The anti-inflammatory effects of inosine are primarily mediated through G-protein-coupled adenosine receptors, mainly adenosine A2A receptors (A2AR) and, to a lesser extent, A3 receptors (A3R), which are found on various immune cells including T lymphocytes. Activation of these receptors has been associated with reduced inflammatory cell recruitment and decreased production of pro-inflammatory cytokines, including TNF-α and several interleukins, limiting excessive immune activation and inflammation.[54,56]

Despite its anti-inflammatory properties, inosine can at the same time support adaptive immune responses and antitumor immunity. The tumor microenvironment is frequently characterized by nutrient deprivation and limited glucose availability due to the high demands of tumor cells. Under these glucose-restricted conditions, inosine can be an alternative energy source for T cells. In vitro studies showed that inosine promotes Th1 differentiation and directly supports CD8+ T-cell proliferation. [57]

1.6.1.2 Bacterial Components

Microbial components, known as microbial-associated molecular patterns (MAMPs), are structures derived from bacteria, fungi, and viruses that are recognized by host pattern recognition receptors (PRRs). Examples include lipopolysaccharides, flagellin, peptidoglycan, formyl peptides, and microbial nucleic acids. [53]

Through PRR engagement, they are essential for immune system education and controlled inflammatory activation. Prominent examples of functionally relevant microbial components include polysaccharide A (PSA), formyl peptides, and heptose-1,7-bisphosphate (HBP). [53]

One example of a microbial MAMP with mostly regulatory immunological effects is polysaccharide A (PSA), a capsular component of Bacteroides fragilis. By stimulating regulatory T cells and anti-inflammatory cytokines like IL-10, it increases immunological

tolerance. Through this mechanism, PSA helps maintain intestinal immune homeostasis and prevents excessive inflammatory responses against commensal bacteria. [53]

Formyl peptides, bacterial-derived molecules, are potent pro-inflammatory MAMPs. They are detected by formyl peptide receptors (FPRs) on innate immune cells, especially neutrophils, and trigger chemotaxis toward sites of microbial presence. This mechanism enables rapid immune surveillance and host defense, representing a classic example of MAMP-mediated immune activation. [53]

Heptose-1,7-bisphosphate (HBP) is a bacterial metabolite derived from lipopolysaccharides that functions as an intracellular MAMP. Once detected within host cells, it activates innate immune signaling pathways and induces inflammatory responses. HBP is an example how intracellular sensing of microbial components supports immune activation and host defense. [53]

1.6.2 Metabolic Interactions

The gut microbiome regulates the metabolism by modulating energy extraction and transforming dietary and host-derived substrates into bioactive metabolites. These microbial processes contribute directly to host metabolic pathways and are increasingly recognized as an important environmental factor influencing metabolic diseases such as obesity and insulin resistance. [58]

In particular, the microbiota enhances the host's ability to harvest energy from otherwise indigestible nutrients and modulates systemic metabolic homeostasis through metabolite production and signaling interactions.[58]

1.6.2.1 Short-Chain Fatty Acids

Short-chain fatty acids are mediators linking microbial fermentation to host metabolism. Through activation of GPR41 (FFAR3) and GPR43 (FFAR2) on enteroendocrine L-cells, they stimulate the release of GLP-1 and PYY, which reduce gastric emptying and decrease food intake. In parallel, SCFAs enhance leptin expression in adipose tissue, reinforcing anorexigenic signaling. Beyond appetite regulation, SCFAs improve insulin

sensitivity mainly in skeletal muscle by increasing glucose uptake and oxidative metabolism, partly via incretin-mediated pathways and AMPK activation. Additionally, they modulate lipid storage and inflammatory tone in adipose tissue and liver, contributing to improved systemic glucose homeostasis. [58–60]

1.6.2.2 Secondary Bile Acids

Microbiota-derived secondary bile acids are endocrine signaling molecules that regulate host metabolism via FXR and TGR5. Activation of FXR in the liver reduces triglyceride synthesis and VLDL secretion, lowers hepatic glucose production, and increases fatty acid oxidation. TGR5 activation in enteroendocrine cells stimulates GLP-1 secretion, enhancing insulin secretion and improving glucose tolerance. TGR5 signaling also increases energy expenditure in peripheral tissues. Together, these pathways link microbial bile acid metabolism to coordinated regulation of lipid metabolism, glucose homeostasis, and energy balance. [49,58]

1.6.2.3 Trimethylamine-N-oxide (TMAO)

TMAO has been associated with numerous metabolic diseases in human observational studies, including obesity, metabolic syndrome, insulin resistance, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, and cardiovascular disease. Circulating TMAO concentrations correlate positively with body mass index, visceral adiposity, liver fat accumulation, and markers of metabolic dysfunction. These studies are largely observational and cannot establish whether TMAO is a causal factor or merely a biomarker of an unfavorable metabolic state. [61]

To investigate whether TMAO actively supports metabolic dysfunction, TMAO levels were experimentally increased in a mouse model. Elevated TMAO impaired pancreatic β-cell function, reduced glucose-stimulated insulin secretion, and worsened glucose tolerance. Conversely, inhibition of TMAO production improved β-cell function and glucose homeostasis. These findings suggest that TMAO may not only be a biomarker of metabolic disease but could also directly contribute to its development.[62]

1.6.3 Intestinal Barrier Function

Microbial metabolites are involved in the regulation of intestinal barrier function by affecting epithelial integrity and mucus production. The intestinal barrier is an interface that enables the absorption of nutrients while limiting the translocation of harmful substances such as pathogens and toxins. A wide range of microbiota-derived metabolites contribute to this regulation and can be signaling molecules or directly influence epithelial cells. Short-chain fatty acids, bile acids, and tryptophan-derived metabolites are among the most extensively studied groups in this context and are associated with effects on tight junctions and epithelial homeostasis. In addition, other metabolites, including polyamines, conjugated fatty acids, and polyphenol-derived compounds, have also been described in relation to intestinal barrier function. [63–65]

1.6.3.1 Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, have been extensively studied in relation to intestinal barrier function. They interact with intestinal epithelial cells and are associated with effects on epithelial integrity. In particular, butyrate is an energy source for colonocytes and has been linked to maintaining epithelial homeostasis. SCFAs influence the expression and localization of tight junction proteins, including zonula occludens-1 (ZO-1), occludin (OCLN), and claudins, regulating paracellular permeability. In addition, SCFAs are associated with increased mucus production and changes in epithelial differentiation. These findings indicate that SCFAs are involved in multiple mechanisms that maintain the intestinal barrier function. [8,9,66]

1.6.3.2 Bile Acids

Bile acids affect the intestinal barrier function, although their effects are not uniform. Depending on the specific bile acid, concentration, and experimental conditions, they may either impair or support barrier integrity. In this context, bile acids are linked to changes in intestinal permeability and the organization of tight junction proteins, including zonula occludens-1, occludin, and claudins. Certain bile acids, such as chenodeoxycholic acid and deoxycholic acid, have been linked to increased permeability and reduced barrier function. In contrast, ursodeoxycholic acid has been reported to preserve or improve barrier integrity under specific conditions. These observations

indicate that bile acids can influence intestinal barrier function in a context-dependent manner.[8,64,67]

1.6.3.3 Tryptophan-derived Metabolites

Tryptophan-derived microbial metabolites, particularly indole and its derivatives, are linked to intestinal barrier function through their effects on epithelial cells. These compounds interact with the aryl hydrocarbon receptor, a signaling pathway that regulates epithelial integrity and barrier homeostasis. Activation of this receptor maintains epithelial function and the expression of barrier-related proteins. In addition, tryptophan-derived metabolites regulate mucosal defense mechanisms, including the production of interleukin-22, which supports epithelial barrier stability. [8]

1.7 Stability, Resilience, and Dysbiosis of the Gut Microbiome

The gut microbiome is a dynamic ecosystem that continuously adapts to internal and external influences while maintaining a certain degree of functional stability. This adaptive capacity, often referred to as resilience, allows the microbiome to recover from transient disturbances. This recovery is not always complete. Under certain conditions, perturbations can lead to persistent alterations in microbial composition and function, resulting in dysbiosis. Dysbiosis does not simply reflect short-term variability, but rather a shift to an alternative, potentially less stable microbial state that may not readily revert to its original configuration. [50]

A driver of such long-lasting perturbations is antibiotic exposure, which is one of the most profound and well-characterised disruptors of microbiome stability. Antibiotics can reduce microbial diversity and alter community structure, often with long-term consequences. While partial recovery may occur, the original composition is not always fully restored, indicating limited resilience of the system under repeated or strong perturbation. This highlights that microbiome stability is conditional and can be permanently altered when ecological thresholds are exceeded.[68–70]

In addition to antibiotics, a wide range of other external factors can contribute to microbiome instability and dysbiosis, particularly in the context of modern Western lifestyles. Commonly used medications, including proton pump inhibitors, selective

serotonin reuptake inhibitors and laxatives, have been shown to significantly alter microbial composition and function. For example, proton pump inhibitor use correlates with reduced microbial diversity and a shift towards oralisation of the gut microbiome, reflecting the displacement of the native intestinal microbial community by bacteria originating from the oral cavity.[50,71,72]

Beyond pharmacological factors, components of processed foods have also been shown to alter the composition of the gut microbiome. Dietary emulsifiers (e.g., carboxymethylcellulose, polysorbate-80, and mono- and diglycerides of fatty acids) and artificial sweeteners can alter microbial community structure and reduce microbial diversity. Repeated exposure to such compounds may contribute to persistent alterations in the gut microbiome. [73,74]

2. Colorectal Cancer: Biological and Clinical Characteristics

Colorectal cancer (CRC) is one of the most common malignancies worldwide and remains a cause of cancer-related mortality. Globally, CRC is the third most common cancer and accounts for approximately 10% of all cancer diagnoses. In Austria, 4,690 new colorectal cancer cases were diagnosed in 2023, highlighting its continued public health relevance. [75,76]

The incidence of CRC increases with age, with the majority of patients being diagnosed after the age of 60 years. Incidence rates are higher in developed than in developing regions, suggesting an important contribution of environmental and lifestyle-related factors to disease development. [75]

CRC typically develops over many years through the progression of precursor lesions to invasive carcinoma. This process is driven by the gradual accumulation of genetic and epigenetic alterations that promote malignant transformation. Consequently, the long preclinical phase of CRC provides an opportunity for prevention and early detection through screening programs.[75]

Numerous modifiable lifestyle factors have been linked to CRC risk, including obesity, smoking, alcohol consumption, physical inactivity, and a high intake of red and processed meat. In contrast, dietary fiber intake and regular physical activity appear to exert protective effects.[75]

The prognosis of colorectal cancer is strongly dependent on the stage at diagnosis. While the overall global 5-year survival rate is estimated to be approximately 60%, survival varies across disease stages. Patients with localized (stage I) disease have a 5-year relative survival rate of approximately 90%, compared with 70–75% for regional (stage II–III) disease and only around 15% for metastatic (stage IV) disease.[77]

Despite the central role of TNM (tumor, node, metastasis) staging in clinical practice, considerable heterogeneity exists among patients within the same disease stage. Patients with apparently similar clinicopathological characteristics may experience different

clinical outcomes, indicating that additional biological factors contribute to disease progression and prognosis. [78]

2.1 Molecular Heterogeneity of Colorectal Cancer

Colorectal cancer (CRC) is not a single disease entity but includes tumors with distinct molecular characteristics. Differences in genetic and epigenetic alterations contribute to considerable variability in tumor biology, disease progression, therapeutic response, and clinical outcome. Understanding this molecular heterogeneity is essential for explaining differences in colorectal carcinogenesis and for the development of more personalized treatment approaches.[79]

2.1.1 Genomic Instability Pathways

The spontaneous mutation rate alone cannot explain the large number of genetic alterations required for colorectal carcinogenesis. Additional mechanisms of genomic instability, including chromosomal instability (CIN), mismatch repair deficiency leading to microsatellite instability (MSI), and epigenetic alterations such as CpG island methylation, are thought to contribute to tumor development.[80]

CIN is characterized by gains or losses of whole chromosomes or chromosomal regions, whereas MSI results from deficient DNA mismatch repair leading to the accumulation of errors within repetitive DNA sequences. In contrast, CIMP is characterized by widespread CpG island hypermethylation, which may induce transcriptional silencing of genes, particularly tumor suppressor and DNA repair genes. Although these mechanisms are commonly described as distinct pathways, they are not entirely mutually exclusive and may overlap. For example, approximately 25% of MSI tumors also have chromosomal abnormalities, while CIMP accounts for a substantial proportion of MSI-positive/CIN-negative tumors, highlighting the molecular complexity of CRC. [80]

These mechanisms increase the likelihood of accumulating genetic alterations, some of which may function as driver mutations by conferring a selective growth advantage and promoting tumor initiation and progression. [80]

2.1.2 Common Driver Mutations in CRC (APC, KRAS, TP53, BRAF)

The genomic and epigenetic alterations described above may promote the accumulation of genetic changes during colorectal carcinogenesis. While many of these alterations are passenger mutations with limited biological significance, a subset provides a selective growth advantage and supports tumor initiation and progression. These so-called driver mutations frequently affect genes involved in regulatory pathways, with commonly altered genes in CRC including APC, KRAS, BRAF, and TP53.[79]

2.1.2.1 APC

Adenomatous Polyposis Coli (APC) is one of the earliest and most frequently altered driver genes in colorectal carcinogenesis and functions as a tumor suppressor gene. APC regulates the WNT/β-catenin signaling pathway, which controls cellular proliferation and differentiation. Under physiological conditions, APC negatively regulates this pathway by promoting β-catenin degradation and limiting pathway activity. Loss of APC function results in intracellular accumulation of β-catenin, leading to transcriptional activation of genes involved in cellular proliferation and tumor development.[80]

2.1.2.2 KRAS

KRAS is one of the most frequently altered oncogenes in colorectal cancer and is mutated in approximately 30–40% of CRC cases. Under physiological conditions, KRAS transmits extracellular growth signals from cell surface receptors to intracellular signaling pathways, including the RAF–MEK–ERK pathway involved in regulating cell proliferation and survival. KRAS mutations reduce the intrinsic GTPase activity of the protein and cause persistent activation of downstream signaling pathways. Constitutive activation of the RAF–MEK–ERK pathway promotes uncontrolled cell growth and supports tumor progression.[79]

2.1.2.3 TP53

Tumor Protein 53 (TP53), often referred to as the “guardian of the genome”, is a tumor suppressor gene with a central role in preserving genomic integrity. Under normal conditions, p53 coordinates cellular responses to DNA damage and other forms of cellular stress by regulating mechanisms involved in DNA repair, cell cycle control, and

programmed cell death. Loss of TP53 activity disrupts these protective mechanisms, allowing damaged cells to continue proliferating and acquire additional genetic alterations. [79–81]

2.1.2.4 BRAF

BRAF is an oncogene involved in intracellular signaling pathways regulating cellular proliferation, differentiation, and survival and is mutated in approximately 8–12% of CRC cases. BRAF is downstream of KRAS within the RAF–MEK–ERK signaling pathway and transmits growth signals. Mutations in BRAF result in persistent activation of downstream signaling pathways, promoting uncontrolled cellular proliferation and tumor progression. BRAF-mutated CRCs are frequently associated with female sex, right-sided tumor location, and distinct molecular characteristics including MSI and CIMP.[82,83]

2.2 Consensus Molecular Subtypes

The Consensus Molecular Subtypes (CMS) classification was developed to systematically address the substantial molecular heterogeneity observed in colorectal cancer (CRC). By integrating several previously independent molecular classification systems, four subtypes were identified: CMS1 (immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal). CMS1 is characterized by microsatellite instability (MSI), hypermutation, and strong immune activation. CMS2 is an epithelial subtype with marked activation of WNT and MYC signalling pathways. CMS3 is associated with epithelial features and metabolic dysregulation, whereas CMS4 is characterized by mesenchymal features, stromal infiltration, angiogenesis, and activation of transforming growth factor beta (TGF-β) signalling. These subtypes differ in their biological characteristics and may influence prognosis and therapeutic response.

2.3 Tumor Location and Clinical Heterogeneity

Colorectal cancer is increasingly recognized as a highly heterogeneous disease, and accumulating evidence suggests that tumor sidedness is an important source of this

heterogeneity. Rather than merely reflecting anatomical differences, left- and right-sided tumors may have functionally divergent disease characteristics with substantial differences in clinical behavior, prognosis, and molecular features. Right-sided tumors correlate with a worse prognosis, while left-sided tumors often show more favorable outcomes. Cohort studies have shown reduced survival in patients with right-sided compared with left-sided colorectal cancer.[84,85]

The observed clinical differences may reflect distinct underlying biological mechanisms. Right-sided tumors are characterized by a higher prevalence of several molecular alterations including microsatellite instability (MSI), BRAF mutations, CpG island methylator phenotype (CIMP), mismatch repair deficiency (dMMR), and increased tumor infiltrating lymphocytes (TILs). In contrast, the association with RAS alterations appeared less pronounced.[86]

Differences between both tumor locations extend beyond genomic alterations and may also involve distinct characteristics of the tumor microenvironment (TME) and extracellular matrix composition. These differences may contribute to altered immune recruitment and treatment response patterns. Right- and left-sided colorectal cancers differ not only in their molecular profiles but also in demographic characteristics, with right-sided tumors occurring more frequently in women. [87]

Interpretation of findings across studies should be performed cautiously, as the distinction between left- and right-sided colorectal cancers has historically not been entirely consistent. [86]

2.4 Clinical implications of Heterogeneity

Clinical and molecular heterogeneity in colorectal cancer has important implications for patient management, as tumors with similar histopathological characteristics may have substantial differences in disease progression, prognosis, and treatment response. Increasing evidence suggests that these differences are driven by distinct molecular alterations and tumor subtypes, highlighting the limitations of a uniform treatment approach. A better understanding of tumor heterogeneity may improve patient stratification and support more individualized treatment strategies. [88,89]

2.4.1 Prognostic Implications

Molecular characteristics contribute to the biological heterogeneity of colorectal cancer and are increasingly recognized as important determinants of clinical outcome. In addition to conventional clinicopathological parameters such as TNM stage, several molecular alterations are linked to differences in metastatic behavior, recurrence rates, and survival. Among these, microsatellite instability (MSI), KRAS mutations, and BRAF mutations are some of the most extensively studied molecular characteristics with prognostic relevance.[90–92]

Microsatellite instability (MSI) is considered one of the most established favorable prognostic molecular characteristics in colorectal cancer. In a population-based cohort study was shown, that MSI-high tumors had a less aggressive phenotype and lower metastatic potential. MSI-high tumors showed less regional lymph node and distant metastases, suggesting that their improved prognosis may partly result from reduced metastatic dissemination. [92,93]

KRAS and BRAF mutations are among the most extensively investigated prognostic biomarkers in colorectal cancer. A recent meta-analysis showed that both KRAS and BRAF mutations are associated with reduced overall survival compared with wild-type tumors. Beyond overall survival, KRAS-mutated tumors were additionally associated with an approximately 1.5-fold increased risk of relapse. Although KRAS mutations occur more frequently, BRAF mutations appear to have an even stronger prognostic impact and are linked to unfavorable clinical outcomes.[92,94]

2.4.2 Predictive Implications

Molecular characteristics are not only relevant for prognostic assessment but also increasingly influence therapeutic decision-making in colorectal cancer. Specific molecular alterations can predict response or resistance to targeted therapies and immunotherapies and contribute to a more personalized treatment approach.[89]

Mutations in signaling pathways can directly affect the efficacy of targeted therapies. For example, KRAS and NRAS mutations correlate with resistance to EGFR antibodies because downstream signaling remains activated despite receptor inhibition. In contrast,

BRAF-mutated tumors may benefit from treatment strategies incorporating BRAF-targeted agents, while additional molecular alterations such as HER2 may also provide therapeutic targets in selected patients.[94,95]

2.5 Limitations of molecular prognostic Biomarkers

One limitation of current molecular prognostic biomarkers in colorectal cancer is the pronounced intratumoral and intertumoral heterogeneity of the disease. Colorectal cancers can differ across tumor regions and over time in their genetic, epigenetic, and microenvironmental characteristics. [96]

Morphologically similar tumor regions may have distinct molecular profiles, whereas genetically similar regions may appear histologically different. Metastatic lesions may differ molecularly from the primary tumor, complicating biomarker-based prognostication and therapeutic stratification. These findings raise concerns regarding the representativeness of single tumor biopsies for molecular characterization.[96]

In addition, transcriptomic analyses have shown that more than half of colorectal cancers display intratumoral heterogeneity with mixed consensus molecular subtype (CMS) signatures. This suggests that many tumors cannot be assigned to a single stable molecular subtype, but rather contain multiple coexisting molecular states. Such heterogeneity may contribute to variable prognosis, treatment resistance, and inconsistent biomarker performance.[97]

Another practical limitation is that current molecular characterization usually relies on invasive tissue biopsies obtained from only a limited tumor region. Given the extensive molecular heterogeneity of colorectal cancer, such samples may not accurately reflect the complete tumor landscape. This has increased interest in less invasive approaches such as stool-based or blood-based biomarkers, which may better capture the dynamic and heterogeneous nature of colorectal cancer while reducing patient burden.[98,99]

3. Gut Microbiome Signatures in Colorectal Cancer

Accumulating evidence indicates that colorectal cancer is associated with substantial alterations in the intestinal microbial ecosystem. Rather than reflecting changes in individual bacterial species alone, colorectal cancer is characterized by broader shifts in microbial community composition and function, commonly referred to as gut microbial dysbiosis. These alterations are thought to promote disease development through interactions with host metabolism, epithelial integrity, immune responses, and the tumor microenvironment. [100–102]

3.1 Gut microbiome Composition and functional Alterations in Colorectal Cancer

Alterations of the gut microbiome are consistently observed in colorectal cancer. Current evidence is largely based on observational studies and does not establish causality. The observed differences in microbial composition may either contribute to tumor development, arise as a consequence of the disease, or reflect shared underlying factors such as lifestyle and metabolic comorbidities. [102,103]

Microbiome changes are not equally present across all stages of colorectal carcinogenesis. Cross-cohort analyses show that the microbiome of patients with adenoma does not differ significantly from that of healthy controls, whereas clear differences are observed in colorectal cancer, indicating that microbiome alterations become more pronounced during the transition from adenoma to carcinoma.[102,103]

3.1.1 Compositional Changes

Large cross-cohort and metagenomic analyses identified reproducible microbial signatures associated with colorectal cancer. The microbial taxa enriched in colorectal cancer are summarized in Table 1. [100,101,103]

Table 1: Bacterial species consistently enriched in colorectal cancer across multiple cohorts and their proposed functional categories. [101]

Species	Family	Functional Category
Fusobacterium nucleatum	Fusobacteriaceae	Proteolytic oral biofilm-associated bacterium [104]
Parvimonas micra	Peptoniphilaceae	oral biofilm-associated bacterium
Peptostreptococcus stomatis	Peptostreptococcaceae	oral biofilm-associated bacterium
Gemella morbillorum	Bacillales	oral biofilm-associated bacterium
Solobacterium moorei	Erysipelotrichaceae	oral biofilm-associated bacterium
Porphyromonas uenonis	Porphyromonadaceae	oral biofilm-associated bacterium
Clostridium symbiosum	Clostridiaceae/Lachnospiraceae	Branch Chain Amino Acid Producer[105]
Hungatella hathewayi	Clostridiaceae/Lachnospiraceae	Glycosaminoglycan (GAG) degrader[106]
Prevotella intermedia	Prevotellaceae	Proteolytic oral biofilm-associated bacterium[107]
Porphyromonas somerae	Porphyromonadaceae	oral biofilm-associated bacterium
Porphyromonas asaccharolytica	Porphyromonadaceae	oral biofilm-associated bacterium
Parvimonas species	Peptoniphilaceae	oral biofilm-associated bacterium
Ruminococcus torques	Clostridiaceae/Lachnospiraceae	Mucin degrader [108]
Porphyromonas uenonis	Porphyromonadaceae	oral biofilm-associated bacterium
Subdoligranulum species	Ruminococcaceae	Butyrat producer [109]

Many of the enriched taxa, including *Fusobacterium, Porphyromonas, Parvimonas, Peptostreptococcus, Gemella, and Solobacterium*, are oral-associated bacteria, suggesting increased colonization by taxa that are not typically dominant members of the healthy colonic microbiome.[100,101,103]

Oral-associated bacteria detected in colorectal tumors may directly originate from the oral cavity. In paired saliva and tumor analyses, identical *Fusobacterium nucleatum* strains were isolated from both sites in the same patients, supporting potential oral-gut transmission. [110]

CRC-associated dysbiosis involves depletion of commensal bacteria that support intestinal homeostasis and colonization resistance. In particular, reduced abundance of Lachnospiraceae has been linked to increased colonic colonization by oral-associated bacteria, suggesting that loss of protective commensals may promote persistence of CRC-associated oral taxa within the colon.[111]

Microbial composition also differs according to tumor location. Right-sided CRC is linked to lower microbial richness and stronger enrichment of oral-associated bacteria compared with left-sided CRC and rectal tumors. Veillonella and Coprobacter were reported to be more abundant in proximal compared with distal tumors. [112]

Microbial composition additionally differs according to tumor stage. Although pooled analyses suggest that global microbiome alterations remain limited in adenomas, individual bacterial taxa already exhibit stage-specific enrichment patterns in early lesions. *Atopobium parvulum* and *Actinomyces odontolyticus* were identified in adenomas and intramucosal carcinomas, whereas *Porphyromonas uenonis, Selenomonas sputigena,* and *Streptococcus anginosus* were enriched in stage III/IV CRC.[101,103]

Reported changes in microbial diversity and richness are heterogeneous across CRC cohorts. Some studys observed increased fecal microbial diversity in CRC patients compared with healthy controls, whereas others reported lower microbial richness in right-sided CRC, suggesting that microbial alterations may differ according to tumor location and microbial niche.[102,103]

3.1.2 Functional Changes

Functional alterations of the gut microbiome are a central feature of colorectal cancer. In addition to taxonomic dysbiosis, CRC-associated microbiota have altered metabolic functions characterized by increased protein fermentation, mucin degradation, and changes in bile acid metabolism, alongside reduced carbohydrate fermentation. These functional alterations are thought to contribute to epithelial injury, inflammation, and colorectal carcinogenesis. [13,17,101,113–115]

Table 2: Summary of microbiome-derived metabolites and their reported alterations in colorectal cancer.

Metabolites/Functional Alteration	
Short-Chain Fatty Acids	↓
Branch-Chain Fatty Acids	↑
Mucus Degradation	↑
Hydrogensulfide	↑
Secondary Bile Acids	↑
Trimethylamine (TMA)	↑
Inosine	↑

3.1.2.1 Alterations in Short-Chain Fatty Acid Concentrations

Reduced fecal short-chain fatty acid (SCFA) concentrations are linked to an increased colorectal cancer (CRC) risk and incidence and have also been observed in individuals at high risk of CRC as well as in adenoma patients.[116,117]

Among individual SCFAs, alterations appear to affect butyrate and acetate more consistently than propionate. While reductions in butyrate and acetate have repeatedly been observed in CRC, alterations in propionate seem less pronounced and remain inconsistent across studies. [118]

The mechanisms underlying reduced short-chain fatty acid (SCFA) concentrations remain incompletely understood. Reduced butyrate concentrations may reflect both a reduced abundance of butyrate-producing bacteria and an increased consumption by cancer tissue. Increased fecal pH is an additional consequence of this shift in SCFA concentrations.[118,119]

The available evidence remains heterogeneous, as some studies did not observe significant differences in SCFA concentrations between CRC patients and controls.[118]

3.1.2.2 Increased Protein Fermentation

Patients with colorectal cancer have a distinct fecal metabolomic profile characterized by increased concentrations of free amino acids and metabolites. Eleven amino acids were reported to be increased by 41–80% in stool samples from colorectal cancer patients compared to healthy controls. Elevated metabolites included proline, alanine, glutamate,

phenylalanine, isoleucine, leucine, valine, serine, threonine, aspartate, benzoic acid, and myristic acid. In addition, the branched-chain fatty acids isobutyric acid and isovaleric acid were also significantly increased. Since branched-chain fatty acids are characteristic end products of bacterial branched-chain amino acid fermentation, these findings collectively suggest increased proteolytic fermentation in the colonic environment of colorectal cancer patients. [120]

The cause of this metabolic pattern remains unclear. This increase could reflect altered dietary protein intake, reduced protein absorption, increased epithelial turnover and tumor-associated tissue breakdown, altered microbial metabolism, or increased substrate availability within the tumor microenvironment. Glutamine was not elevated whereas glutamate was increased, which may indicate enhanced glutamine use and conversion to glutamate within the tumor-associated metabolic network. [120]

3.1.2.3 Mucus Barrier Dysfunction

Physiological mucus degradation is an essential component of intestinal homeostasis and host–microbiome interactions. Accumulating evidence suggests that this physiological mucus homeostasis becomes disrupted in colorectal cancer. CRC is associated with impaired mucus barrier integrity, altered microbial colonization, and changes in mucin glycosylation patterns, including truncated glycan structures and altered sialylation. These alterations may shift normally physiological microbe–mucus interactions toward excessive mucus degradation, increased bacterial–epithelial contact, and chronic inflammation.[121–123]

The colonic mucus layer does not only function as a passive physical barrier but it is also a habitat and nutrient source for specialized commensal bacteria. In particular, mucin-degrading bacteria possess glycosyl hydrolases, sulfatases, and mucinases that enable the enzymatic degradation of complex mucin glycans. The released carbohydrates function as metabolic substrates for both mucin-degrading and neighboring commensal microorganisms, supporting the development of complex microbial ecosystems within the mucus layer. At the same time, the glycosylation pattern of mucins strongly

influences bacterial adhesion and colonization, as specific microbes are adapted to recognize and use distinct glycan structures. Consequently, the mucus barrier is a highly dynamic host–microbiome interface whose integrity depends on a tightly regulated balance between mucus production and controlled microbial degradation.[123–125]

Among the best-characterized mucin-degrading bacteria are *Akkermansia muciniphila, Akkermansia glycaniphila, Bacteroides thetaiotaomicron, Bifidobacterium* spp., *Parabacteroides distasonis, and Ruminococcus* species. Mucin degradation itself is not inherently pathological, as several of these organisms are considered physiologically beneficial commensals. Akkermansia muciniphila in particular has frequently been associated with metabolic health and intestinal homeostasis and produces short-chain fatty acids. Recent evidence suggests that excessive or dysregulated mucus degradation may compromise the integrity of the mucus barrier and promotes inflammation, pathogenic overgrowth, and tumor-promoting host–microbiome interactions. Consequently, the role of mucin-degrading bacteria in colorectal cancer appears highly context-dependent and may differ depending on microbial composition, dietary conditions, and mucus barrier integrity.[123,126]

In addition to altered microbial mucus degradation, colorectal cancer is also linked to changes in mucin expression and glycosylation patterns. Physiological mucins are heavily O-glycosylated, which is essential for mucus stability, hydration, viscoelasticity, and resistance to enzymatic degradation. During colorectal carcinogenesis both quantitative and qualitative alterations in mucin glycoconjugates occur, including shortened glycan chains, altered sialylation patterns, and the increased expression of truncated tumor-associated glycans such as Tn-, STn-, and T-antigens. Moreover, aberrant mucin expression patterns are observed during tumor progression. These glycosylation changes may alter microbial adhesion, mucus degradability, immune interactions, and epithelial barrier stability, which may promote chronic inflammation and tumor progression. [124,125,127,128]

3.1.2.4 Sulfur Metabolism

Hydrogen sulfide (H_2S) is increasingly recognized as an important signaling molecule in colorectal cancer. Current evidence suggests that its effects follow a bell-shaped concentration–response relationship. While low physiological concentrations are not considered tumor-promoting, moderately elevated H_2S levels may support tumor-associated signaling and metabolic activity. Excessively high H_2S concentrations become toxic even for cancer cells. Consequently, colorectal tumors appear to benefit from maintaining H_2S within a specific intermediate concentration range.[17,129,130]

Altered sulfur metabolism in colorectal cancer is currently understood as a dysregulation of H_2S homeostasis resulting from both microbial and host-associated mechanisms. Elevated luminal H_2S concentrations are thought to arise at least partly from increased microbial sulfur metabolism within the colon. The observation that sulfur-metabolizing bacteria are associated especially with distal colorectal cancer further supports a potential contribution of bacterial sulfur metabolism to CRC pathogenesis, as the distal colon is a site of microbial fermentation and sulfur metabolism. [17,129,130]

Elevated H_2S concentrations in colorectal cancer do not appear to originate exclusively from bacterial metabolism. CRC cells themselves may additionally increase H_2S production through upregulation of H_2S-producing enzymes. This suggests, that tumor cells actively try to maintain a favorable H_2S environment. Altered sulfur metabolism could be caused by a disturbed balance between microbial H_2S production, endogenous H_2S synthesis, and reduced detoxification pathways. [17,129,130]

3.1.2.5 Altered Bile Acid Metabolism

Colorectal cancer is associated with increased bile acid abundance and alterations in bile acid composition involving both primary and secondary bile acids. Increased fecal bile acid levels have not only been observed in CRC patients but also in individuals at increased risk for CRC, suggesting that alterations in bile acid metabolism may occur before tumor development. In addition to elevated total bile acid levels, increased

concentrations of secondary bile acids, particularly deoxycholic acid (DCA), have frequently been reported.[131,132]

Bile acid profiles additionally differ according to tumor location. 12 out of 14 measured bile acids were significantly increased in right-sided colon carcinoma compared with left-sided tumors. In particular, amino acid-conjugated bile acids, taurine-conjugated secondary bile acids and ursodeoxycholic acid (UDCA), a secondary bile acid, were increased in right-sided tumors.[133]

Several confounding factors should be considered, since increased bile acid levels are frequently accompanied by Western dietary patterns, low fiber intake and decreased butyrate production.[134]

3.1.2.6 Changes in Choline Metabolism

Colorectal cancer correlates with alterations in microbial choline metabolism. A large multicohort metagenomic analysis demonstrated enrichment of genes involved in microbial choline degradation in CRC patients, suggesting an increased microbial capacity for choline conversion pathways. In particular, the abundance of *cutC*, encoding choline trimethylamine-lyase, was consistently increased across all cohorts. Since this enzyme catalyzes the conversion of choline into trimethylamine (TMA), these findings indicate that CRC-associated microbiota may have enhanced TMA/TMAO-producing potential.[135]

Experimental evidence further suggests that increased TMAO levels may contribute to tumor-promoting processes. In animal models, TMAO administration increased tumor and stem-cell proliferation while reducing apoptosis and impairing intestinal barrier integrity. A potential mechanism may be the inhibition of the farnesoid X receptor (FXR), disrupting negative feedback regulation of bile acid synthesis and intestinal homeostasis. Reduced FXR activity may disturb signaling pathways involved in the control of cellular proliferation and differentiation promoting tumor-associated processes.[136]

3.1.2.7 Alterations of Inosine Metabolism in Colorectal Cancer

Recent evidence suggests that inosine metabolism is altered in colorectal cancer. Metabolomic analyses showed that activation of the TGF-β signaling pathway induces metabolic reprogramming of purine metabolism, resulting in increased intracellular inosine levels. TGF-β upregulates the expression of laccase domain-containing 1 (LACC1), a regulator of inosine metabolism. Elevated inosine levels promote epithelial–mesenchymal transition (EMT) and enhance the migratory capacity of colorectal cancer cells, suggesting a potential role in tumor progression and metastatic dissemination.[137]

The biological effects of inosine appear to be highly context-dependent. In contrast to its pro-migratory effects in tumor cells, inosine has been shown to promote anti-tumor immunity. Experimental studies showed that inosine induces polarization of macrophages toward the M1 phenotype and enhances the anti-tumor activity of these cells. In addition, previous studies showed that effector CD8+ T cells can use inosine as an alternative carbon and energy source under glucose-restricted conditions, supporting T-cell proliferation and effector function. These findings indicate that inosine may simultaneously exert tumor-promoting effects within cancer cells while supporting anti-tumor immune responses.[57,138]

It is tempting to speculate that immune cells may have evolved mechanisms to exploit metabolites generated during pathological cell proliferation as both metabolic resources and indicators of tissue stress. Whether inosine fulfills such a function in the tumor microenvironment remains unclear.

3.2 Tumor-Associated Microbiome

The tumor microbiome differs from the surrounding gut microbiome. While the gut microbiome is primarily located within the intestinal lumen, tumor-associated microbes are found within and around tumor tissues, making them an integral component of the tumor microenvironment (TME). This localization enables direct interactions with cancer cells and immune components. [139,140]

Tumors are no longer considered sterile environments but instead host structured microbial communities with tumor-type-specific bacteria. The development of this

microbiome is driven by multiple mechanisms, including invasion through disrupted mucosal barriers, migration from adjacent tissues, and systemic dissemination via blood or lymphatic circulation. [139,140]

In contrast to the gut microbiome, the tumor microbiome is characterized by low microbial biomass and pronounced heterogeneity. It differs from both luminal microbiota and adjacent normal tissue, suggesting selective enrichment within tumors and not a random distribution. [139–143]

Similar microbial strains have been detected in both primary and metastatic lesions, indicating that microbes can persist and migrate alongside tumor cells during disease progression. Overall, the tumor microbiome is a dynamic, tumor-associated ecosystem shaped by local tumor conditions and host–microbe interactions.[139–143]

3.2.1 Association of the Tumor Microbiome with Clinical Outcome

Evidence shows that the tumor microbiome closely correlates with clinical outcomes in colorectal cancer, including overall survival, recurrence, and disease progression. In particular, *Fusobacterium nucleatum* is one of the most consistently reported taxa associated with adverse prognosis. Increased intratumoral levels of *Fusobacterium nucleatum* correlate with shorter overall survival, higher recurrence rates, and reduced recurrence-free survival. *Fusobacterium nucleatum* is enriched in tumors of patients who relapse after chemotherapy, suggesting a role in treatment failure. [143–145]

3.2.2 Mechanistic Role of the Tumor Microbiome in Colorectal Cancer

Tumor-associated microbes can influence colorectal cancer progression through defined molecular interactions with host cells and activation of intracellular signaling pathways. A central example is *Fusobacterium nucleatum*, which has been shown to interact directly with epithelial cells via its adhesin FadA. FadA has been reported to bind to E-cadherin on host cells, a mechanism by which bacterial attachment may influence oncogenic signaling pathways involved in colorectal carcinogenesis. [145]

Fusobacterium nucleatum activates innate immune signaling pathways, particularly through Toll-like receptor 4 (TLR4) and MYD88-dependent signaling, resulting in downstream changes in gene expression. These interactions link bacterial presence to

host cellular signaling networks and may contribute to tumor development and progression.[145]

3.3 Gut Microbiome Signatures associated with Disease Course and Survival

The development of colorectal cancer is strongly linked to lifestyle-related factors such as diet, obesity and medication use. Given that these factors also influence the gut microbiome composition, it is plausible that the microbiome not only reflects cancer risk but may also correlate with disease course after diagnosis.[146,147]

A question is whether microbiome characteristics remain relevant once cancer has already developed. In recent years, several studies have begun to address this by investigating associations between gut microbial composition and clinical outcomes in colorectal cancer patients. Prospective cohort analyses have demonstrated that baseline microbiome features are associated with disease-free survival, indicating that the gut microbiome may carry prognostic information beyond established clinical parameters. In addition, microbiome-based stratification approaches have identified distinct patient subgroups with differing survival outcomes, further suggesting a potential link between microbial community structure and disease course.[146,147]

Most available evidence is derived from observational studies. Although statistical analyses can account for known confounders (e.g., age, sex, comorbidities, and medication use), residual confounding by unknown or unmeasured factors cannot be excluded.[146,147]

3.3.1 Microbiome Features associated with improved clinical Outcomes

Several studies have associated increased microbial diversity with improved prognosis in colorectal cancer patients. A prospective cohort study found that higher alpha diversity was associated with improved disease-free survival, with the strongest association observed in rectal cancer patients.[147]

Several bacterial taxa associated with favorable prognosis belong predominantly to short-chain fatty acid-producing bacteria, particularly butyrate-producing species. An increased

prevalence of *Clostridiales, Coprococcus, Blautia, Roseburia, Faecalibacterium prausnitzii, and Fusicatenibacter saccharivorans* was associated with prolonged survival.[141,146,147]

3.3.2 Microbiome Features associated with negative clinical Outcome

Unfavorable prognosis in colorectal cancer has frequently been associated with enrichment of oral-associated, inflammatory, and mucus-degrading bacterial taxa, including *Fusobacterium, Peptostreptococcus, Clostridium* spp., and *Akkermansia muciniphila*, together with depletion of beneficial short-chain fatty acid-producing bacteria. Several of these taxa are linked to inflammatory signaling, epithelial barrier disruption, and tumor-promoting microenvironments. Tumor-associated *Clostridium* species were associated with poorer prognosis. The same study further linked unfavorable microbial profiles to increased hypoxia and activation of inflammatory pathways including NF-κB, TNF-α, IL-6, and toll-like receptor signaling. [141,147]

3.4 Potential prognostic Microbiome Biomarkers in Colorectal Cancer

Given the considerable interindividual variability of the gut microbiome, the use of individual bacterial species as prognostic biomarkers may be limited. Instead, the characteristic microbiome signature observed in colorectal cancer, including oralization of the gut microbiome, depletion of butyrate-producing bacteria, and functional alterations, may be a more promising biomarker candidate. Such a signature is likely to capture disease-associated changes more comprehensively than individual microbial taxa.[148]

Microbiome-derived metabolites may also have biomarker potential. Their interpretation can be challenging, as measured concentrations are influenced not only by microbial production but also by host uptake and further metabolism. As a result, microbiome signatures may currently be the most promising microbiome-based biomarker approach in colorectal cancer.[149]

3.5 Limitations

Despite the growing evidence linking gut microbiome alterations to clinical outcomes in colorectal cancer, several limitations should be considered. Most available studies are observational and primarily demonstrate associations rather than causal relationships. The gut microbiome is influenced by numerous host- and environment-related factors, including age, diet, obesity, medication use, smoking, physical activity, comorbidities, and other lifestyle variables. [150]

Consequently, it remains difficult to determine whether the reported microbial signatures directly contribute to disease progression or simply reflect differences in overall health status. While a prognostic biomarker does not necessarily need to be causally involved in disease progression, its clinical utility depends on whether it provides prognostic information beyond established clinical risk factors. Future studies should evaluate whether microbiome-derived signatures retain their prognostic value after adjustment for relevant confounding variables and whether they can improve risk stratification compared with existing prognostic models.[150]

4. Gut Microbiome as a Marker of Treatment Response in Colorectal Cancer

The response to cancer therapy varies between patients, even when similar treatment regimens are applied, highlighting a challenge in gastrointestinal oncology. Emerging evidence suggests that, beyond tumor-intrinsic factors, host-associated elements such as the gut microbiome play a crucial role in modulating therapeutic efficacy. The gut microbiota has been increasingly recognized as an active regulator of treatment outcomes, influencing both immune-mediated and cytotoxic therapies. [151]

4.1 Impact of the Gut Microbiome on Chemotherapy Efficacy

Microbiota-mediated effects on chemotherapy efficacy can arise through both direct interactions with anticancer agents and indirect effects on host-related pathways. Direct microbiome–drug interactions may alter the pharmacological properties of therapeutic compounds through mechanisms such as microbial drug activation or inactivation, enzymatic degradation, intracellular drug accumulation, or changes affecting drug availability. In addition, the gut microbiota may influence treatment response through host-mediated mechanisms, including the production of bioactive metabolites, modulation of immune signaling pathways, alterations in host gene expression, and changes within the tumor microenvironment. Together, these interconnected processes contribute to the complex relationship between the gut microbiota and variability in treatment efficacy and toxicity.[50,152,153]

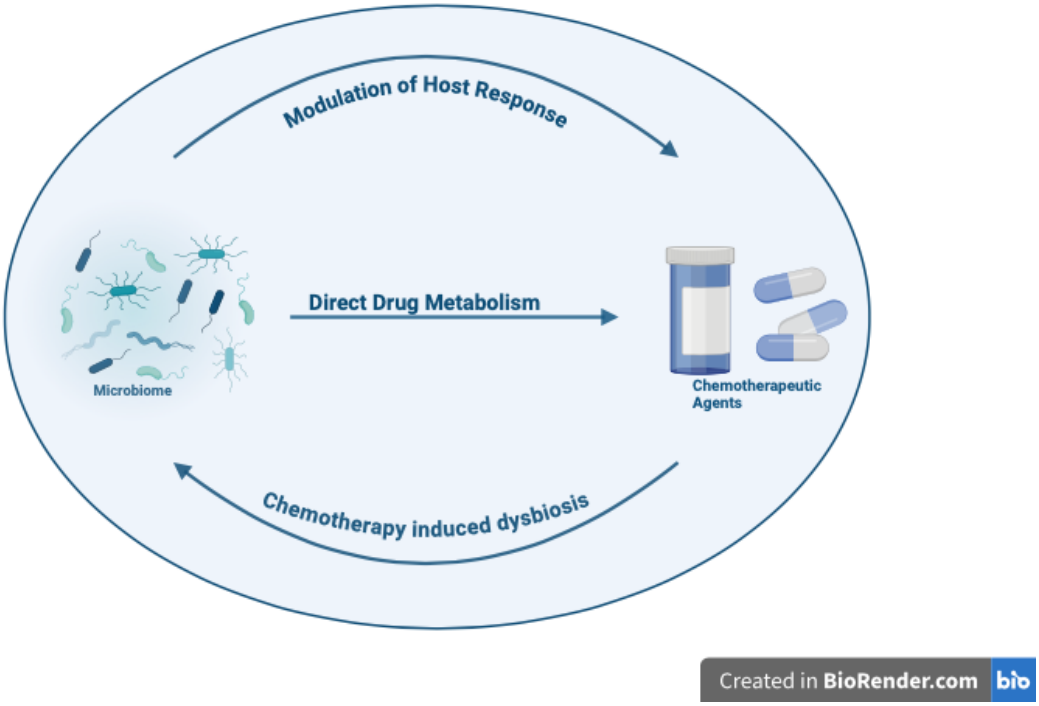

Figure 5: Bidirectional interactions between the gut microbiome and chemotherapy. The microbiome can influence treatment outcomes through direct drug metabolism and modulation of host immune responses, while chemotherapeutic agents can alter the composition and function of the gut microbiome, resulting in chemotherapy-induced dysbiosis.

Since microbiome–therapy interactions may differ between individual therapeutic agents and treatment regimens, the following sections focus on representative examples to illustrate mechanisms underlying microbiome-dependent modulation of treatment responses.

4.1.1 Microbiome-dependent Modulation of Cytotoxic Drugs

The gut microbiome is an additional metabolic system that complements host-mediated drug metabolism. In addition to classical hepatic phase I and phase II reactions, microbial enzymes contribute to the biotransformation of xenobiotics and influence the pharmacokinetics of anticancer drugs. This interplay is described as microbiota–host co-metabolism.[154,155]

Microbial enzymes are widely distributed across the gut microbiome and contribute to xenobiotic metabolism through diverse biochemical reactions. The abundance and distribution of these enzymes vary between individuals, which further contributes to variability in drug metabolism. [154,156]

A central mechanism of microbiome-dependent modulation of cytostatic drugs is the direct enzymatic transformation of chemotherapeutic agents by gut bacteria. These transformations can lead to either inactivation or modification of drugs, altering their bioavailability and therapeutic efficacy. [155,157]

Prominent examples of cytostatic drugs affected by microbiome-dependent metabolism include gemcitabine, 5-fluorouracil, and irinotecan. [155,157]

4.1.1.1 Gemcitabine

Gemcitabine is subject to bacterial inactivation via cytidine deaminase (CDD), an enzyme expressed by intratumoral bacteria, particularly members of the Gammaproteobacteria. CDD catalyzes the deamination of gemcitabine into 2′,2′-difluorodeoxyuridine (dFdU), an inactive metabolite lacking cytotoxic activity. This microbial transformation reduces the availability of active gemcitabine within the tumor microenvironment. In a mouse model, antibiotic treatment restored sensitivity to gemcitabine, providing direct evidence for bacteria-mediated drug inactivation. [157,158]

4.1.1.2 5-Fluorouracil

Gut bacteria metabolize 5-fluorouracil (5-FU) via pathways associated with nucleotide metabolism, converting it into the inactive metabolite dihydrofluorouracil. This transformation is mediated by bacterial taxa including members of the Proteobacteria and Firmicutes and results in reduced drug bioavailability and efficacy. [154,155]

4.1.1.3 Irinotecan

Irinotecan is converted in the liver into its active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), which is detoxified through glucuronidation to form 7-ethyl-10-hydroxycamptothecin glucuronide (SN-38G). In the intestine, bacterial β-glucuronidases cleave the glucuronide group and regenerate SN-38, restoring the active

compound. This microbial reactivation increases local exposure to SN-38 in the gut and is strongly associated with gastrointestinal toxicity. [154,155,159]

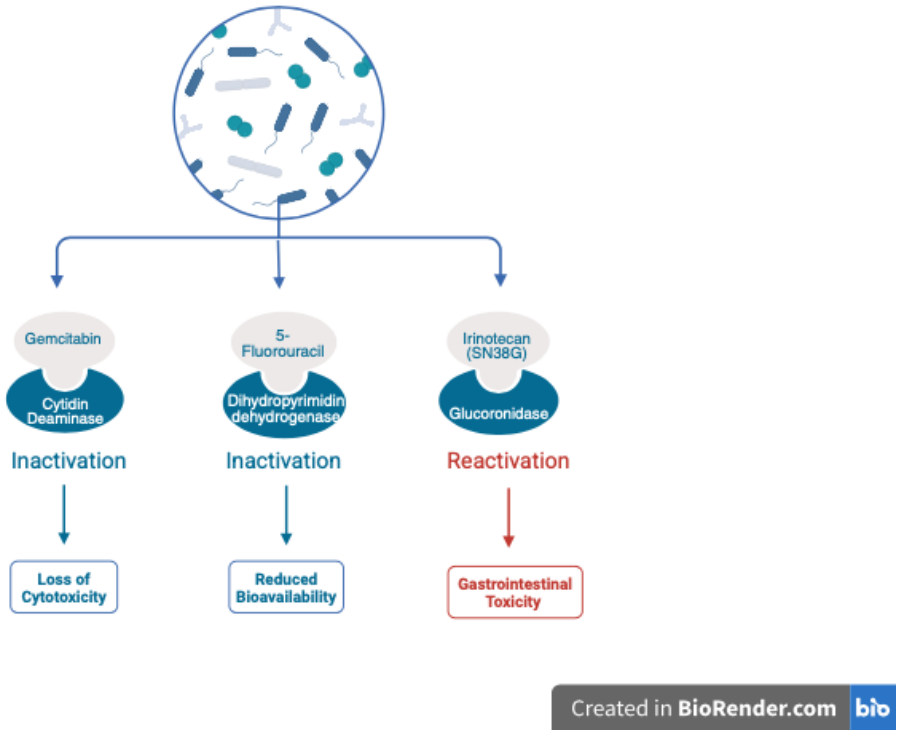

Figure 6: Overview of microbiome-mediated modulation of chemotherapeutic agents. Specific gut microbial enzymes can alter drug activity through metabolic transformation. Bacterial cytidine deaminase inactivates gemcitabine, resulting in reduced cytotoxic efficacy; bacterial dihydropyrimidine dehydrogenase contributes to 5-fluorouracil inactivation and decreased bioavailability; and bacterial β-glucuronidase reactivates the irinotecan metabolite SN-38 from its inactive glucuronidated form (SN-38G), leading to increased gastrointestinal toxicity. These interactions illustrate how the gut microbiome can influence both treatment efficacy and therapy-associated adverse effects.

4.1.2 Microbiome-mediated Modulation of immune Responses to Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs) are an important therapeutic option in metastatic colorectal cancer particularly in patients with mismatch repair deficiency (MMR-D) or a microsatellite instability-high (MSI-H) phenotype. Currently, anti-PD-1 antibodies such as pembrolizumab and nivolumab are approved for patients with metastatic MSI-H/MMR-D CRC. Despite substantial clinical benefit in a subset of patients, treatment responses remain heterogeneous, with reported objective response rates ranging from approximately 30% to 50%. [160]

Increasing evidence suggests that the gut microbiome is an important determinant of immune checkpoint inhibitor (ICI) efficacy. Several studies show that alterations in gut microbial composition correlate with differences in treatment response, suggesting that microbiome dysbiosis may contribute to primary resistance to immunotherapy. [161–163]

Microbiome-derived inosine enhances checkpoint inhibitor efficacy through modulation of T-cell activation. *Bifidobacterium pseudolongum* was identified as one of the bacterial species associated with improved immunotherapy efficacy and showed that inosine acted as the functional metabolite mediating this effect. These experimental findings are consistent with clinical observations showing that *Bifidobacterium pseudolongum* is enriched in responders compared with non-responders.[162]

Roseburia intestinalis, a butyrate-producing bacterium, enhances anti-PD-1 efficacy through activation of cytotoxic CD8+ T cells.[163]

Antibiotic exposure reduces the clinical benefit of immune checkpoint inhibitors, suggesting that disruption of the gut microbiota may impair antitumor immune responses. Fecal microbiota transplantation (FMT) from responding patients into germ-free or antibiotic-treated mice restored the efficacy of PD-1 blockade, supporting a causal role of the microbiome in treatment response. [161]

4.2 Therapy-Driven Microbiome Changes

Gastrointestinal toxicity is one of the most significant limitations of chemotherapy in clinical oncology. Among these adverse effects, gastrointestinal mucositis is particularly relevant, as it leads to epithelial damage, inflammation, and severe impairment of the intestinal barrier. Clinically, mucositis is linked to symptoms such as abdominal pain, diarrhea, and ulceration, and may ultimately compromise treatment continuity and patient outcomes.[164,165]

The pathophysiology of chemotherapy-induced mucositis has traditionally been explained by mechanisms such as oxidative stress, inflammatory signaling, and epithelial cell death. These models primarily focus on host-driven processes and do not fully

account for additional biological systems that may influence disease progression and severity. [164,165]

Recent evidence suggests that the intestinal microbiome is an additional and previously underappreciated factor in the development of chemotherapy-induced mucositis. Chemotherapy not only damages host tissues but also disrupts the ecological balance of the gut microbiota, contributing to intestinal dysfunction and increased susceptibility to infection. [164,165]

In a clinical study chemotherapy was shown to induce pronounced compositional and functional alterations in the gut microbiome of patients with non-Hodgkin lymphoma. These findings show that chemotherapy-driven dysbiosis involves both taxonomic shifts and substantial changes in microbial metabolic capacity.[164]

Specifically, chemotherapy was associated with distinct shifts at the phylum level, characterized by a significant decrease in Firmicutes and Actinobacteria, alongside a marked increase in Proteobacteria following treatment. These changes indicate a reduction in dominant commensal bacterial groups and a concurrent expansion of taxa commonly associated with dysbiosis. [164]

Beyond taxonomic shifts, chemotherapy was associated with substantial alterations in the functional capacity of the gut microbiome. Specifically, a reduced microbial potential for nucleotide metabolism, energy metabolism, and the metabolism of cofactors and vitamins was observed, indicating a diminished metabolic capacity within the intestinal environment. [164]

In contrast, pathways related to glycan metabolism, signal transduction, and xenobiotic biodegradation were increased, suggesting an adaptive microbial response to altered nutrient availability and drug exposure. Given the concurrent reduction in Firmicutes, which include short-chain fatty acid–producing taxa, these changes may also be associated with a decreased production of microbial metabolites such as short-chain fatty acids, potentially affecting epithelial homeostasis and local inflammatory processes. [164]

These findings may not be restricted to the specific chemotherapeutic regimen studied but could extend to other anticancer agents. Similar alterations of the gut microbiome have been observed in preclinical models using different chemotherapeutic drugs, suggesting that chemotherapy-induced dysbiosis may be a broader phenomenon rather than a compound-specific effect.[152,159,165,166]

4.3 Clinical Implications and future Perspectives

As in many other areas of oncology, colorectal cancer management is increasingly moving toward precision medicine. While current treatment decisions already incorporate molecular characteristics such as KRAS, BRAF, and MSI status, future approaches are likely to integrate a broader range of patient-specific information. In particular, comprehensive molecular profiling of tumors together with characterization of the gut and tumor-associated microbiome may improve prognostic assessment and enable more individualized therapeutic strategies.[167]

The rapid development of multi-omics technologies and artificial intelligence-based machine learning approaches may further enhance risk stratification by integrating genomic, transcriptomic, metabolomic, and microbial data into predictive models. Such approaches could help identify patients with distinct prognostic profiles or differential responses to specific therapies more accurately than current biomarkers alone.[168]

Beyond its role as a biomarker, the gut microbiome may also emerge as a therapeutic target. Future interventions may aim to actively modulate the microbiome in order to improve treatment efficacy and reduce therapy-related toxicity. Potential strategies include engineered probiotics, targeted prebiotics, bacteriophage-based therapies, and fecal microbiota transplantation. Although many of these approaches remain experimental, they highlight the potential for microbiome-informed precision oncology, in which both tumor biology and host–microbiome interactions contribute to personalized treatment decisions.[167,169]

Conclusion

The association of microbiome signatures with tumor stage, tumor location, and clinical outcome suggests that the gut microbiome may have potential as a prognostic biomarker in colorectal cancer. This is supported by consistent compositional and functional alterations observed in colorectal cancer, including oralization of the gut microbiome and depletion of butyrate-producing bacteria, for example *Fusobacterium nucleatum, Parvimonas micra, Peptostreptococcus stomatis, Faecalibacterium prausnitzii,* and *Roseburia spp.*.

Whether these microbiome signatures are an independent prognostic factor remains unclear. It is currently unknown to what extent the observed associations reflect information already captured by established prognostic factors, such as tumor stage, molecular characteristics, and the overall health status of the patient. Further large prospective cohort studies are needed to determine the independent clinical value of microbiome-based biomarkers and their potential role in routine clinical practice.

Declaration of Authorship

I declare that this master's thesis is my own original work and that I have only used the sources cited in the thesis. All figures were created by me using BioRender. Artificial Intelligence was only used for translation purposes.

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