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REVIEW

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Implications of gut microbiota-mediated epigenetic modifications in intestinal diseases

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ABSTRACT

Intestinal diseases are highly prevalent, affecting millions worldwide and significantly contributing to global morbidity. The treatment of complex disorders, such as inflammatory bowel disease (IBD) and colorectal cancer (CRC), remains challenging due to multifactorial etiologies, diverse patient responses, and the limitations of current therapeutic strategies. Although the gut microbiota clearly plays a role in regulating the onset of intestinal diseases, few studies have explored the epigenetic factors by which the microbiota contributes to disease development. Here, the latest insights into the molecular mechanisms underlying the bidirectional influence between gut microbiota and epigenetic modifications are discussed, including DNA methylation, histone modifications, non-coding RNAs, and *N*6-methyladenosine (m^6A). Importantly, mechanistic studies based on animal models or human cells have demonstrated that the gut microbiota, and other environmental factors, influence targeted gene expression and activate immune pathways through host epigenetic dysregulation, which are closely associated with the development of IBD and CRC. Furthermore, potential microbiome interventions, including probiotics, prebiotics and postbiotics, fecal microbiota transplantation (FMT), dietary modifications, and phage therapy, have been proposed as innovative therapeutic strategies to correct these abnormal epigenetic patterns associated with the diseases. Overall, addressing microbiome dysbiosis and its epigenetic consequences presents a promising frontier in the treatment of intestinal diseases, offering the potential to not only restore microbial balance but also provide more targeted and personalized therapeutic strategies for better patient outcomes.

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Introduction

The mammalian intestinal tract hosts a myriad of diverse microorganisms, encompassing bacteria, archaea, fungi, and protozoa, collectively known as the gut microbiota. Often hailed as our secondary genome, the gut microbiome (Box 1) harbors an estimated gene content surpassing that of the human genome by a factor of 100. Consequently, it has earned the epithets of the 'forgotten organ' and a 'superorganism.[2–5] The development and function of the immune system in germ-free (GF) mice are deficient, highlighting the importance of the **commensal** (Box 1) relationship between the host and gut bacteria in forming and maintaining a healthy gut immune system.[12] In a healthy state, the microbiota supports crucial functions such as aiding in the breakdown of complex carbohydrates like fiber, producing anti-inflammatory short-chain fatty acids (SCFAs) for energy.[13] The gut microbiota not only aids in digesting nutrients but also shaping host immunity by protecting against pathogen invasion and the overgrowth of harmful organisms.[14] In turn, skewing symbiotic communities, or 'dysbiosis,' is closely related to inflammatory diseases. Indeed, a hallmark of dysbiosis is an imbalance in the proliferation of certain strains, characterized by a decline in beneficial probiotics and an increase in harmful pathogenic bacteria. For example, patients with inflammatory bowel disease (**IBD**) (Box 1) exhibit an overall

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reduction in microbial diversity, along with specific declines in certain taxa, including Firmicutes and Bacteroidetes, as well as *Lactobacillus* and *Eubacterium*.[15] Microbiota dysbiosis is increasingly recognized as a significant factor in various disease states. Disruption of the gut microbiota compromises the intestinal barrier, leading to increased intestinal permeability ('leaky gut'), facilitating the development and progression of IBD and/or colorectal cancer (**CRC**) (Box 1).[16,17] Changes in gut microbiota composition can diminish the production of beneficial metabolites like SCFAs which are crucial for gut health and immune regulation.[18] Thus, keeping a balanced microbial ecosystem may offer new possibilities for diagnosis and treatment of various diseases, especially complex gastrointestinal disorders like IBD and CRC.

Epigenetics, denoting heritable changes in gene expression, empowers mammalian cells to orchestrate their transcriptional program sans altering the genetic code. Epigenetic modifications primarily encompass DNA modifications, non-coding RNAs (ncRNAs) and histone modifications, playing pivotal roles in elucidating the interplay between disease and environment when deciphering the genetic underpinnings of ailments.[19,20] Notably, distinct epigenetic markers can serve as indicators of exposure to the gut microbiota, aiding in the identification of individuals predisposed to complex diseases when coupled with genetic data.[21,22] **Host epigenome** (Box 1), particularly DNA methylation, serves as a crucial biomarker for the prospective diagnosis and screening of intestinal diseases, significantly aiding in the advancement of early clinical transformation.[23] The *Sept09* blood-based DNA methylation biomarker has been employed in the diagnosis and screening of CRC.[24] Differentially methylated regions, including *Vmp1*, *Itgb2*, and *Txk*, have been identified between IBD cases and controls.[25] These epigenetic markers consequently emerge as potential therapeutic targets.

Gut microbiota can significantly influence host health by modulating epigenetic mechanisms such as DNA methylation and histone modification. These epigenetic changes can alter gene expression, impacting pathways related to inflammation and metabolism. As a result, the interplay between gut microbiota and epigenetics is increasingly recognized as a crucial factor in the development of various diseases. However, little is known about the extent to which microbiome-induced epigenetic dysregulation contributes to the progression of IBD and CRC, as well as the specific mechanisms involved, and there is a lack of systematic integration on this topic. This review delves into the

Box 1. Glossary

Commensal
Derived from the Latin word *mensa*, meaning 'together at the table', this term typically denotes microorganisms that exist in symbiotic or mutually advantageous relationships with their mammalian hosts.[1]

Gut microbiome
The gut microbiome represents the entirety of genetic sequences, including homologous sequences, harbored by the microbial communities within host intestinal environment at a specific time and under specific conditions.[2–5]

Host epigenome
Host epigenome refers to the phenomenon where the genetic sequence remains unchanged, yet the patterns of gene expression are altered, leading to phenotypic changes driven by environmental factors or the gene's chromosomal positioning. In simpler terms, it can be understood as the environment dictating the expression of genes. Epigenome includes chromatin remodeling, DNA methylation, histone modification, RNA methylation, and the regulation of non-coding RNAs (ncRNAs).

DNA methylation
DNA methylation is an epigenetic modification where DNA methyltransferases (DNMTs) catalyze the transfer of a methyl group from S-adenosylmethionine (SAM), acting as a methyl donor, to the cytosine of a CpG dinucleotide within the DNA sequence.[6]

Histone modification
Histone modification is a crucial epigenetic regulatory mechanism that involves the addition or removal of various chemical groups on specific amino acid residues of histones, such as histone methylation, histone acetylation, histone lactylation, histone crotonylation. These modifications can alter the structure and function of chromatin, thereby influencing gene expression.

m^6A
The N6-methylation of adenosine, known as m^6A modification, has been recognized as the most prevalent and abundant mRNA modification in eukaryotes, with an occurrence rate of 0.1% to 0.6% (m^6A/A). m^6A regulates a variety of cellular processes by influencing the transcription, maturation, localization, function, and metabolism of different RNA classes.[7]

miRNAs
MicroRNAs (miRNAs, usually 19–24 nucleotides in length) are non-coding RNAs that mediate post-transcriptional gene silencing by binding to and guiding target transcripts into RNA-induced silencing complexes.[8]

LncRNAs
LncRNAs are defined as transcripts longer than 500 nucleotides that lack apparent protein-coding potential, though some lncRNAs have been shown to encode micropeptides.[9]

IBD
Inflammatory bowel disease (IBD) is an idiopathic inflammatory disorder primarily involving the ileum, rectum, and colon. It clinically presents with symptoms such as diarrhea, abdominal pain. This disease category includes ulcerative colitis (UC) and Crohn's disease (CD).[10]

CRC
Colorectal cancer (CRC) ranks as the second leading cause of cancer-related mortality globally, with a rising incidence among individuals under the age of 50. CRC is a progressive malignancy driven by the accumulation of genetic and epigenetic changes within colonic epithelial cells, leading to neoplasia and subsequent malignant transformation.[11] As discussed in this article, the onset of CRC is partially attributable to epigenetic modifications induced by dysbiosis of the gut microbiota.

interactions between gut microbiota and epigenetic modifications implicated in intestinal diseases (focus on IBD and CRC) risk, including DNA/histone modification, non-coding RNAs, and *N*6-methyladenosine (m^6A). We systematically summarized the epigenetic modifications induced by changes in gut microbiota and environmental factors (such as air pollution, smoking, alcohol consumption, and diet) that promote key IBD- and CRC-related genes and pathways in both human and animal models. Finally, we discuss contemporary therapeutic approaches targeting epigenetic alterations through modulating gut microbiota, like probiotics, prebiotics, postbiotics, dietary interventions, fecal microbiota transplantation (FMT) and phage therapy. These insights augment our comprehension of the pathophysiology of IBD and CRC, along with avenues for disease prevention and management.

How are gut microbiota and epigenetic modifications connected?

Even though monozygotic twin mice share the same genotype, differences in the microbiota or epigenome can create differences in susceptibilities to diseases, demonstrating the influences of potential environmental factors on bacterial composition and disease susceptibility.[26,27] The co-existence of commensal microbes in high abundance and proximity to intestinal epithelial cells (IECs) underscores the significant role played by microbiota as a major source of environmental stimuli capable of shaping host cellular function.[28] Indeed, epigenetic modifications mediated by microbiome represents a key entrance that allows the microbiome to reprogram the genome in response to environmental stimuli, resulting in triggering multiple cellular functions (Figure 1).

DNA methylation, histone methylation/acetylation

DNA methylation serves as a biochemical signaling mechanism that orchestrates cellular functions by altering the expression of gene through its ability to modulate DNA conformation/stability and chromatin structure.[29] Histones are spools around which DNA wraps, regulating gene expression.[30,31] A plethora of post-translational modifications can impact histones, with acetylation and methylation being the most extensively investigated. These diverse modifications typically entail the covalent attachment to lysine (K) residues on histone tails, thereby shaping chromatin structure and governing gene expression. In mammals, the dynamic and reversible nature of epigenetic modification is governed by specialized modifying enzymes, including DNA and histone methyltransferases (DNMTs and HMTs) as 'writers', histone acetyltransferases (HATs), alongside 'erasers' such as histone demethylases (HDMs) and deacetylases (HDACs).[32]

The gut microbiota influences host physiology by modulating DNA methylation. For instance, exposure of immature enterocytes to *Lactobacillus acidophilus* and *Bifidobacterium* leads to the identification of over 200 regions exhibiting differential DNA modification.[33] In another study, conventionally raised (CNV) mice display significantly diminished global methylation levels within their IECs compared to GF mice.[34] Additionally, exposure to commensal microbiota induces DNA hypomethylation in ten-eleven translocation 2/3 (TET2/3), pivotal epigenetic regulatory elements, thereby shaping gene expression patterns.[34]

Gut microbiota regulates host DNA methylation primarily through two mechanisms. First, microbial metabolites such as SCFAs (e.g., butyrate and propionate),[35] folate, and S-adenosylmethionine (SAM) can serve as direct methyl donors or modulate the activity of methylation-related enzymes (Figure 1a). For example, folate produced by *Bifidobacterium* and *Lactobacillus* synthesizes SAM through one-carbon metabolism, promoting DNA methylation.[36] Methyl donors such as methionine (MET), SAM, and folate are integral components of one-carbon metabolism. Key enzymes or coenzymes, including vitamins B_2, B_6, and B_{12}, are essential for facilitating methyl group transfer in one-carbon metabolism.[37] Secondly, microbiota or its metabolites directly regulate DNMTs and TETs. For example, studies show that the gut microbiota regulates TET1 expression to influence DNA hydroxymethylation, modulating the epigenetic program of innate lymphoid cell differentiation, thereby affecting ILC1 expansion and intestinal homeostasis.[38]

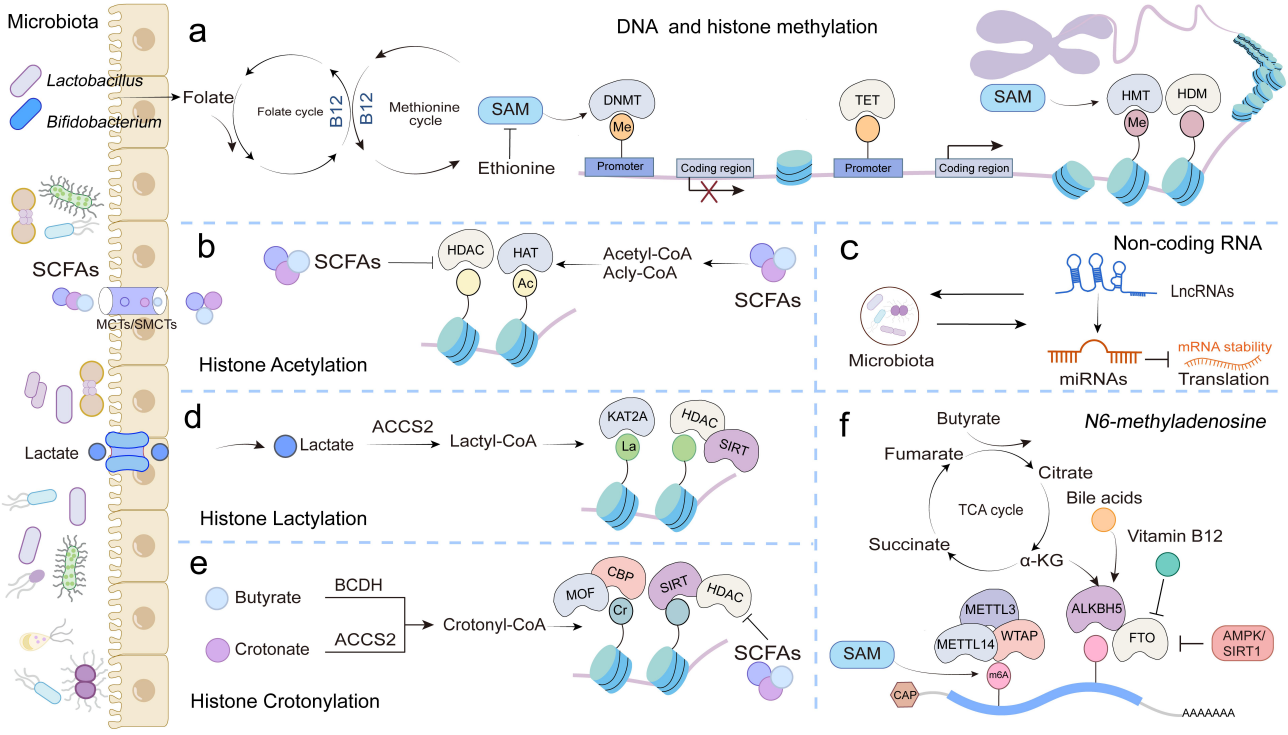

Figure 1. The gut microbiota interacts with the mammalian epigenome by generating epigenetic substrates or acting as regulators for chromatin-modifying enzymes. a, DNA methylation and histone methylation. DNA methylation is generally linked with repression of transcription initiation at promoters. Folate produced from *Lactobacillus*, *bifidobacterium* can synthesize methyl group donor S-adenosylmethionine (SAM) by the folate and methionine cycles to DNA/histone (a) methylation and N^6-methyladenosine (m^6A) (f). b, histone acetylation. Extracellular short-chain fatty acids (SCFAs) enter colon cells via monocarboxylate transporters (MCTs) and sodium-dependent monocarboxylate transporters (SMCTs). SCFAs can influence acetylation, deacetylation and crotonylation (f) of histone via inhibiting the activity of histone deacetylases (HDACs). Acetyl-CoA and acyl-CoA derived from SCFAs metabolism can be utilized for histone acetylation. c, non-coding RNAs. Gut microbiota can influence the expression of non-coding RNAs, in turn, alterations of non-coding RNAs can also impact the abundance of gut microbiota. d, histone lactylation. Lactate is transported across the plasma membrane by monocarboxylate transporters (MCTs). Acetyl-CoA synthetase 2 (ACCS2) and lysine acetyltransferase 2A (KAT2A) function as lactyl-CoA synthetase and lactyltransferase, whereas HDACs or SIRT function as erasers. e, histone crotonylation. Butyrate and crotonate are metabolized into crotonyl-CoA by butyryl-CoA dehydrogenase (BCDH) and ACCS2, respectively, which promotes lysine crotonylation (kcr) of histone. f, m^6A. Butyrate enters the tricarboxylic acid (TCA) cycle to produce the intermediate α-KG, which contributes to RNA demethylation. Bile acids and vitamin B_{12} can influence the expression of the *alkb* dioxygenase family. Additionally, gut microbiota can regulate FTO expression through the AMPK/SIRT1 signaling pathway. AMP-activated protein kinase, AMPK; CBP, CREB-binding protein; DNMT, DNA methyltransferase; FTO, obesity-associated protein; HAT, histone acetyltransferase; HDM, histone demethylases; HDAC, histone deacetylase; HMT, histone methyltransferases; METTL3/14, methyltransferase like 3/14; SAM, S-adenosylmethionine; SIRT, sirtuin; TET, ten-eleven translocation; WTAP, wilms tumor 1 associated protein.

The gut microbiota also modulates post-translational modifications of histone proteins. Distinct alterations in histone modifications, particularly histone H3 at lysine 4 trimethylation (H3K4me3) and H3K27me3, are observed in GF and antibiotic-treated (ABX) mice.[39] Moreover, histone H4 acetylation is diminished in the epithelial cells of GF mice.[40] SCFAs serve as pivotal substrates and cofactors for epigenetic enzyme activity (Figure 1b). Butyrate, derived from Clostridia bacteria, notably functions as a potent endogenous histone deacetylase inhibition (HDACi). For example, microbiota-derived butyrate inhibits tuft cell expansion by regulating histone deacetylase 3 (HDAC3), which is essential for tuft cell hyperplasia, type 2 immunity, and intestinal differentiation.[41] Similarly, butyrate ameliorates colitis and arthritis by promoting B10 cell differentiation through HDAC inhibition and p38 MAPK activation, independently of the G-protein-coupled receptor pathway.[42] On the other hand, butyrate can be metabolized intracellularly to generate acetyl-CoA, which serves as an essential substrate for HATs.[43] Early cell culture studies suggest that butyrate may serve as a carbon donor for histone acetylation, as evidenced by ^{14}C incorporation into histones and

detection of ^{13}C-labeled acetate from histone hydrolysis.[44] To support the metabolic role of microbiota in histone acetylation, isotope tracing experiments have demonstrated that histone acetyl groups incorporate carbon derived from butyrate and dietary fiber.[40] These findings highlight a direct link between gut microbial metabolites, cellular metabolism, and epigenetic regulation, wherein different acylation modifications are driven by the cellular concentrations of their respective metabolic substrates.

Non-covalent modifications

Non-covalent modifications, particularly those mediated by non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are key players in initiating and perpetuating epigenetic alterations.[45] These ncRNAs serve as crucial regulators of the epigenetic landscape and wield functional significance in modulating gene expression in various diseases.

miRNAs

MiRNAs have garnered increasing attention for their interactions with the host and its microbiota (Figure 1c). Colonization of GF mice with microbiota from pathogen-free counterparts reveals dysregulated miRNA expression profiles in the ileum and colon.[46] Similarly, FMT-induced shifts in the microbiota could alter fecal miRNA profiles, implicating the gut microbiota in the regulation of intestinal miRNA expression.[47] Furthermore, the probiotic *Lactobacillus paracasei* LC01 (*L.casei* LC01) has been shown to inhibit intestinal permeability of IECs by downregulating miR-144 expression, thereby improving intestinal homeostasis.[48] Although the precise mechanisms by which the microbiota induce miRNA expression alterations remain unclear, they are believed to be primarily influenced by microbiota-derived metabolites, such as SCFAs.[49] Studies have demonstrated that increasing concentrations of butyrate and acetate upregulate DNA methylation levels at miR-378a promoter, consequently affecting miR-378a expression.[50] However, further investigation is warranted to elucidate the specific steps in miRNA production regulated by the gut microbiota or the corresponding metabolites, such as pre-miRNA splicing, transport, and/or RNA polymerase activity.

Interestingly, a bidirectional crosstalk exists between gut microbiota and miRNAs, whereby miRNAs can also influence bacterial abundance by entering bacteria and regulating bacterial gene transcripts. For instance, miR-1226-5p and miR-515-5p can penetrate *Fusobacterium nucleatum* (*F. nucleatum*) and *Escherichia coli* (*E. coli*), modulating their growth through regulation of bacterial gene expression.[51] Similarly, gga-miR-222a has been shown to penetrate *Bacteroides fragilis* NCTC 9343, leading to an increase in its abundance during the logarithmic growth phase.[52] And, the downregulated miR-30a-5p specifically inhibited the growth of *Lactobacillus reuteri* in both mouse and human feces after treatment with raffinose.[53] While these findings suggest that miRNAs can influence bacterial abundance, further extensive studies are required to delineate the specific mechanisms whereby individual miRNAs affect different gut microbiota.

LncRNAs

Similarly, transcript alterations of lncRNAs also link to the gut microbiota (Figure 1c). Compared to GF mice, the lncRNA expression profile in mice colonized with specific gut microbiota exhibited significant alterations, with most of the differentially expressed lncRNAs being strain-specific.[54] One of the genes, lncRNA Snhg9, is highly expressed in GF mice. Microbiota regulates host lipid metabolism by repressing lncRNA Snhg9 in intestinal epithelial cells, thereby modulating the PPARγ/SIRT1/CCAR2 axis and influencing lipid absorption and obesity.[55] Interestingly, in the context of mild colonic inflammation, the microbiota effectively alleviates the progression of CRC in mice by suppressing the lncRNA *Snhg9*/SIRT1/CCAR2/p53 axis.[56] These findings suggest that gut microbiota could shape the expression of lncRNAs, thus influencing host metabolism. Moreover, the expression of the oncogenic lncRNA LINC00152 is significantly increased in HCT116 cells following infection by *Salmonella typhimurium* SL1344 or exposure to lipopolysaccharides (LPS) derived from other enteric pathogenic bacteria. In turn, elevated LINC00152

expression could increase the abundance of LPS in tumor tissue.[57] Similarly, *F. nucleatum* induces inflammation by targeting the lncRNA EN01-IT1 /miR-22-3p axis in neonatal necrotizing enterocolitis (NEC).[58] During tuberculosis infection, *Bacteroides fragilis* (*B. fragilis*) directly regulates lncRNA-CGB, while the interaction between lncRNA-CGB and EZH2 negatively regulate H3K27Me3 epigenetic programming, ultimately boosting IFN-γ expression.[59] Collectively, through lncRNAs, gut microbiota may affect a larger number of genes than expected.

Histone lactylation

Recently, histone lactylation has emerged as a novel histone modification, highly sensitive to lactate production through glycolysis.[60] Intracellular and extracellular lactate have been found to modulate the levels of histone lysine lactylation (Kla).[60] Lactate is conveyed into the blood via multiple monocarboxylate transporters (MCTs) or the G protein-coupled receptor *GPR81*. In cell-free systems, histone lactylation has been shown to depend on the activity of lysine acetyltransferase (KAT) enzyme P300 and lactyl-CoA, with lactyl-CoA serving as the lactyl group donor for lysine lactylation.[60] A recent study reported that acetyl-CoA synthetase 2 (ACSS2) is identified as a lactyl-CoA synthetase that, upon epidermal growth factor receptor (EGFR) activation, undergoes ERK-mediated phosphorylation, translocates to the nucleus, and forms a complex with KAT2A. This coupling enables histone lactylation, promoting tumor-associated gene expression, immune evasion, and brain tumor growth.[61]

There is relatively less evidence linking gut microbiota to histone lactylation. However, based on current clues, we deduce a close regulatory relationship between them (Figure 1d). Lactylation levels are influenced by lactate concentration. Gut microbiota dysbiosis may activate the warburg effect (glycolysis) in host cells, enhancing lactate dehydrogenase (LDHA) activity and leading to lactate accumulation. This high-lactate environment promotes histone lactylation, thereby regulating the polarization state of macrophage, such as the transition from pro-inflammatory M1 to anti-inflammatory M2 phenotype.[60] A recent study reported that tumor-resident *E. coli* promotes colorectal cancer liver metastasis by enhancing lactate production, which induces M2 macrophage polarization through RIG-I lactylation, suppressing NF-κB signaling and *Nlrp3* transcription, thereby modulating regulatory T cell (Treg) and $CD8^+$ T cell functions.[62] In turn, compelling evidence has revealed that lactate can stimulate histone H3K9ac and histone H3K18lac while concurrently eliciting modifications in the abundance of gut microbiotas and metabolic products in mice colitis.[63] Therefore, although evidence supports the hypothesis that gut microbiota regulates histone Kla, more data are required to better understand the complexity and relationships between gut microbiota and histone Kla, including specific mechanisms and potential impacts on disease development.

Histone crotonylation

Histone lysine crotonylation (Kcr) refers to the transfer of crotonyl groups onto lysine residues of histones.[64] Typically, Kcr regulates gene transcription by marking and influencing the activity of promoters and enhancers.[65] Known histone crotonyltransferases (HCT), also referred to as crotonylases, include CREB-binding protein (CBP)/P300 and MOF, whilst histone decrotonylases (HDCR) identified so far include HDAC and sirtuin 1–3 (SIRT1–3).[66,67]

Consistently, the alteration of Kcr is also regulated by the gut microbiota (Figure 1e). The depletion of the gut microbiota leads to global changes in colonic histone crotonylation. Meanwhile, studies have identified class I histone deacetylases HDAC1, HDAC2, and HDAC3 as the primary executors of histone decrotonylation. Notably, known HDAC inhibitors, including gut microbiota-derived butyrate, influence histone decrotonylation.[68] SCFAs are not only HDAC inhibitors but also serve as metabolic precursors of crotonyl-CoA, directly participating in histone crotonylation, such as crotonate.[69] Crotonate regulates the levels of Kcr by influencing intracellular crotonyl-CoA, with the transition of crotonate into crotonyl-CoA being mediated by ACSS2.[70] Moreover, butyrate is converted to glutaryl-CoA and subsequently to crotonyl-CoA via the β-oxidation pathway, catalyzed by butyryl-CoA dehydrogenase (BCDH).[71] In summary, the gut microbiota dynamically regulates the process of histone crotonylation by inhibiting

HDACs or providing crotonyl-CoA precursors through SCFAs. Future studies could further explore the targeted intervention potential of specific microbiota or their metabolites in crotonylation modification.

m^6A

Studies have shown that changes in the gut microbiota are associated with cecal m^6A modification between GF and normal mice. Further studies revealed that the absence of microbiota downregulates the methyltransferase *Mettl16*, leading to reduced m^6A methylation of its target mRNA, encoding S-adenosylmethionine synthase *Mat2a*, while specific bacterial species, such as *Akkermansia muciniphila* and *Lactobacillus plantarum*, regulate distinct m^6A modifications.[72] Furthermore, compared to specific pathogen-free (SPF) mice, GF mice exhibit significant differences in m^6A modification patterns in brain tissue, along with differential expression of methyltransferase-like 3 (METTL3) and METTL14 and demethylases (AlkB homolog 5, ALKBH5 and Fat mass and obesity-associated protein, FTO).[73] Additionally, the level of m^6A modification is also regulated by specific microorganisms. The global m^6A level in THP-1 cells significantly increased after heat-killed *Salmonella typhimurium* infection, which depends on Wilms Tumor 1 Associated Protein (WTAP) expression.[74] In MAC-T cells treated with *E. coli*, significant differences are observed in the total number of m^6A modification peaks compared to the control group.[75] *Lactobacilli* and *Bifidobacterium* species have also been shown to elevate m^6A levels in the total RNA of gut tissues, ensuring normal intestinal development.[76] In turn, when the activity of m^6A-modifying enzymes changes, the composition of the gut microbiota also changes accordingly. METTL14-deficient mice exhibit changes in the taxonomic composition of gut microbiota, including *Enterobacteriaceae*, *Lachnospiraceae*, *Helicobacteraceae*, and *bacteroidaceae* compared with control group.[77] Moreover, FTO-deficient mice harbor a specific bacterial signature associated with the suppression of inflammation, characterized by signification alteration in adundance of *Helicobacter*, *Lactobacillus*, and *Porphyromonadaceae*.[78]

Similar to DNA methylation, the influence of gut microbiota metabolites on methyl donor levels is one of the key factors driving m^6A modification changes (Figure 1f). For example, vitamin B_{12} functions as a cofactor for enzymes that perform methyl transfer reactions, which are essential for the deposition of methyl groups on adenosine in mRNA.[79,80] Studies by Mosca and his colleagues have shown that in neuronal cells with vitamin B_{12} deficiency, the expression of FTO is significantly up-regulated, concomitant with global m^6A hypomethylation of mRNAs.[81] Deoxycholic acid (DCA) regulates the expression of miR-92b-3p through an m^6A-dependent mechanism, thereby inactivating the PI3K/AKT pathway and inhibiting the progression of gallbladder cancer (GBC).[82] Butyrate supplementation in polycystic ovary syndrome (PCOS) mice improves ovarian function by downregulating METTL3 expression, thereby reducing *Fosl2* m^6A methylation levels and lowering the expression of inflammatory cytokines IL-6 and TNF-α.[83] Notably, gut microbial metabolites have been reported to regulate m^6A modification by controlling α-ketoglutarate (αKG) levels.[84] It is known that ferrous iron and αKG are co-substrates of FTO and ALKBH5, which oxidize the N-methyl group of m^6A. When butyrate enters tricarboxylic acid (TCA) cycle, the αKG concentration increases, effectively decreasing the level of m^6A. A recent study found that the activation of the *Alkb* dioxygenase family depends on the ability of the primary substrate to induce a metal-centered conformational transition in αKG, which exposes the Fe(II) atom to incoming oxygen, thereby initiating the secondary reaction.[85] Finally, the activation of gut microbiota-induced signaling pathways may also be a potential factor influencing m^6A level changes. *Lactobacillus sakei* can activate the AMPK/SIRT1 pathway to promote FTO degradation by enhancing FTO sumoylation mediated by RANBP2, thereby influencing m^6A levels.[86] Therefore, these findings suggest that the gut microbiota can regulate the host m6A modification profile by modulating methyl donor availability, αKG levels, or signaling pathways through its metabolites.

Environmental and lifestyle factors in microbiota-epigenetic interactions

Understanding how external factors shape gut microbiota composition and epigenetic modifications is

crucial for unraveling their roles in health and disease. Environmental influences such as pollution, smoking, alcohol drinking, and diet use have been shown to disrupt microbial homeostasis, leading to alterations in host epigenetic landscapes. These changes may contribute to immune dysregulation, chronic inflammation, and disease susceptibility. A more cohesive discussion on these interactions will provide deeper insights into the mechanisms through which environmental exposures impact gut microbiota and epigenetic regulation, ultimately influencing disease progression and therapeutic outcomes.

Air pollution and gut microbiota dysbiosis

Air pollution is one of the most significant environmental threats to global public health, causing approximately 4.2 million premature deaths annually due to outdoor air pollution.[87] Since infancy, air pollution has exerted a significant impact on the composition and diversity of the gut microbiota. At the age of 6 months, particulate matter 10 (PM_{10}) exposure showed positive associations with the relative abundance of *Dialister*, *Dorea*, *Acinetobacter*, and *Campylobacter*, whereas $PM_{2.5}$ exposure was positively associated with Actinobacteria.[88] Distinct environmental pollutants may exert selective effects by promoting or suppressing the growth of particular bacterial genera. High levels of ozone (O_3) exposure have been associated with reduced gut bacterial diversity and an increased abundance of *Bacteroides caecimuris*.[89] In murine models exposed to $PM_{2.5}$, an increased abundance of pro-inflammatory bacteria such as *Fusobacterium nucleatum* and a concomitant reduction in butyrate-producing species like *Faecalibacterium prausnitzii* have been observed, which may contribute to impaired intestinal barrier integrity.[90] Furthermore, exposure to environmental toxicants, including bisphenols, phthalates, and per- and polyfluoroalkyl substances (PFAS), has been implicated in the development of obesity and insulin resistance, potentially mediated by gut microbiota dysbiosis and changes in circulating secondary bile acid profiles. Elevated exposure to chemical pollutants has been linked to an increased relative abundance of specific bacterial genera, such as *Anaerostipes*, *Alistipes*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, among others.[91]

The strong association between environmental pollutant exposure and changes in the gut microbiota suggests a potential mechanism through which environmental factors, such as air pollution, may contribute to disorders, such as IBD. Prolonged exposure to high levels of nitrogen dioxide and $PM_{2.5}$ is associated with a higher risk of early-onset Crohn's disease (CD).[92] Individuals residing in areas with higher concentrations of SO_2 also increases the risk of developing UC compared to those living in regions with lower levels of the pollutant.[92] The impact of air pollution on IBD may be mediated by microbiota and specific epigenetic modifications. An epigenetic Mendelian randomization (MR) analysis has identified five and twenty-two methylated CpG sites associated with exposure to $PM_{2.5}$ and NO_2, respectively, with particularly notable methylation alterations in the CXCR2 and MHC class III regions, which exhibit a significant correlation with Ulcerative colitis (UC).[93] In mouse models, prolonged exposure to elevated levels of PM results in increased expression of pro-inflammatory cytokines and significant alterations in the composition and function of the colonic microbiota, which are characterized by the overgrowth of Firmicutes and *Verrucomicrobia*, alongside a reduction in Bacteroidetes and SCFA-producing bacteria.[94] Crucially, exposure of the gut to PM leads to a reduction in butyrate levels, which are essential for colonic cells and mucosal immune cells. Depletion of butyrate usually triggers weakened barrier function and higher susceptibility to mucosal inflammation.[94] Moreover, special emphasis should be placed on emerging trace pollutants, including metals, microplastics, and disinfectants, which are being detected in soil, water, and air in ever-increasing quantities. For example, a recent prospective cohort study by Zhou et al., involving over 22,000 participants, has revealed that exposure to drinking water contaminated with metals (such as mercury and manganese), disinfectants, or fertilizers significantly elevates the risk of developing IBD. Long-term consumption of water contaminated with excessive microplastics can damage the intestinal barrier, disrupt microbial balance, and induce metabolic alterations, leading to intestinal inflammation.[95]

Meanwhile, a recent study demonstrates that residents living in close proximity to industrial

areas emitting air pollutants, such as manganese, naphthalene, antimony, organotin compounds, nonylphenol, dichloromethane, and vanadium, face an elevated risk of developing CRC.[96] Prolonged exposure to air pollutants like $PM_{2.5}$ and NO_2,[97] as well as contaminants such as microplastics and chlorination byproducts in drinking water, has been linked to higher risk of CRC.[98] It is imperative to formulate public health policies that aim to lower levels of environmental pollutants and minimize their effects on microbial communities and resulting inflammatory responses.

Smoking and alcohol consumption

Smoking, as a distinct form of inhaled environmental exposure, is arguably the most extensively studied factor in shaping the progression of IBD. CD patients who smoke exhibit significant anti-inflammatory and immunosuppressive effects, which are associated with impaired regulation of adaptive responses, potentially rendering cells more vulnerable to chronic inflammation and oxidative damage.[99] Smoking has been linked to changes in gut microbiota, with smokers having higher levels of Bacteroidetes compared to non-smokers with IBD. Smoking cessation, however, induces an increase in Firmicutes and Actinobacteria at the phylum level, alongside a reduction in Bacteroidetes and Proteobacteria, indicating that smoking significantly alters the composition of the gut microbiome.[100,101] Moreover, smoking-induced inflammation leads to epigenetic changes like DNA methylation, histone modification, and ncRNAs. A study by Zhang et al. has found that current and former smokers have a higher risk of developing CD ($p = 7.09 \times 10 - 10$) and UC ($p < 2 \times 10 - 16$) compared to non-smokers in a prospective cohort analysis with MR analysis. Subsequently, genome-wide methylation and colocalization analyses identify DNA methylation changes at the *Dnmt3a*, *Ahrr*, and *Lta*/*Tnf* loci as potential key epigenetic sites through which smoking influences IBD susceptibility.[102] In terms of histone modifications induced by smoking, tobacco promotes histone hyperacetylation by upregulating HATs and downregulating SIRT 17. This can increase the transcription of pro-inflammatory genes associated with the NF-κβ signaling, such as IL-1β, TNF-α and IL-6, ultimately triggering chronic inflammation.[103] In addition, the influence of smoking on miRNAs in IBD has been more thoroughly investigated. Smoking can significantly alter the expression of several inflammation-related miRNAs, most notably miR-21, miR-132, miR-223, and miR-195.[104] Smoking increases the risk of intestinal fibrosis and strictures in patients with IBD by reducing miR-200 levels, which typically prevent EMT associated with fibrosis.[105] While the exact impact of smoking on IBD progression is not fully understood, quitting smoking is beneficial for treating IBD. A recent multicenter study has shown that persistent smokers have a higher rate of CD relapse compared to those who quit or never smoked.[106]

While chronic alcohol intake is typically linked to hepatic damage, the frequency of alcohol consumption represents a significant factor influencing gut microbiota composition. Chronic or excessive alcohol consumption significantly reduces the alpha diversity of the gut microbiota and alters the Firmicutes-to-Bacteroidetes ratio, a shift that has been implicated in metabolic disorders.[107] Evidence from animal models indicates that alcohol is rapidly absorbed in the small intestine and metabolized into acetate in the liver, which is then transported to the gut through the bloodstream. Elevated levels of acetate have been shown to disrupt gut microbial composition and metabolic function.[108] Fecal microbiota transplantation from patients with alcohol use disorder (AUD) into mice results in gut microbial dysbiosis and metabolic disturbances. A decrease in ethanol production by specific bacterial genera, coupled with reduced lipid catabolism, has been associated with diminished hepatic β-hydroxybutyrate (BHB) synthesis, potentially compromising the neuroprotective role of BHB.[109] Moreover, using a rodent model of prenatal alcohol exposure, this study demonstrated that alcohol consumption during pregnancy alters gut microbiota composition and reduces bacterial diversity in both dams and offspring, with distinct patterns of microbial disruption.[110] Although alcohol is not a primary inducer of IBD, alcohol and its metabolite acetaldehyde can damage intestinal epithelial cells and disrupt tight junctions, thereby compromising the intestinal barrier function. This increased intestinal

permeability facilitates the translocation of bacterial products, such as LPS, into the mucosal layer, triggering immune responses. Overall, smoking and alcohol are more controllable and avoidable factors compared to other environmental exposures, making them potential targets for influencing the susceptibility or progression of IBD.

Dietary factors and microbiota-epigenetic crosstalk

Diet shapes the complex interactions between the host and its commensal gut bacteria, further enhancing the plasticity of host–microbiota associations and ultimately influencing host health. Diet strongly affects gut microbiota composition. For example, in mice fed a 'Western diet' (a diet rich in saturated fats and simple carbohydrates but low in dietary fiber), an increased abundance of Firmicutes and a reduced abundance of Bacteroidetes and Actinobacteria have been observed in the colon. These changes have been associated with increased permeability of the inner mucus layer and reduced growth rates.[111] Diet shapes the gut microbiota by selectively promoting the growth of microbes capable of utilizing specific nutrients, particularly indigestible glycans, thereby altering both their composition and growth dynamics.[112] Unlike the human genome, which encodes only a limited number of glycan-degrading enzymes, the gut microbiome harbors a vast array of CAZymes, enabling certain bacteria such as *Bacteroides*, *Bifidobacterium*, and *Ruminococcus* to serve as primary degraders and gain a competitive advantage by utilizing dietary glycans.[113] Moreover, dietary antigens and bioactive compounds can indirectly influence the gut microbiota by modulating host metabolism and immune responses. For instance, aryl hydrocarbon receptor (AhR) activity is essential for maintaining intraepithelial lymphocytes in the gut, and its deficiency leads to an increased bacterial burden, particularly involving members of the Bacteroidetes phylum.[114] Vitamin D is essential for immune defense against pathogens and the maintenance of beneficial symbiotic bacteria in the intestinal mucosa. Vitamin D intake is associated with reduced levels of circulating LPS, a decrease in the abundance of *Faecalibacterium* and *Bifidobacterium*, and an increase in the abundance of *Prevotella*.[115]

Many studies currently focus on the connection between mammalian diet and epigenetics to elucidate the potential impact of dietary exposures on metabolic diseases. The availability of nutrients, shaped by diet and energy balance, influences the levels of specific energy metabolites that serve as essential cofactors for epigenetic enzymes, thereby impacting the epigenetic regulation of gene expression. For example, a diet deficient in methyl groups and folate significantly reduces SAM levels in the livers of male rats and mice, leading to global DNA hypomethylation.[116] Similarly, the inclusion of components such as betaine, cycloleucine, and curcumin in the diet has been shown to influence m^6A RNA methylation patterns, thereby altering gene expression and overall phenotype.[117] Cycloleucine is a competitive and reversible inhibitor of methionine adenosyltransferase, which suppresses methylation by reducing SAM levels.[118] Curcumin alters the expression profile of microRNAs both in vivo and in vitro, and the formation of m^6A is regulated by microRNAs through the binding of METTL3 to mRNA substrates.[119] In particular, diet directly influences the epigenetic modifications through the metabolism of gut microbiota. Dietary fiber in the diet is fermented by the gut microbiota to produce SCFAs, which, as mentioned earlier, enhance histone acetylation by inhibiting HDACs. In addition, high-fat feeding increases the abundance of homogentisic acid (HGA) through gut microbiota metabolism, and HGA elevates the m6A levels of *Ehmt2* in white adipose tissue, which reduces *Ehmt2* protein expression and H3K9me2 levels, leading to metabolic disorders in mice.[120] In conclusion, this evidence demonstrates a direct link between diet, the gut microbiota, and epigenetics, suggesting that dietary interventions may be an effective approach for disease treatment (the role of dietary interventions in disease will be discussed in subsequent chapters).

Antibiotic exposure and microbiota-epigenetic crosstalk

The widespread use of antibiotics has led to their common exposure as early as the

beginning of life, which may result in unintended adverse effects. While antibiotics are primarily administered to combat bacterial infections, accumulating evidence suggests that their impact extends beyond microbial eradication to include profound and lasting effects on host gene expression through epigenetic mechanisms. An early study suggested that prenatal antibiotic exposure may be associated with birth weight and linked to differential DNA methylation at five loci: *Igf2* ($p = 0.05$), *H19* ($p = 0.15$), *Plagl1* ($p = 0.01$), *Meg3* ($p = 0.006$), and *Peg3* ($p = 0.08$).[121] Studies have shown that early-life antibiotic exposure reduces gut microbial diversity and alters the abundance of specific bacterial taxa, including a decrease in *Bacteroides fragilis* and *Bifidobacterium*, and an increase in *Bacteroides vulgatus*, *Klebsiella*, and *Enterococcus*.[122,123] This dysbiosis is associated with increased intestinal permeability, heightened inflammation and antimicrobial peptide expression, as well as decreased levels of SCFAs.[124] The reduction in SCFAs may influence the expression of immune-related genes such as FOXP3 by altering histone acetylation levels, thereby impairing the differentiation of regulatory T cells.[125] Importantly, epidemiological studies have indicated that early-life exposure to antibiotics is associated with an increased risk of developing IBD later in life,[126] potentially due to antibiotic-induced gut microbiota dysbiosis during critical developmental periods. Furthermore, antibiotic-induced gut microbiota dysbiosis significantly alters bile acid metabolism in conventional mice, and the resulting imbalance in bile acids profoundly affects host m^6A methylation levels.[127] In summary, early-life antibiotic exposure-induced disruptions in the gut microbiota and epigenetic regulation may represent a critical window into understanding antibiotic-associated metabolic dysregulation, warranting further in-depth investigation.

Interplay between gut microbiota, epigenetics, and diseases: causation or correlation?

In the preceding section, we discussed the potential interplay between the gut microbiota ('invironmental'), the external environment ('environmental'), and host epigenetics. However, the extent to which microbiota-induced epigenetic dysregulation contributes to the progression of IBD and CRC remains poorly understood. Although substantial evidence links these epigenetic alterations to disease development, the available data are fragmented and lack a comprehensive synthesis. Herein, we provide a systematic overview of recent advances in understanding the role of microbiota-induced epigenetic regulation in IBD and CRC, focusing on the underlying mechanisms involved.

Microbiome influences host epigenome: key environmental factors in IBD origins

The most prominent feature of IBD is the occurrence of chronic inflammation in the gastrointestinal tract and gut dysbiosis.[128] For instance, the phylum Firmicutes, particularly *Faecalibacterium prausnitzii*, *Christensenellaceae*, and *Coriobacteriaceae* is frequently found in lower relative abundance in the stool of patients with CD.[129–131] In contrast, members of *Actinomyces*, *Veillonella*, and *Escherichia coli* are often found to be elevated in patients with CD compared to healthy individuals.[131] For patients with UC, both *Bacteroides* and *Akkermansia* are reduced, while *Escherichia coli* appears to be increased.[131] Specific pathobionts can also influence IBD in specific environments. The Enterobacteriaceae family, particularly certain adherent-invasive *Escherichia coli* (AIEC) strains,[132] *Mycobacterium avium subspecies. paratuberculosis*,[133] and *F. nucleatum* [134] are considered potential contributors to the pathogenesis of CD. However, the mechanism by which the host integrates commensal microbial signals during the pathogenesis of IBD remains unclear.

The development of IBD is undoubtedly influenced by a multitude of aspects, including environmental factors (diet, air pollution, and smoking) as well as 'invironmental' factors (intestinal microbiome), all of which interact in complex ways with host genetics, epigenomics, and environmental influences.[135–138] Recent research has shown a strong link between IBD progression and changes in DNA methylation patterns.[139] A study in Scottish adults has identified significant differences

in methylation levels of key genes related to IBD, with some of these differences confirmed independently in a group of treatment-naïve pediatric CD patients from North America.[25,140] A recent prospective case-control study of 295 IBD cases in Europe has identified 137 differentially methylated sites. Consistent patterns are observed for *Vmp1/Mir21* and *Rps6ka2* across Scandinavia and the UK, indicating stability in the IBD-specific methylome within regions sharing an ancestral lineage. The study also reveals significant methylation differences in the pro-inflammatory gene *Osm*, which is upregulated in IBD.[23] In addition, a whole-genome methylation analysis of 229 CD patients requiring intestinal resection revealed a strong association between postoperative recurrence and five specific methylation sites, particularly in *Whsc1* and *Efna3*.[141] These evidence highlight that epigenetic changes may provide important insights into the gene-environment interactions underlying the complex disease of IBD.

Since the onset of IBD is properly complicated, it is hard to explore the reality of gut microbiota and epigenetics in intestinal diseases. Howsoever, in this article, epigenetic modifications deem to be influenced by external environmental factors, and the gut microbiota is one such factor that can impact epigenetics. Essentially, dysbiosis initiates epigenetic alterations, which then alter gene expression and immune function, ultimately affecting disease development (Figure 2 and Tables 1–2).

Microbiota-induced DNA/histone modification changes are involved in IBD pathogenesis

Recently, the rapidly evolving field of epigenetics has provided new insights into the mechanisms through which environmental changes trigger 'disease memory' and determine the phenotype and function of cells in IBD (Figure 2a,b). A related study has identified significant epithelial DNA methylation differences between CD patients and healthy individuals, which are associated with microbiota composition.[142] In a mouse model of acute inflammation induced by dextran sulfate sodium (DSS), exposure to the microbiota leads to alterations in DNA methylation and chromatin accessibility at regulatory elements, resulting in changes in the expression of genes associated with inflammation. This process is primarily associated with microbiota-induced TET2/3-dependent demethylation of enhancers.[34] Concomitantly, alterations in microbiota-induced histone methylation, particularly H3K4me3, have been observed in CD patients compared to healthy individuals. Intriguingly, a proportion of the genes sensitive to symbiotic bacteria also exhibit similar H3K4me3 signatures in mouse IECs as observed in CD patients.[143] Importantly, specific bacteria can influence IBD progression through epigenetic mechanisms. The enrichment of *Fusobacterium* has been found to increase the DNA methylation level in portions of gene promoters in UC patients.[144] In trinitrobenzene sulfonic acid (TNBS)- and DSS-induced mouse models of colitis, *Bifidobacterium longum* (*B. Longum*) and *Bacteroides thetaiotaomicron* (*B. thetaiotaomicron*) influence Treg cell differentiation and the production of anti-inflammatory cytokines IL-22 by modulating DNA methylation levels at the *Foxp3* promoter.[145,169] Notably, DNA or histone modification changes, in turn, affect gut microbiota colonization aggravating colitis. For example, AIEC adheres to enterocytes through the interaction between type 1 pili and CEACAM6 receptors on CD ileal mucosa. Particularly, abnormal DNA methylation upregulates CEACAM6 expression, allowing AIEC to colonize in intestinal more efficiently and inducing colitis.[146] Moreover, another study has shown that HDAC1 expression is required to prevent AIEC colonization, whilst HDAC5 expression favored AIEC encroachment.[170]

As previously mentioned, epigenetic regulation triggered by gut microbiota dysbiosis contributes to the onset of IBD primarily through two mechanisms. The first involves gut microbial metabolites influencing the expression of epigenetic modification enzymes. For example, reduction in SCFAs, triggers abnormal DNA/histone modification and augments intestinal inflammation susceptibility, thereby escalating the risk of IBD.[171] Butyrate can promote epigenetic remodeling in intestinal immune cells by acting as a HDACi and reduce intestinal inflammation by inhibiting NF-κB pathways.[165] It is worth mentioning that butyrate also reduces the risk of IBD by upregulating the expression of lncRNA Ly6C, a novel

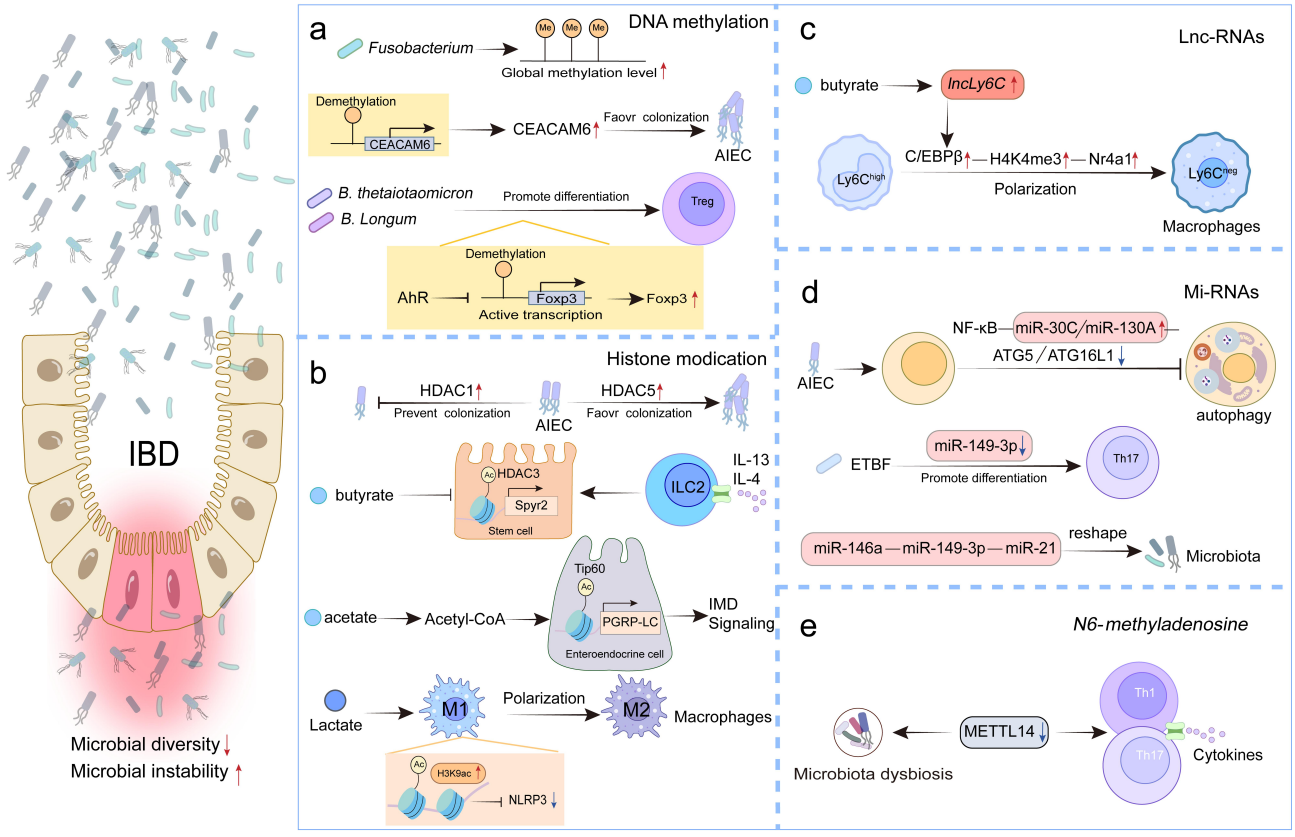

Figure 2. Epigenetic modifications induced by gut microbiota promote inflammatory bowel disease (IBD). Gut microbial dysbiosis is a co-evolving hallmark of IBD, characterized by decreasing microbial diversity. Gut microbiota can regulate DNA methylation (a), histone modification (b), lncRNAs (c), miRNAs (d), N^6-methyladensine (m^6A) (e), in turn, alterations in epigenetic modifications can reshape the composition of the gut microbiota or influence the colonization of specific bacteria. These interactions exacerbate gut inflammation by promoting type 1/17 T helper (Th1/17) cell immune responses, influencing macrophage polarization states, or inhibiting autophagy. Red upward arrow indicates an increase in gene expression level, while blue downward arrow indicates a decrease in gene expression level. AIEC, adherent-invasive *Escherichia coli*; *B. Longum*, *Bifidobacterium longum*; *B. thetaiotaomicron*, *bacteroides thetaiotaomicron*; ETBF, Enterotoxigenic *bacteroides fragilis*; AhR, aryl hydrocarbon receptor; C/EBPβ, CCAAT/enhancer binding protein β; H3K4me3, histone 3 at lysine 27 (H3K4) trimethylation; H3K9ac, histone 3 at lysine 9 (H3K9) acetylation; H3K18lac, histone 3 at lysine 18 (H3K18) lactylation; HDAC1/3/5, histone deacetylase1/3/5; IMD pathway, immunodeficiency pathway; *lncLy6C*, lncRNA *lncLy6C*; NF-κB, nuclear factor-kappa B; treg, regulatory T cell; Th1/17, T helper 1/17 cell.

ultraconserved lncRNA (Figure 2c). This upregulation subsequently promotes the expression of C/EBPβ and H4K4me3 on the *Nr4a1* promoter. Of note, elevated *Nr4a1* expression induces Ly6Chigh monocytes to differentiate into Ly6Cint/neg macrophages, and compared to Ly6Chigh macrophages, Ly6Cint/neg macrophages play a crucial role in inhibiting inflammation.[166] Microbe-derived acetate promotes chromatin remodeling in enteroendocrine cells by entering through the Tarag transporter and modulating histone acetylation, thereby enhancing immunodeficiency (IMD) signaling through a Tip60–steroid hormone axis that links metabolism and intestinal innate immunity.[172] Moreover, *B. thetaiotaomicron* increases the levels of AhR ligands such as indole-3-acetic acid (IAA) and indole-3-propionic acid (IPA), thereby enhancing AhR activation, which is associated with altered transcription factor expression profiles in T cells.[145] Studies have shown that AhR can selectively bind to unmethylated xenobiotic response elements (XREs), suggesting that AhR may function as a reader of DNA methylation and play a role in stress response pathways.[173] In contrast, dysregulated tryptophan metabolism in patients with IBD leads to reduced levels of AhR ligands, exacerbating intestinal inflammation. Microbial-derived inositol-1,4,5-trisphosphate (InsP3), produced from phytate metabolism, counteracts the inhibitory effect of butyrate

Table 1. Effects of gut microbiota on epigenetic regulation of IBD and CRC.

Diseases	Related microbiota	Epigenetic changes	Mechanism	Level of evidence
IBD	Global microbiota	DNA methylation	Overall methylation differences between CD and healthy controls	CD patients[142]
		Histone methylation	Induce alterations in H3K4me3 in IECs	CD patients and mouse model[143]
	Fusobacterium	DNA methylation	Accelerate DNA methylation in specific groups of genes	UC patients[144]
	Bacteroides thetaiotaomicron	DNA demethylation	Increase FOXP3 expression and demethylate several CpG sites in *Foxp3* promoter.	DSS- mouse colitis[145]
	Adherent-invasive *Escherichia coli*	DNA methylation	Hypomethylation in *CEACAM6* promoter correlates with high expression	Mouse model[146]
		Histone acetylation	HDAC1 expression is central to prevent AIEC colonization, HDAC5 expression favors AIEC encroachment	Mouse model[147]
		miR-30C, miR-130A	Inhibit autophagy by upregulating miR-30C, miR-130A by reducing the expression of ATG5 and ATG16L1	Cell lines[148]
	Enterotoxigenic *Bacteroides fragilis*	miR-149-3p	MiR-149-3p facilitates T-helper type 17 cell differentiation	Cell lines[149]
CRC	Global microbiota	DNA methylation	Hypermethylation in multiple gene promoter	Mouse model[150]
	Fusobacterium nucleatum & Hungatella hathewayi	DNA methylation	Hypermethylation in gene promoter by regulating DNMT	Cell lines, mouse model[151]
	Fusobacterium nucleatum	miR-21	Increase CRC cell proliferation by activating TLR4/MyD88/NF-κB and upregulated miR-21	Cell lines, mouse model[152]
		miRNA-4802, miRNA-18a*	Mediate CRC chemoresistance by TLR4/MyD88/miRNA-4802&miRNA-18a*/ATG7&ULK1 axis	Cell lines, mouse model[153]
		miRNA-1322	Induce M2 macrophage polarization by NF-κB/miRNA-1322/CCL20 axis	Cell lines[154]
		miR-4474, miR-4717	Involve the progression of CRC by miR-4474&miR-4717/CREBBP axis	Cell lines[155]
		lncRNA (*EVADR*)	Promote CRC cell metastatic by lncRNA (*EVADR*)/YBX1 axis	Cell lines[156]
		lncRNA1 (*KRT7-AS*)	Promote CRC cell metastasis by NF-κB/lncRNA1 (*KRT7-AS*)/KRT7	Cell lines[157]
		lncRNA *ENO1-IT1*	Promote glycose metabolism by SP1/lncRNA *ENO1-IT1*/KAT7 axis	Cell lines[158]
		N6-methyladenosine	Promote CRC cell metastasis by YAP/FOXD3/METTL3/KIF26B	Mouse model, cell lines[158]
	Enterotoxigenic *Bacteroides fragilis*	DNA methylation	Hypermethylation in gene promoter	Mouse model[159]
		miR-149-3p	Promote CRC cell proliferation by miR-149-3p/PHF5A/KAT2A axis	Cell lines[160]
		lncRNA1 (*BFAL1*), miR-155-5p, miR-200-3p	Promote CRC cell proliferation by lncRNA1 (*BFAL1*)/miR-155-5p/miR-200-3p/RHEB/mTOR axis	Cell lines[161]
	Butyrivibrio fibrisolvens	Histone acetylation	Function as histone deacetylase inhibition to induce apoptosis	AOM/DSS mouse model[162]
	pks+ E.coli	miR-20a-5p	Promote cell proliferation by c-Myc/miR-20a-5p/SENP-1/p53 axis	CRC mouse model[163]
	Clostridium butyricum	*N6*-methyladenosine	Reduce epithelial-mesenchymal transition by decreasing the expression of METTL3 and VEGFR2	Mouse model, cell lines[164]

Acronym (in the order they appear in the table): IBD, inflammatory bowel disease; CRC, colorectal cancer; CD, Crohn's disease; H3K4me3, histone H3 trimethylation at lysine 4; IECs, intestinal epithelial cells; UC, ulcerative colitis; FOXP3, forkhead box P3; DSS, dextran sulfate sodium; CEACAM6, carcinoembryonic antigen-related cell adhesion molecule 6; HDAC, histone deacetylase; AIEC, adherent invasive *Escherichia coli*; ATG5, autophagy related 5; ATG16L1, autophagy related 16 like 1; TLR4, toll-like receptor 4; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ATG7, autophagy related 7; ULK1, unc-51 like autophagy activating kinase 1; CCL20, C-C motif chemokine ligand 20; CREBBP, CREB binding protein; lncRNA (EVADR), lncRNA (endothelial cell vasculature-associated death receptor); YBX1, Y-box binding protein 1; lncRNA1 (KRT7-AS), lncRNA 1 (keratin 7 antisense); lncRNA ENO1-IT1, lncRNA ENO1 intronic transcript 1; SP1, specificity protein 1; KAT7, lysine acetyltransferase 7; YAP, yes-associated protein; FOXD3, forkhead box D3; METTL3, methyltransferase-like 3; KIF26B, kinesin family member 26B; PHF5A, PHD finger protein 5A; KAT2A, lysine acetyltransferase 2A; lncRNA1 (BFAL1), lncRNA 1 (brain and fat associated long non-coding RNA 1); RHEB, ras homolog enriched in brain; mTOR, mechanistic target of rapamycin; AOM, azoxymethane; *pks+ E.coli*, polyketide synthase-positive *Escherichia coli*; c-Myc, cellular Myc; SENP-1, sentrin-specific protease 1; VEGFR2, vascular endothelial growth factor receptor 2.

on intestinal HDAC3, thereby inducing HDAC3 activation.[174] Epithelial-specific deletion of the epigenetic enzyme HDAC3 inhibited tuft cell expansion and impaired type 2 immune responses during helminth infection.[41] Intriguingly, lactate has been reported as elevated in CD and UC patients.[175] Although it is not known whether elevated lactate affects the progression of Kla in human IBD, in mice with colitis, the engineered Saccharomyces cerevisiae with high lactate production has shown good anti-inflammatory activity and improved histological damage.[63] Lactate mitigates DSS-induced colitis by promoting H3K9ac, H3K18lac, and inhibiting the secretion of inflammatory cytokines, whilst also increasing the diversity of microbiota.[63]

The second mechanism lies in the regulation of epigenetic substrates by the concentrations of gut microbial metabolites. It has been observed that several common methyl donors involving in methylation of DNA/histone and m^6A, such as

Table 2. Effects of metabolites on epigenetic regulation of IBD and CRC.

Diseases	Related metabolites	Epigenetic changes	Mechanism	Level of evidence
IBD	Butyrate	Histone H3 acetylation	Histone deacetylase inhibition (HDACi)	DSS- mouse colitis[165]
		LncLy6C, H4K4me3	Influence macrophage polarization states through lncLy6C/C/EBPβ/Nr4A1 axis	Mouse model[166]
	Lactate	H3K9 acetylation, H3K18 lactylation	Promote H3K9 acetylation, H3K18 lactylation, and inhibit the production of pro-inflammatory cytokines in macrophages	DSS- mouse colitis[63]
CRC	Propionate	Histone methylation	Induce apoptosis by HECTD2/EHMT2/TNFAIP1 axis	Cell lines[167]
	Butyrate	miR-92a	Induce apoptosis by c-Myc/miR-92a/p57 axis	Cell lines[168]

Acronym (in the order they appear in the table): HDAC, histone deacetylase; DSS, dextran sulfate sodium; LncLy6C, lncRNA Ly6C; H4K4me3, histone H4 trimethylation at lysine 4; C/EBPβ, CCAAT/enhancer-binding protein beta; Nr4A1, nuclear receptor subfamily 4 group A member 1; HECTD2, HECT domain E3 ubiquitin ligase 2; EHMT2, Euchromatic histone-lysine N-methyltransferase 2; TNFAIP1, TNF alpha induced protein 1; c-Myc, cellular Myc.

folate, vitamin B12 and choline, exhibit diminished levels in IBD. Studies have shown that IBD patients are often at risk of folate and vitamin B12 insufficiencies.[176] Supplementing the maternal diet with methyl donors, including folate, vitamin B12, and choline, has been shown to increase offspring susceptibility to DSS-induced colitis, partially due to aberrant methylation of anti-inflammatory genes.[177] Further investigation should focus on elucidating the impact of distinct metabolites on specific epigenetic modifications, while also conducting clinical trials to validate the clinical relevance of these findings.

Gut microbiota-miRNAs interactions in IBD

Both the gut microbiota and miRNAs have been the focus of recent studies aimed at comprehending their roles in IBD. We have previously discussed roles of gut microbiota in miRNA expression, whilst the miRNAs, in turn, actively contribute to the modulation of the microbiota, thereby leading to alterations in intestinal homeostasis. Thus, pathogenic bacteria may induce IBD via altering miRNA expression. Indeed, there have been reports implicating the influence of AIEC on the host miRNAs as a potential major contributor (Figure 2d). Specifically, AIEC infection has been shown to inhibit autophagy in IECs expression by activating nuclear factor-kappa B (NF-κB)-miR-130A/miR-30C axis. Mechanistically, elevated miR-130A/miR-30C targets and reduces the expression of ATG5 and ATG16L1, the autophagy-associated genes, leading to an increase in the numbers of intracellular AIEC and the aggravation of intestinal inflammation.[178,179] Likewise, Enterotoxigenic *Bacteroides fragilis* (ETBF) is shown negatively correlated with miR-149-3p. Reduced miR-149-3p facilitates type 17 T helper cells (Th17) differentiation, which exacerbate intestinal inflammation.[160]

Conversely, host-derived miRNAs can specifically regulate the composition of gut microbiota and serve as potential biomarkers of IBD. A compelling study has shown that mice with a targeted knockout of Dicer exhibit reduced fecal miRNAs and increased susceptibility to colitis compared to wild-type mice, suggesting the host can exert an active influence on the composition of gut microbiota via manipulating miRNAs.[51] Furthermore, low levels of miR-149-3p, miR-21, and miR-146a increase susceptibility to DSS-induced colitis, which may be associated with microbiota dysbiosis.[180–182] Overall, the compiled information suggests that the crosstalk between the gut microbiota and miRNAs axis leads to intestinal epithelial dysfunction, autophagy alterations, and immune hyperactivation, all of which contribute to the development of IBD.

Gut microbiota-m^{6}A interactions in IBD

Unfortunately, research exploring the interrelation between gut microbiota and m^{6}A modifications impacting IBD progression remains quite limited, with most studies focusing on murine models (Figure 2e). In a related study, MEETL14 deficiency in mice can induce more severe colitis. Further investigations have found that it is characterized by heightened infiltration of inflammatory cells, increased levels of Th1 and Th17 cytokines,

and is accompanied by dysbiosis in the gut microbiota.[77] Furthermore, METTL3 expression is enhanced in LPS-stimulated macrophages, whilst METTL3 overexpression in macrophages could mitigate LPS-induced inflammation through regulating the NF-κB signaling.[183] In summary, the interaction between microbiota and m^6A in IBD is still a complex and intricate field that should require significant efforts to unravel.

Associations between gut microbial dysbiosis, epigenetic alterations, and CRC development

The etiology of CRC is multifaceted; however, it has been established that ecological imbalances in the gut microbiome contribute substantially to CRC progression.[184,185] Of particular significance is the increased presence of oral pathogens, such as *F. nucleatum*, *Parvimonas micra*, *Peptostreptococcus stomatis*, *Prevotella anaerobius*, *Porphyromonas asaccharolytica*, *Solobacterium moorei*, and *Prevotella intermedia*.[186] Other studies have also demonstrated an enrichment of specific colon-resident bacterial strains in CRC patients, including enterotoxigenic *Bacteroides fragilis*,[187] pks+ *Escherichia coli*,[188] *Streptococcus gallolyticus*,[189] and *Morganella morganii*.[190] These findings suggest that the dysbiosis are associated with the onset of CRC, although it remains unclear whether they act independently or collaborate with other microorganisms to accelerate its development.

Furthermore, specific microbial metabolites, such as SCFAs, act as signaling molecules that modulate the host epigenome thereby intervening in the cancer process.[191] Cancer process is often closely linked to the silencing of tumor suppressor genes and the unchecked activation of oncogenes, involving the intricate regulation of epigenetic factors such as DNA and histone methylation, as well as non-coding RNAs. Additionally, aberrant epigenetic signals can influence the colonization of the gut microbiota.[192] The bidirectional epigenome-microbiome axis not only influences gene expression profiles but also affects immune responses and metabolic pathways; this dynamic regulatory interplay may be crucial for understanding the pathogenesis of CRC (Figure 3 and Tables 1–2).

Gut microbiota-DNA methylation interaction in CRC

Upon the transfer of freshly obtained fecal samples from both CRC patients and their healthy counterparts to GF mice, the former group displays augmented colon epithelial renewal, an elevated occurrence of precancerous lesions, and heightened levels of peripheral blood DNA methylation.[193] Particularly, multiple gene promoters, such as *Sept9*, *Wif1*, and *Penk*, exhibit hypermethylation in CRC but not in normal tissues.[193] Despite the extensive understanding of the methylation signature in CRC, there are few studies investigating the specific bacteria involved in regulating CRC methylation as well as the underlying mechanisms governing this regulatory process. To this end, a population-based study reveals that a substantial presence of *F. nucleatum* in CRC tissues exhibits associations with elevated levels of CpG island methylator phenotype (*CIMP*)-high, and poor patient prognosis.[194] Importantly, through multi-omci profiling, Wang and his colleagues have shown that the increase in *Fusobacterium* is closely associated with the decrease in 4-Hydroxybutyrate acid (4-HB), a derivative of butyrate.[195] Hence, *Fusobacterium* on host DNA methylation may be mediated by butyrate. Additionally, Xia and his colleagues have shown that *F. nucleatum* and *Hungatella hathewayi* (*H. hathewayi*) facilitate hypermethylation of tumor suppressor gene promoters by upregulating DNMT expression.[196]

Concomitantly, preliminary experiments delving into the effects of ETBF on the epigenome are underway. A related study has demonstrated that ETBF inoculation in $Apc^{min/+}$ mice results in the recruitment of DNMT1, potentially mediated by mismatch repair proteins.[197] *Bacteroides fragilis* toxin (BFT) is the virulence factor secreted by ETBF. It has been demonstrated that BFT stimulation results in increased chromatin accessibility, primarily associated with enhancer regions and binding sites of AP-1/ATF family transcription factor which are also enriched for common DMRs in CRC.[198] Current studies are limited to a few bacterial species, lacking a comprehensive understanding of the microbiome's impact on DNA methylation in CRC. Other symbiotic microbes may also be significant. Microbiome analysis can

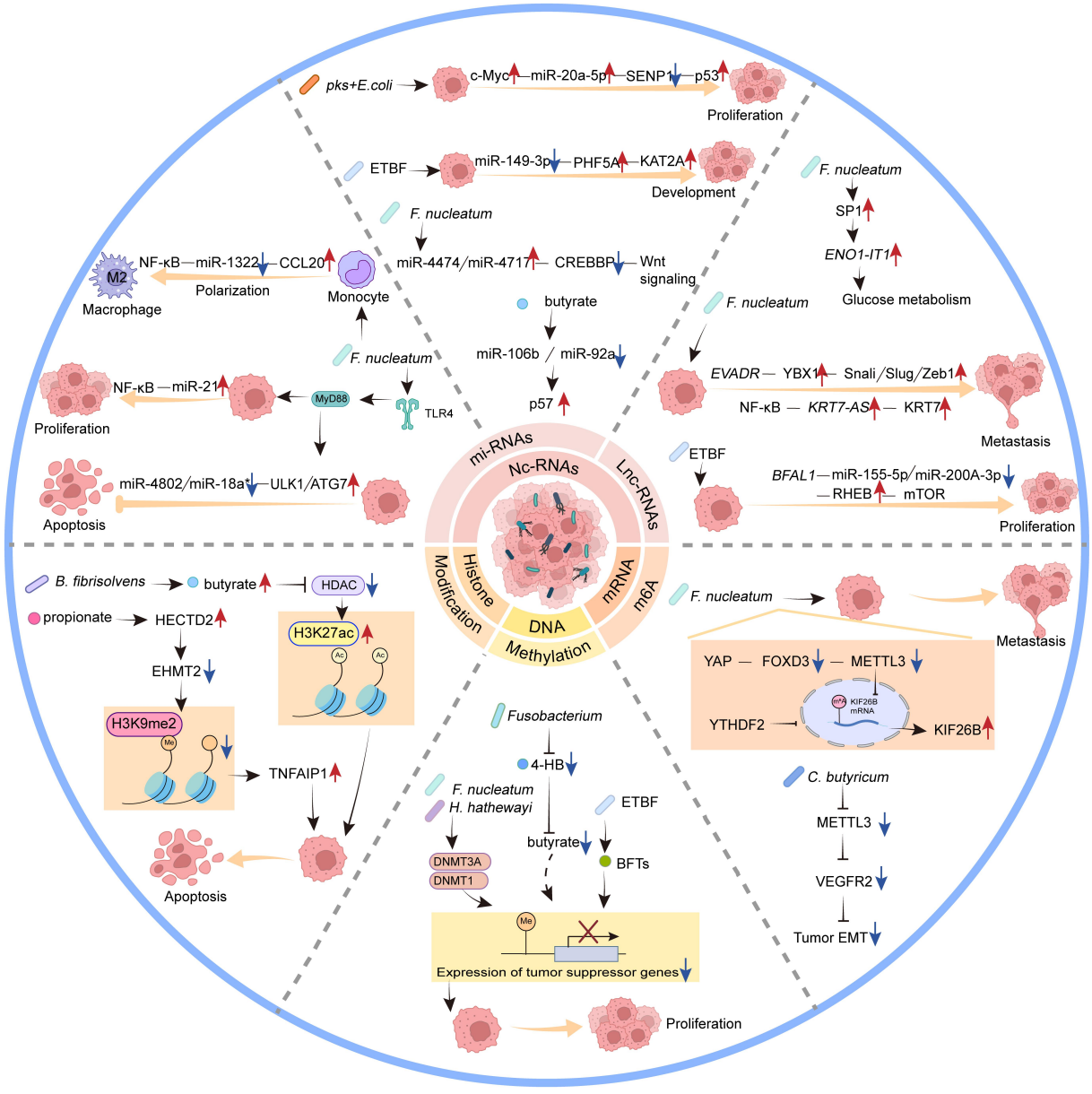

Figure 3. Gut microbial dysbiosis contributes to the development of colorectal cancer (CRC) through epigenetic mechanisms. Enrichment of cancer-inducing pathogenic microorganisms, such as enterotoxigenic *bacteroides fragilis* (ETBF), *fusobacterium nucleatum* (*F. nucleatum*), and *pks*$^{+}$ *Escherichia coli* (*pks*$^{+}$ *E.Coli*), has been observed in CRC. These pathogenic microorganisms or metabolites present in the gut are involved in tumor initiation and progression by interacting with epigenetic modifications, including DNA methylation, histone acetylation, non-coding RNAs, and N^6-methyladensine (m^6A). Red upward arrow indicates an increase in gene expression level, while blue downward arrow indicates a decrease in gene expression level. *B. fibrisolvens*, *butyrivibrio fibrisolvens*; BFT, *bacteroides fragilis* toxin; *C. butyricum*, *Clostridium butyricum*; ETBF, enterotoxigenic *bacteroides fragilis*; *F. nucleatum*, *fusobacterium nucleatum*; *H. hathewayi*, *hungatella hathewayi*; *pks*$^{+}$ *E.Coli*, *pks*$^{+}$ *Escherichia coli*; *BFAL1*, *bacteroides fragilis*-associated lncRNA1; CREBBP, CREB-binding protein; DNMT1/3A, DNA methyltransferase 1/3A; EHMT2, euchromatic histone-lysine N-methyltransferase 2; EMT, epithelial-mesenchymal transition; *ENO1-IT1*, lncRNA enolase1-intronic transcript 1; *EVADR*, endogenous retroviral-associated adenocarcinoma lncRNA; FOXD3, forkhead box D3; HDAC, histone deacetylases; HECTD2, HECT domain E3 ubiquitin protein ligase 2; H3K27ac, histone 3 at lysine 27 (H3K27) acetylation; KIF26B, kinesin family member 26B; *KRT7-AS*, lncRNA Keratin7-antisense; KRT7, Keratin7; RHEB/mTOR, MTORC1 binding/mammalian target of the rapamycin; TLR4, toll-like receptor 4; TNFAIP1, tumor necrosis factor α-induced protein 1; YAP, yes associated protein; YBX1, Y-box binding protein 1; VEGFR2, vascular endothelial growth factor receptor 2; 4-HB, 4-hydroxybutyrate acid.

improve identification of CpG methylation targets linked to cancer risk and enhance environmental risk markers.

Gut microbiota-histone modifications interaction in CRC

While SCFAs, particularly butyrate, are recognized for supporting colonocyte function and lowering CRC risk, exploring their gene regulatory mechanisms provides a novel approach to CRC intervention. Microbial metabolism-derived colonic SCFAs enhance epithelial homeostasis gene expression pathways and attenuate carcinogenic processes through direct histone modifications. Nshanian et al. investigated the epigenetic regulatory roles of SCFA metabolites, propionate and butyrate, in colorectal cancer and normal cells. Using multi-omics approaches, the authors expanded on the non-HDACi functions of SCFAs and mapped the genome-wide localization of propionyl and butyryl H3K18 and H4K12 in CRC cells. These findings underscore the interplay between diet, microbial metabolism, and epigenetics in shaping tissue physiology and cancer susceptibility.[199] Furthermore, a more recent study that has examined the impact of propionate on CRC considering specific histone modifications. Ryu and his colleagues have shown that propionate suppresses CRC growth by promoting the proteasomal degradation of EHMT2 through upregulation of HECTD2. Through more site-specific analysis, the downregulation of EHMT2 is found to reduce the H3K9me2 level on *Tnfaip1* promoter, which in turn, causes the upregulation of TNFAIP1 and subsequent induction of apoptosis in CRC cells.[167] These findings suggest that combining histone modification inhibitors with the microbiome could lead to new therapeutic approaches for CRC. Further research is needed to understand the specific molecular pathways connecting the microbiota, metabolites, and histone modification enzymes.

Gut microbiota-miRNAs interaction in CRC

Intriguingly, the importance of miRNAs in the host-microbiota interactions and their impact on CRC has begun to be appreciated. As mentioned earlier, *F. nucleatum* is a carcinogenic microorganism intricately linked with CRC, as it actively facilitates the initiation and progression of CRC through various mechanisms, including miRNome. In a compelling study, *F. nucleatum* was shown to activate NF-κB signaling and increased expression of miR-21 by triggering TLR4-MyD88 signaling cascade, resulting in promoted CRC progression in mice.[152] High levels of *F. nucleatum* DNA and miR-21 are among the features indicating poor prognosis in CRC patients. Notably, miR-21 is identified as an oncogenic microRNA, with elevated expression observed in various solid and hematological malignancies, encompassing CRC.[200] Furthermore, CRC resistance to chemotherapy is associated with *F. nucleatum*. Specifically, the infection of *F. nucleatum* results in the suppression of miR-4802 and miR-18a by TLR4-MyD88 signaling. Subsequently, the reduced levels of miR-4802 and miR-18a alleviate their suppression on autophagy genes *ULK1* and *ATG7*, inducing autophagy and ultimately leading to CRC chemoresistance.[153] Analogously, *F. nucleatum* has been shown to promote CRC metastasis by miRNome. *F. nucleatum* infection decreases miRNA-1322 expression by activating the NF-κB signaling. In turn, the low expression of miR-1322 promotes its target gene, *CCL20* expression, a chemokine that is differentially upregulated and associated with CRC, consequently inducing M2 macrophage polarization and enhancing tumor metastasis.[154] In addition, *F. nucleatum* can also inhibit CREB-binding protein (CREBBP) expression through facilitating miR-4474 and miR-4717 expression. CREBBP promotes the progression of CRC by activating the Wnt/β-catenin signaling, a signaling pathway associated with CRC.[201]

In general, colibactin-producing *pks*$^+$ *Escherichia coli (pks*$^+$ *E.coli)* promote cancer by regulating inflammation or DNA damage. However, a study from Dalmasso's group extends this paradigm. They have shown that colibactin-producing *pks*$^+$ *E.coli* induces c-Myc and miR-20a-5p expression, which targets *SENP1*, a negative regulator of p53 small ubiquitin-like modification (SUMOylation). Consequently, elevated p53 SUMOylation, recognized as a key contributor to

cell senescence, induces the expression of carcinogenic growth genes, thereby promoting tumor cell proliferation.[163] Another bacterium strongly links with the occurrence of CRC, ETBF, also facilitates colorectal carcinogenesis by reducing miR-149-3p expression and subsequently increasing PHF5A-mediated alternative RNA splicing of *Kat2a*.[160] The interaction between the microbiome and host miRNAs plays a significant role in CRC progression. Evaluating these multi-omics biomarkers could provide a new approach for early cancer diagnosis or patient monitoring. However, current research primarily focuses on a limited number of specific microorganisms and miRNAs, similar to studies on DNA methylation.

Gut microbiota-lncRNAs interaction in CRC

Although supporting evidence is limited, lncRNAs have been recognized as another contributor in the host-microbiota interactions to regulate CRC carcinogenesis. For example, it has been demonstrated that *B. fragilis*-associated lncRNA1 (*BFAL1*) is highly expressed in tumor tissues and ETBF-infected cells. Mechanistically, *BFAL1* mediates ETBF-induced tumor growth by competitively sponging miR-200a-3p and miR-155-5p to activate RHEB-mTOR signaling.[161] Another recent study has revealed that the endogenous retroviral-associated adenocarcinoma lncRNA (*EVADR*) interacts with YBX1, and facilitates the translation of epithelial-mesenchymal transition (EMT)-related transcription factors by guiding YBX1 to polyribosomes, thereby promoting CRC metastasis.[156] Similarly, *F. nucleatum* activates the NF-κB pathway, and subsequently increases the expression of lncRNA Keratin7-antisense (*KRT7-AS*) to facilitate CRC cells migration.[157] Interestingly, the potential correlation between *F. nucleatum* and glucose metabolism has been explored in CRC, which also involves the regulatory role of lncRNAs. Specifically, *F. nucleatum* activates the transcription of lncRNA enolase1-intronic transcript 1 (*ENO1-IT1*) by upregulating transcription factor SP1, and elevated *ENO1-IT1*, in turn, acts as a guiding module for KAT7 histone acetyltransferase, directing the histone modification pattern on its target genes, and consequently influencing CRC progression.[202] Hence, it is obvious that gut microbiota could influence the expression of lncRNAs, which subsequently target related genes and signaling pathways, ultimately altering CRC development.

Metabolites regulated-ncRNAs regulate CRC

Finally, host ncRNAs can be regulated by metabolites in CRC, such as butyrate. In addition to its ability to inhibit HDAC activity, butyrate exerts another anti-cancer mechanism by modulating ncRNAs in cancer cells. It has been reported that butyrate influences the expression of 44 distinct miRNAs in CRC cell lines, chiefly targeting members of the miR-106b family.[49] Further investigation of differentially expressed miRNAs in a butyrate-treated CRC cell line reveals that butyrate inhibits miR-92a expression in cMyc-dependent manner, allowing the CDK inhibitor p57, a target of miR-92a, to regulate cell-cycle progression in CRC.[203] However, the focus on butyrate as a single metabolite may overlook other microbial metabolites that could also regulate ncRNAs in CRC, warranting broader investigation into metabolite-ncRNA interactions.

Gut microbiota-m^{6}A interaction in CRC

Increasing evidence suggests that the crosstalk between m^{6}A modification and microbiota is implicated in CRC carcinogenesis. A recent study has shown that *F. nucleatum* induces low m^{6}A levels in CRC cells. Specifically, *F. nucleatum* inhibits forkhead box D3 (FOXD3), a transcriptional regulation factor of METTL3, by activating YAP signaling. Thus, decreased expression of METTL3 impedes the YTHDF2-mediated degradation of *Kif26b*, a pivotal gene in CRC aggressiveness and metastasis.[204] Moreover, *Clostridium butyricum* (*C. butyricum*) has been found to possess an inhibitory effect on tumor cell proliferation.[205,206] Importantly, *C. butyricum* has been shown to exert an inhibitory effect on CRC in an m^{6}A-dependent fashion. *C. butyricum* treatment could down-regulate METTL3 expression in CRC cells to inhibit EMT-related genes (*Snali*, *Slug*, *Zed1*) and VEGFR2 expression, causing reduced EMT and vasculogenic mimicry (VM) formation.[207] Likewise, butyrate could inhibit CRC

development by inhibiting METTL3 expression and related cyclin E1.[208] Thus, these findings suggest that microbiota-mediated m^6A modification could be a significant contributor influencing CRC progression. Nevertheless, further study is warranted to explore the potential interactions between gut microbiota and RNA m^6A modification, aiming to enhance our comprehension of their respective roles in the advancement of CRC.

Microbiota or its metabolites influence epigenetics: promising therapeutic approaches for IBD and CRC

With the growing understanding of the role of the gut microbiome in disease development, the modulation of gut microbiota to restore microbial homeostasis emerges as a promising strategy for disease prevention and treatment.[209] Moreover, the intersection of microbiome analysis and epigenetic research not only deepens our understanding of disease mechanisms but also opens new avenues for targeted therapeutic interventions.[210,211] Research on probiotics, prebiotics, postbiotics, diet, FMT and bacteriophages (phages) is currently underway. (Figure 4 and Table 3).

Probiotics

Several lines of evidence have been shown that probiotics possess the capability to modulate gut microbiota composition, bolster mucosal barrier integrity, lower serum cholesterol levels, and stimulate the host immune system.[218,219] The most widely studied and used probiotics are *lactobacilli* and *bifidobacterial*. For example, *Lactobacillus reuteri* FPHC2951 (*LR* FPHC2951), isolated from the feces of exclusively breastfed healthy infants, was found to alleviate DSS-induced IBD symptoms, enhance the expression of interleukin-10 (IL-10) mRNA, and increase the abundance of *Verrucomicrobiaceae Akkermansia*.[220] A secreted protein named LPH was identified from the secretome of *Lactobacillus* and demonstrated to alleviate colitis and colorectal cancer by regulating intestinal homeostasis through the release of NOD2 ligands.[221] Furthermore, in the spontaneous CRC mouse model, supplementation with *Bifidobacterium breve* CCFM683 effectively alleviates CRC progression by promoting tumor cell apoptosis through the conjugated linoleic acid (CLA)- peroxisome proliferator-activated receptor gamma (PPAR-γ) axis.[222] In CRC mice, supplementation with *Bifidobacterium animalis* ssp. *lactis* BX-245 (BX-245) significantly reduced intestinal barrier permeability, alleviated barrier damage, and increased serum IL-2 and IFN-γ levels, thereby effectively reducing the tumor burden.[223]

Importantly, probiotics have the capacity to modulate epigenetic enzymes or factors, thereby influencing aberrant epigenetic mechanisms in the treatment of diseases. The administration of *Lactobacillus casei* (*L. casei*) *LH23* reduces nitric oxide and inflammatory factors levels by suppressing the overactivation of JNK/p38 signaling, thereby mitigating DSS-induced colitis. Notably, supplementation of *L. casei LH23* elevates the level of H3K9ac.[212] Similarly, the administration of *Lactobacillus fermentum* (*L. fermentum*) results in a significant reduction in the expression of miR-155 and miR-223, contributing the amelioration of the microbiota dysbiosis and the restoration of intestinal immune function in mice with colitis.[224] A recent study has been shown that *Lactobacillus plantarum l168* and its metabolite indole-3-lactic acid (ILA) treatment significantly mitigate CRC oncogenesis by regulating T cell immunity in mice models.[214] Mechanistically, ILA promotes the production of IL12a in dendritic cells through augmentation of H3K27ac in enhancer regions of IL12a, thereby contributing to the initiation of $CD8^+$ T cell-mediated immunity against tumor development.[214] Alternatively, an increased *Bifidobacterium* population reduces inflammation in TNBS-induced colitis by epigenetic mechanism that regulate the tolerogenic T cell differentiation. Specifically, treatment with *Bifidobacterium* causes demethylation of several CpG sites of *Foxp3* promoter to enhance immunosuppressive function of Tregs, thereby ameliorating colorectal colitis in rats.[169] Collectively, the findings above emphasize the potential of probiotics in IBD/CRC treatment or prevention by reshaping the composition of gut microbiota and affecting epigenetic programming.

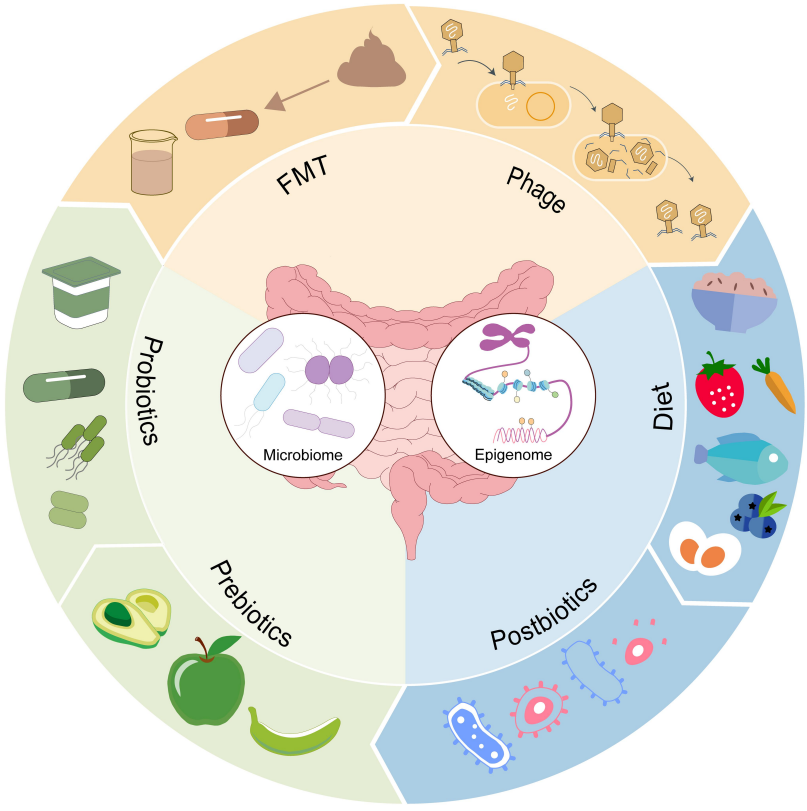

Figure 4. Clinical strategies of gut microbiota in the prevention and treatment of diseases. Strategies for modulating epigenetic changes by regulating gut microbiome composition may include probiotics, prebiotics, postbiotics, diet, fecal microbiota transplantation (FMT) and phage therapy.

Table 3. Probiotics, prebiotics, postbiotics in IBD/CRC prevention.

Probiotic	Function	Epigenetic mechanism	Level of evidence
Lactobacillus casei LH23	Amelioration of intestinal inflammation	Inhibit JNK/p38 signal pathway, enhance histone H3K9ac	DSS- mouse colitis[212]
Lactobacillus fermentum	Amelioration of intestinal inflammation and gut dysbiosis	Reduction the expression of miR-155 and miR-223	DSS- mouse colitis[213]
Lactobacillus plantarum I168/indole-3-lactic acid (ILA)	Amelioration of intestinal inflammation, tumor growth, and gut dysbiosis	Activation $CD8^+$ T cells by increasing H3K27ac binding at the enhancer regions of IL12a	CRC mouse model[214]
Bifidobacterium longum	Amelioration of intestinal inflammation	Activation regulatory T cells by demethylating the *Foxp3* promoter	TNBS-mouse colitis[169]
Prebiotics/postbiotics			
ω-3 polyunsaturated fatty acids	Inhibition of cell growth	Induction of apoptosis by inhibiting *Bcl2*	CRC mouse model[215]
Non-starch polysaccharides	Amelioration of tumor growth, and gut dysbiosis	Change the m^6A methylation by affecting the methyl donor supply	CRC mouse model[216]
SCFAs	Amelioration of intestinal inflammation, tumor growth, and gut dysbiosis	Histone deacetylase inhibition	AOM/DSS-mouse model[165]
P40 (isolated from Lactobacillus casei/paracasei and L rhamnosus phylogenomic)	Promote differentiation of Tregs and protect against colitis	Induce the sustained H3K4me1/3 and *Tgfb* expression	DSS- mouse colitis[217]

Acronym (in the order they appear in the table): JNK, c-Jun N-terminal kinase; DSS, dextran sulfate sodium; H3K9ac, histone H3 acetylation at lysine 9; H3K27ac, histone H3 acetylation at lysine 27; IL12a, interleukin 12 subunit alpha; TNBS, trinitrobenzene sulfonic acid; Foxp3, forkhead box P3; Bcl2, B-cell lymphoma 2; m^6A, *N*6-methyladenosine; SCFAs, short-chain fatty acids; AOM, azoxymethane; H3K4me1, histone H3 monomethylation at lysine 4; H3K4me3, histone H3 trimethylation at lysine 4; Tgfb, transforming growth factor beta.

Prebiotics and postbiotics

Prebiotics refer to organic substances that, while not digested by the host, selectively promote the metabolism of beneficial microbes in the body, thereby improving health. Recently, it is reported that specific prebiotics might disrupt the epigenetic regulation of gene expression, thus contributing to their beneficial effects. Of these, significant attention has been drawn to ω-3 polyunsaturated fatty acids (ω-3 PUFA), which are abundant in fatty fish and nuts. Multiple preclinical experimental studies have consistently shown the potential of ω-3 PUFA to confer protective effects against the initiation and advancement of inflammation and CRC.[225–227] Specially, ω-3 PUFA is found to inhibit the anti-apoptotic gene BCL-2 expression by increasing *Bcl-2* promoter methylation in rat AOM-induced carcinomas.[215,228] Additionally, a proposition that non-starch polysaccharides (NSPs) could potentially modify m^6A level through their influence on the production of methyl donors, thereby mitigating CRC.[229]

Postbiotics encompass the soluble byproducts and metabolites that are produced by the gut microbiota. SCFAs, which are generated through the fermentation of probiotics, represent one of the most prominent instances of postbiotics. In a broader context, the rising interest in dietary HDACi, particularly butyrate, stems from their potential to influence epigenetic mechanisms, offering more targeted therapeutic strategies for disease prevention and treatment. For example, in mouse models, butyrate restricts colorectal cancer proliferation by inhibiting the Wnt/β-catenin signaling cascade through the reduction of HDAC activity.[230] Abundant butyrate inhibits HDAC3/8 in $CD8^+$ T cells, inducing H3K27 acetylation and expression of the *Tbx21* promoter. TBX21 transcriptionally represses PD-1, alleviating $CD8^+$ T cell exhaustion and enhancing the sensitivity of microsatellite stable (MSS) CRC mice to anti-PD-1 therapy.[231] Moreover, SCFAs protect against tumor growth in both mice and humans by inhibiting HDAC, thereby preventing the activation of calcineurin and nuclear factor of activated T cells C3 (NFATc3).[232] Notably, although SCFAs are generally regarded as beneficial, patients with IBD exhibit poor tolerance to fiber-rich diets, suggesting that SCFAs may exert opposing effects under inflammatory conditions. Accordingly, in macrophages treated with TLR agonists, SCFAs inhibit HDAC, suppressing the transcription of c-FLIP and IL-10, thereby triggering caspase-8-dependent NLRP3 inflammasome activation and exhibiting pro-inflammatory effects opposite to those observed under homeostatic conditions.[233] Alternatively, a soluble protein derived from *Lactobacillus rhamnosus GG*, referred to as p40, has demonstrated the ability to alleviating intestinal inflammation.[234–236] Importantly, p40 has been shown to expand Tregs and alleviate gut colitis in adult mice by inducing epigenetic imprint in TGFβ. Specifically, p40 could stimulate *Setd1β* expression, resulting in an increase in H3K4me3 marks at the *Tgfb1* promoter, thereby upregulating TGFβ expression in IECs.[237] Finally, whilst the evaluation of probiotics and prebiotics necessitates meticulous dosage scrutiny for potential adverse effects, they hold the promise of mitigating and managing various digestive disorders, encompassing colitis and gastrointestinal cancers.

FMT

FMT is an emerging microbiome-modulating therapy that has shown efficacy in select clinical settings. It involves transferring a diverse yet incompletely characterized microbial community from a thoroughly screened healthy donor, free of known pathogens, to a recipient. Sequence analyses of fecal samples from individuals with IBD have revealed correlations between the presence or absence of specific bacterial strains or fungi, suggesting that microbiota transplantation from healthy donors could potentially alleviate the disease.[238] A study comparing allo-FMT and auto-FMT via nasoduodenal infusion following bowel cleansing found no statistically significant differences in clinical or endoscopic remission of UC. However, a trend suggested improved remission rates in those receiving allo-FMT compared to auto-FMT, with treatment response appearing to correlate with microbiota composition.[239] A high baseline abundance of *Candida* is associated with clinical response to FMT in UC, while its post-FMT reduction reflects an improvement in disease

severity. However, in low-*Candida* recipients, FMT led to increased inflammation, accompanied by reduced bacterial diversity and elevated *Candida* abundance.[240] This suggests that variations in microbiota composition between donors, as well as temporal shifts in the microbiota of individual donors, may unpredictably influence the efficacy of FMT.

In addition to affecting changes in the gut microbiota composition, the impact of FMT on other aspects of host physiology, such as the epigenome, should not be overlooked. When GF mice received fresh fecal transplants from CRC patients and their healthy controls, they exhibited colonic epithelial turnover, increased precancerous lesions, and elevated DNA methylation in intestinal tissues and blood.[150] A comparison of peripheral blood mononuclear cell DNA methylation results in patients with metabolic syndrome before and 6 weeks after FMT revealed that the *Prevotella* ASV introduced by FMT significantly affected the methylation of the AFAP1 gene, which is associated with insulin resistance.[241] Another study on patients with nonalcoholic fatty liver disease (NAFLD) found multiple differentially methylated CpG sites in the liver of patients receiving FMT, such as the *Tars* gene.[242] Additionally, in patients with systemic lupus erythematosus (SLE), FMT treatment increased the global DNA methylation levels in peripheral blood, along with elevated serum levels of SAM. Notably, methylation levels in the promoter region of interferon-related genes, such as *Ifih1*, were increased, suggesting that FMT may alleviate SLE symptoms by inhibiting the aberrant expression of these genes.[243] Therefore, a comprehensive understanding of FMT-induced epigenetic modifications may contribute to optimizing its therapeutic efficacy. Although clinical trials have demonstrated some benefits of FMT in IBD, the outcomes are generally less pronounced compared to its remarkable efficacy in treating recurrent *Clostridioides* difficile colitis.[244,245] The microbial composition varies significantly among different donors, leading to inconsistent FMT efficacy. Therefore, screening high-efficacy donors based on microbiome sequencing and tailoring personalized FMT strategies according to the gut microbiota composition of IBD patients are essential. Additionally, multi-omics analyses (including transcriptomics, epigenomics, and metabolomics) are crucial for elucidating the long-term effects of FMT in IBD.

Dietary interventions

For the prevention of disease, diet is an important factor that cannot be ignored.[246] It is well known that the incidence of IBD rises with the increasing popularity of Western diets, leading to speculation that diet may be linked to the development of the disease. An overabundance of calories and macronutrients has been found to increase susceptibility to inflammation and infection, thereby raising the risk of autoinflammatory diseases such as IBD. Low-carbohydrate and fiber-deficient diets have been demonstrated to significantly reduce colonic cancer-protective fecal metabolites, exacerbate colitis, and prolong symptoms such as bloody stools.[247] Since milk and dairy products contain high levels of casein and gastro-resistant proteins, they can exacerbate gut dysbiosis, inflammation, and intestinal permeability in mice.[248] Consequently, milk and dairy products are often excluded from most IBD diets, with the exception of yogurt. In a double-blind, placebo-controlled clinical trial, yogurt was administered to IBD patients, resulting in beneficial increases in the average counts of Lactobacillus, Bifidobacterium, and Bacteroides in the feces of the treated group.[249] At the same time, high fat intake should be avoided, as a high-fat diet may lead to the accumulation of secondary bile acids (such as deoxycholic acid), which can inhibit the growth of Bacteroidetes and Firmicutes, resulting in dysbiosis.[250] Conversely, plant-based dietary patterns have been proposed as effective methods for the sustained management of inflammation over the long term.[251] Similarly, in a prospective, single-center, two-year clinical trial, semi-vegetarian diet (SVD) has been shown to offer preventive effects against the recurrence of IBD in individuals who have attained remission.[252] Some of the reasons for this are that plants contain natural products that have preventive and therapeutic effects against diseases.[253,254] More importantly, dietary fiber reduces gut transit time while increasing the synthesis of SCFAs like acetate, butyrate, and propionate, which support intestinal barrier function and

play a positive role in regulating gut inflammation. Certain dietary components, such as polyphenols from fruits and vegetables, can shape the gut microbiota composition, modulate key mucosal markers involved in epithelial barrier integrity repair, alleviate metabolic inflammation, and improve glucose homeostasis.[255] Although it is widely recognized that dietary fiber may reduce the risk of IBD, a prospective cohort epidemiological study observed no clear association between total fiber intake or fiber from specific sources and the development of CD and UC.[256] Conversely, a randomized controlled trial involving 113 UC patients found that, compared to standard drug treatments, multi-donor FMT combined with an anti-inflammatory diet effectively induced deep remission in patients with mild-to-moderate UC, with the benefits lasting for over a year of continued anti-inflammatory dietary intervention.[257]

Specially, in certain cases, dietary alterations can impact tumor-suppressor genes or inflammatory genes by perturbing epigenetic events. To this, several micronutrients are especially important for immunonutrition, such as vitamin D, a fat-soluble vitamin, is present in fatty fish, red meat, liver, and egg yolks, and can also be acquired through dietary supplements. Beyond regulating skeletal, calcium, and phosphorus metabolism, vitamin D plays a critical role in gut defense mechanisms, modulating adaptive and innate immune systems, and preventing microbial invasion of the epithelium.[258] Importantly, study has revealed a negative association between circulating vitamin D and *Sfrp2* promoter methylation in CRC patients.[259] The SFRP2 protein acts as a Wnt antagonist, binding to Wnt proteins and inhibiting signal stimulation.[260] Furthermore, it has been reported that vitamin D regulates histone demethylation in CRC through JMJD3, an H3K27me3-specific demethylase.[261] Alternatively, vitamin D can enhance the sensitivity of CRC cells by increasing miR-1278 expression.[262] Finally, clinical data has shown a positive correlation between the lncRNA MEG3 and serum vitamin D concentrations in CRC patients.[263] Other vitamins, such as vitamins A, C, and E, have been shown to possess potent antioxidant and anti-inflammatory effects.[264] Importantly, clinical trials involving CRC patients and controls with acute non-tumorous diseases have indicated a negative correlation between antioxidant capacity and CRC risk, suggesting that vitamin supplementation may reduce the risk of tumor development.[265]

A famous real-world example is the Mediterranean diet (MedDiet), considered the healthiest dietary pattern in Europe. The MedDiet emphasizes the consumption of large amounts of fresh vegetables, fruits, whole grains, nuts, seeds, and legumes. These foods are rich in fiber, vitamins, minerals, and antioxidants. Research has shown that the MedDiet is associated with higher levels of total SCFAs, which potentially regulate the methylation of inflammation-related genes.[266] Based on this, we recommend adopting a strategy of smaller, more frequent meals, maintaining adequate hydration, avoiding excessive caffeine and alcohol intake, incorporating vitamin and mineral supplements, eliminating dairy products in cases of lactose intolerance, limiting the consumption of excessive fats and carbohydrates, and ensuring regular physical activity. Finally, larger-scale, long-term clinical studies should be conducted to explore the effects of different dietary regimens on patients with various types of gut diseases, while considering individualized interventions. At the same time, modern nutrition science, microbiomics, and personalized genomics should be integrated to develop precision medicine-based dietary intervention strategies.

Phage therapy

Due to the growing issue of antibiotic resistance in bacteria, phages have resurfaced as a potential alternative therapeutic tool targeting specific gut microbiota. Phages, also known as bacteriophages, are self-replicating viruses that infect bacteria with high host specificity.[267] Phage communities are also a major component of the human gut virome, accounting for approximately 90% of the total viral population.[268] Importantly, there are differences in the gut virome between healthy individuals and those with IBD. In a healthy environment, phages of the crass-like and Microviridae families are the most stable colonizers.[269] In fecal samples from IBD patients, a higher prevalence of temperate phages targeting *F. prausnitzii* was observed.[270] This shift in phage

communities can also be observed in a mouse colitis model, characterized by a reduction in *Clostridium difficile*-like phages, alongside an increase in tail-like phages and phages targeting *Alistipes* and *Streptococcus*.[271] However, the relationship between the differences in the virome between IBD patients and healthy individuals and the pathogenesis of IBD remains inconclusive. The underlying reasons for changes in gut phage abundance and diversity are still unclear.

Given that phages can specifically target and eliminate harmful bacteria while preserving beneficial ones, and that they can adapt to bacterial resistance over time, their ability to replicate rapidly and disrupt biofilms makes them a promising tool for treating bacterial infections. Moreover, the advantage of strategically designing various phage combinations to suppress pathogenic strains lies in the prevention of bacterial resistance to treatment. Each phage component targets specific bacterial species through distinct mechanisms, while minimizing off-target ecological disruption to the surrounding microbiome.[272,273] A microbiome analysis conducted on a cohort of IBD patients from four distinct geographical regions ($n = 537$) has revealed a significant correlation between *Klebsiella pneumoniae* (Kp) and the severity of IBD. Subsequently, researchers have developed a lytic combination composed of five phages that effectively targeted both sensitive and resistant Kp strains, successfully reducing inflammation in colitis-prone mice. Finally, proof-of-concept studies conducted in an artificial human gut and among healthy volunteers demonstrate that the phages remained viable and were well tolerated.[274] Similarly, in mice with orthotopic colorectal tumors or spontaneously formed colorectal tumors, oral or intravenous administration of irinotecan-loaded dextran nanoparticles covalently linked to azide-modified phages significantly inhibits the growth of *F. nucleatum* and enhances the efficacy of first-line chemotherapy for CRC.[275]

These findings are undoubtedly encouraging for the development of novel phage therapies related to IBD and CRC; however, they also present certain challenges. The main challenges include immunogenicity, bacterial resistance, bioavailability, and safety concerns. Engineer phage surface structures to reduce their immunogenicity or utilize phages that are naturally less immunogenic. PEGylation (adding polyethylene glycol) can also be employed to shield phages from the immune response.[276] To combat bacterial resistance, it is important to develop rationally designed phage cocktails that include multiple phages targeting different bacterial strains or to use them in combination with antibiotics to reduce the likelihood of resistance development. Additionally, personalized phage therapy is emerging as an alternative strategy for patients with infections resistant to all known antibiotics. The primary challenge of personalized phage therapy lies in the significant diversity of phages, making it labor-intensive to identify suitable phages that can target resistant infections. Therefore, developing high-throughput platforms for rapid identification of phages tailored for personalized therapy is essential.[277] However, there are still concerns regarding the safety and durability (both short-term and long-term) of this approach, particularly in immunocompromised patients. In summary, the ability of bacteriophages to adapt and overcome bacterial resistance further reinforces their viability as a therapeutic option for intestinal diseases. To better define the role of phages in the treatment of intestinal diseases, larger-scale randomized controlled trials are needed.

Conclusion

Limitations

Overall, gut microbiota and epigenetic modifications are intricately connected, with microbiota-derived metabolites significantly influence key epigenetic processes such as DNA methylation, RNA m6A and histone modifications, thereby shaping cellular function and health. Notably, non-covalent histone modifications, like lactylation and crotonylation, emerge as critical mediators through which gut microbiota regulates gene expressions, impacting diverse physiological outcomes. Nevertheless, current research faces several limitations, with the primary issue being the uncertainty regarding causality. Whether the microbiota directly causes these epigenetic modifications is still undetermined. Many studies struggle to differentiate correlation from causation due to their models and

experimental designs. To better infer causal relationships, research must embrace more rigorous and innovative design and methodology. Firstly, controlling variables and using reverse experimental designs is essential. Secondly, longitudinal studies to monitor gut microbiota changes and associated epigenetic alterations will aid in establishing a causal timeline, distinguishing between long-term and short-term effects.

When studying microbiome function, issues related to sample selection and animal models are inevitably encountered. Due to ethical considerations and numerous uncontrollable variables, fecal samples are chosen for most microbiological analyses. This might not accurately reflect the actual function of gut microbiota and could potentially introduce bias into clinical diagnoses. The sample sizes in many studies are limited, and variations in experimental conditions, equipment, and reagents across different batches can lead to batch effects, increasing the randomness and error in results, making them difficult to reproduce. On the other hand, rodents associated with the human microbiota have emerged as a fundamental element in microbiome research, but the recipient rodents lack the ecological components such as lifestyle, that originally lead to dysbiosis in human, thereby disease-associated alterations are not replicated in rodent model.[278] For example, IBD is exclusive to humans, as analogous disorders observed in non-human primates exhibit significant disparities.[279] Consequently, utilizing animal-based environmental and nutritional models for investigating the etiology of IBD presents inherent limitations.

Beyond the inherent limitations of the samples and models, the current understanding of gut microbiota functions remains incomplete. Most research to date has predominantly focused on bacteria, with a notable paucity of studies addressing fungi and viruses. The primary reason is likely that, compared to bacterial community, the gut fungal community, which constitute approximately 0.1% of the entire microbiome, are lower in both abundance and diversity.[280] Given the low fungal diversity in individual fecal samples, which compromises the authenticity of experimental results. The choice of amplicon (18S, ITS1, or ITS2), amplicon primers, and reference databases significantly influences the identification of fungi. Indeed, fungi also play a profound role in maintaining gut homeostasis and influencing the development of disease.[281] Although a definitive conclusion remains elusive, some evidence suggests that fungi may influence the progression of IBD, reflected in alterations of the mycobiome and abnormal immune responses to fungi in IBD patients, and the tangible impact of different fungal species on the severity of IBD.[282] Moreover, some evidence suggest that specific fungi species do influence CRC. For example, *Candida* species and *Aspergillus rambellii* have a well-established link with human colon tumors, and xenotransplantation with *Aspergillus rambellii* can accelerate tumor growth.[283,284]

Compared to bacteria and fungi, the gut virome has been relatively understudied. In addition to their difficulty in detection, viral genomes do not contain the diverse array of common marker gene sequences found in bacterial genomes.[285] However, advancements in technology have facilitated more sophisticated analyses of the gut virome. In a study published in 2020 by Gregory and his colleagues, the assembly of 2,697 gut metagenomes from 32 studies revealed a total of 33,242 viral populations.[286] Particularly, a connection between the gut virome and IBD has been proposed. A more recent study by Tian and his colleagues, employing virus-like particle (VLP) metagenomic and bulk metagenomic sequencing technologies, identified 139 differentially abundant viral markers in IBD patients from China.[287] Simultaneously, experiments involving fecal viral particle transplantation (FVT) in mouse models demonstrated that mice receiving the gut virome from IBD patients (IBD-FVT mice) exhibited more severe colitis symptoms.[287] This finding highlights the potential of FVT as a viable therapeutic strategy for individuals with IBD. However, the limitations of viral community detection technologies, being confined to either VLP or bulk metagenomics, and the incomplete nature of human gut virome databases restrict the exploration of gut virome functions within specific disease contexts. Overall, the limitations of microbiome research methodologies, including sample collection, analytical techniques, and experimental models, may obscure the identification and functional characterization of specific microorganisms, resulting in misinterpretations of the findings.

Conclusion and future perspectives

To address these limitations, it is essential to implement standardized research methods. Establishing consistent standard operating procedures across all stages – sample collection, storage, DNA extraction, sequencing, and data analysis – can help minimize the impact of technical variations on the results. Secondly, increasing sample sizes and fostering multicenter collaborations are crucial. Larger sample sizes can effectively reduce the impact of individual variability and enhance statistical reliability. Multicenter collaborations allow for validation of results across different environments, helping to identify broadly applicable conclusions. Additionally, encouraging open data sharing and establishing public databases for gut microbiome data can facilitate cross-validation and further analysis, enhancing data reuse and result verifiability. Regarding the issue of animal models, the use of mouse models remains essential; however, incorporating 3D organoid models that more closely replicate human tissue characteristics can provide valuable insights.

Significant advancements have been made in the development of methodologies aimed at elucidating the pivotal roles of the microbiome and understanding the epigenome in both health and disease. In this review, we concentrate primarily on the gut microbiome-epigenetic axis, as it lies at the core of the pathogenesis of intestinal diseases. The prospects for developing therapeutic strategies targeting the gut microbiome are promising, as probiotics, prebiotics, postbiotics, dietary interventions, FMT and phage therapy offer significant potential for disease treatment and prevention. Moreover, avoiding environmental pollution plays a pivotal role in disease prevention, particularly regarding the impacts of smoking on IBD. Unfortunately, focusing solely on the role of the gut microbiome is insufficient. Because various environmental influences from the perinatal period to adulthood, such as diet, smoking, pollution, and antibiotics, along with factors like breastfeeding, geographic location, physical activity, modern lifestyle, socioeconomic status, psychological state, educational level, and mode of birth, can indeed affect the risk and natural history of these complex, immune-mediated diseases.[288] However, due to our limited understanding of gut diseases, we have not thoroughly integrated every clue regarding their impact. In the era of omics data explosion, these technologies are instrumental in enhancing our comprehensive understanding of the key mechanisms involved in disease progression. Therefore, future research should employ multi-omics big data approaches, necessitating the integration of diverse multi-layered information, including clinical data, environmental exposures, microbial composition, genetics, epigenetics, and immune function. In particular, the next-generation long-read metagenomic technologies, such as PacBio HiFi and Oxford Nanopore, can more accurately assemble complex microbial genomes and resolve repetitive regions and structural variations within the genome. Another recent advancement is single-cell spatial omics. Through spatial transcriptomics and metabolomics, we can better identify the distribution of genes and metabolic products within specific regions. Single-cell microbiomics allows the acquisition of genomic, transcriptomic, and epigenomic information at the level of individual microbial cells. Through microfluidic sorting and single-cell sequencing technologies, it is possible to identify and characterize rare species and their functional traits within complex microbial communities. This approach is particularly well suited for studying microbial community heterogeneity and aids in understanding the role of specific microorganisms in complex environments.[289,290]

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Author contributions

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