Dietary metabolites derived from gut microbiota: critical modulators of epigenetic changes in mammals

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The mammalian gastrointestinal tract harbors trillions of commensal microorganisms, collectively known as the microbiota. The microbiota is a critical source of environmental stimuli and, thus, has a tremendous impact on the health of the host. The microbes within the microbiota regulate homeostasis within the gut, and any alteration in their composition can lead to disorders that include inflammatory bowel disease, allergy, autoimmune disease, diabetes, mental disorders, and cancer. Hence, restoration of the gut flora following changes or imbalance is imperative for the host. The low-molecular-weight compounds and nutrients such as short-chain fatty acids, polyamines, polyphenols, and vitamins produced by microbial metabolism of nondigestible food components in the gut actively participate in various epigenomic mechanisms that reprogram the genome by altering the transcriptional machinery of a cell in response to environmental stimuli. These epigenetic modifications are caused by a set of highly dynamic enzymes, notably histone acetylases, deacetylases, DNA methylases, and demethylases, that are influenced by microbial metabolites and other environmental cues. Recent studies have shown that host expression of histone acetylases and histone deacetylases is important for regulating communication between the intestinal microbiota and the host cells. Histone acetylases and deacetylases influence the molecular expression of genes that affect not only physiological functions but also behavioral shifts that occur via neuroepigenetic modifications of genes. The underlying molecular mechanisms, however, have yet to be fully elucidated and thus provide a new area of research. The present review provides insights into the current understanding of the microbiota and its association with mammalian epigenomics as well as the interaction of pathogens and probiotics with host epigenetic machinery.

INTRODUCTION

The human gut microbiome is a multifaceted ecosystem that harbors a stunning number of microbes – approximately 100 trillion – representing about 5000 species.[1] An estimated 90% of cells present in the human body are of prokaryotic origin, belonging to some 40 000 bacterial strains in 1800 genera.[2,3] The collective number of these microbes is far greater than the total number of host cells, and both the number and the diversity of these microbes play an important role in the establishment and maintenance of body health. Gut microorganisms co-evolve with their host and are imperative for the development of a healthy gut. They are also important for normal daily gastrointestinal tract functions such as digestion, absorption, and immune function as well as for protection against colonization by pathogens.[4] These microbes also synthesize important

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compounds like K and B vitamins, break down cholesterol, produce short-chain fatty acids (SCFAs) like butyrate, and digest dietary polysaccharides that would otherwise be left unmetabolized.[5] A well-balanced gut microflora also prevents colonization of the gut by pathogens and is thus essential for the well-being of an individual.[6] The interaction between the gut microbiota and the host promotes the mutual cooperation and functional stability of this complex gut ecosystem. Many human diseases, such as asthma, diabetes, obesity, autism, cancer, allergy, and inflammatory bowel disease, are influenced by complex interactions between mammalian genes and the environment.[7–9]

Epigenetics is the study of mitotically and meiotically heritable changes in gene function that are independent of DNA sequence.[10] The epigenome or the overall epigenetic state of an organism is just as important to normal development as is the contribution of the parent genome. Currently, epigenetics is considered to be at the epicenter of modern medicine because it helps to explain the relationship between individual genotype and the environment during all stages of life, and epigenetic perturbations may lead to serious health issues.[11,12] Epigenomic reprogramming of the cell genome and post-translation modification of gene expression are essential mechanisms for the development, regeneration, and postpartum life of higher eukaryotic organisms. These mechanisms influence cell proliferation, cellular stress events, aging and DNA repair, lifelong circadian drifts, equilibrium between mitosis and apoptosis, modification of bacterial and host cell quorum sensing, host bacteria crosstalk, gene regulation, pathogen virulence, DNA replication scheduling, and DNA repair.[13]

Environmental factors (nutrients, toxins, infections, and hypoxia) can have profound effects on the epigenetic signature of higher organisms and may trigger susceptibility of these organisms to disease.[14] Epigenetic mechanisms integrate environmental changes at the cellular level, thereby enabling cellular plasticity. The main epigenetic modifications that alter the accessibility of DNA to transcriptional machinery and influence gene expression are acetylation, methylation, phosphorylation, and biotinylation. These modifications occur on either the DNA itself or on the histone octamer around which DNA is coiled. Although DNA must be tightly compacted in order to fit into the nucleus, it must also be temporarily accessible to transcriptional machinery for expression of proteins to reveal their phenotypic character. Therefore, mechanisms of chromatin uncoiling and recoiling that are activated by the addition and removal of specific chemical groups known as *epigenetic marks* are thought to regulate chromatin architecture, which influences gene expression in response to environmental factors.

The methylated DNA, acetylated proteins, micro RNA (miRNA), specific substrates, cofactors, and enzymes involved in various biochemical reactions associated with epigenomic processes could serve as biomarkers for detecting the progression of various diseases.[12,15] Recently, it has been established that various infectious agents like Epstein-Barr virus, hepatitis viruses B and C, human papilloma virus, polyomaviruses, *Streptococcus bovis*, *Chlamydia pneumoniae*, *Campylobacter rectus*, *Helicobacter pylori*, and others induce changes at the epigenetic level that result in the onset and progression of certain diseases.[16] Similarly, the global DNA methylation patterns associated with greater susceptibility to cardiovascular disease as a result of abnormal lipid metabolism and inflammatory responses have been linked to a microbiome dominated by bacteria in the phyla Firmicutes.[17] However, the mechanism of these epigenetic modifications and the various signaling cascades associated with them are still uncertain. This review has a twofold objective. First, it highlights the role of various enzymes associated with epigenetic changes, such as histone acetyltransferases (HATs), histone deacetylases (HDACs), DNA methyltransferases (DNMTs), and kinases. Second, it examines the potential effect of gut microbes and their secondary metabolites/nutrients on the epigenome via epigenetic modification and RNA interference.

REPROGRAMMING OF THE EPIGENOME BY THE GUT MICROBIOTA

Chromatin undergoes sequence-independent epigenetic modifications like DNA methylation, histone acetylation, phosphorylation, biotinylation, and RNA interference, all of which turn genes on and off without changing their original sequence. DNA methylation is generally associated with the suppression of gene transcription, whereas histone methylation may mediate either transcription activation or transcription repression, depending on which amino acid residue of histone is methylated. Acetylation and phosphorylation of histones typically enhance gene expression, in contrast to biotinylation, which usually represses gene expression. Micro RNA, via RNA interference, suppresses the expression of epigenetic-associated and other genes, either by binding directly with the respective messenger RNA (mRNA) sequences of the genes or indirectly by binding with different histone modifiers. The various enzymes associated with epigenetic modifications include methyltransferases, deacetylases, acetyltransferases, phosphotranferases, BirA ligase, and serine/threonine protein kinases. These key epigenetic players

Table 1 **Enzymes associated with important epigenetic modifications**

Enzyme	Epigenetic modification	Consequence
Histone acetyltransferases (HATs) GNAT (Gcn5 and PCAF) MYST (MOZ), (TIP60), (MORF), and (HBO1) CBP/p300 and SRC	Histone acetylation	Transcriptional activation by promoting open conformation of chromatin and gene-specific recruitment of coactivators[19,20]
Histone deacetylases (HDACs) Classes I, II, and IV (Rpd3/Hda1 family) Class III (sirtuin family)	Histone deacetylation	Gene repression by promoting chromatin condensation[21]
Histone methyltransferases (HMTs)	Methylation at histone lysine residues	Transcriptional activation via H3K4me3 Transcriptional repression via H3K9me and H3K27me[22]
Histone demethylases (HDMs) KDM1–KDM6	Histone demethylation at lysine residues	Transcriptional activation or repression, depending upon lysine residue[23]
DNA methyltransferases (DNMTs) DNMT1, DNMT3a, DNMT3b, and DNMT2	Maintenance of and de novo DNA methylation	Suppression of gene expression[24]
DNA demethylases (DNDMs) TET and IDH families	DNA demethylation	Activation of gene expression[25,26]
Holocarboxylase synthetase, biotinidase, and BirA ligase	Histone biotinylation	Transcriptional activation[27]
Kinases	Histone phosphorylation	Increase in gene expression[28]
miRNAs		
miR-29 family	DNA methylation	Induction of tumor suppressor genes[29]
miR-101 and miR-137	Histone methylation	Repression of methyltransferase EZH2[30]
miR-449a	Histone demethylation	Repression of HDAC-1[31]
miR-744, miR-1186, and miR-466d-3p	Accessibility to transcriptional machinery	Induction of gene expression[32]

Abbreviations: miR, micro RNA; PCAF, protein (CBP)-associated factor).

have been found to be directly or indirectly influenced by the presence of some low-molecular-weight compounds/nutrients of gut microbial origin.[18] Of these enzymes, HATs and HDACs are best understood (Table 1).[19–32]

ACETYLATION AND DEACETYLATION

Four families of HATs have been characterized in humans, the first being the general control nonderepressible 5 (GNC5)-related *N*-acetyltransferase (GNAT), which includes GNC5 and its ortholog p300/CREB-binding protein (CBP)-associated factor (PCAF). The second family, MYST, includes monocytic leukemia zinc finger protein (MOZ), the 60-kDa Tat-interactive protein (TIP60), MOZ-related factor (MORF), and HAT bound to ORC1 (origin recognition complex 1) (HBO1).[19] The third family, p300/CBP, consists of p300 and CBP, and the fourth, the steroid receptor coactivator (SRC) family, consists of SRC-1, nuclear receptor coactivator ACTR, SRC-3, and TATA box-binding (TBP)-associated factor (TAF).[20]

Eighteen HDACs have been characterized. These have been further categorized into 4 groups on the basis of their homology and subcellular location in yeast.[21] The deacetylase activity of HDACs in groups I, II, and IV, which belong to the Rpd3/Hda1 family, is zinc dependent, whereas that of the HDACs in group III, categorized in sirtuin family, requires the presence of cofactor nicotine adenine dinucleotide.[33] Histone acetyltransferases facilitate the addition of acetyl moieties on lysine residues of histone tails, resulting in transcriptional activation, whereas HDACs remove these moieties, resulting in transcriptional repression. Histone deacetylases carry out deacetylation by interacting with different transcriptional factors, and reports have suggested that microbial metabolites derived from dietary intake, bacterial lipopolysaccharide (LPS), and endogenous hormones have an enormous influence on HDAC activities.[22,34]

Interestingly, HDACs, in comparison with HATs, seem to be more strongly influenced by a number of microbial metabolites. For example, SCFAs, such as butyrate and propionate, produced by gut microbes like *Faecalibacterium prausnitzii* and *Eubacterium hallii*, respectively, inhibit HDAC enzymes and alter the expression of specific genes via conformational changes in the active site of HDAC, resulting in HDAC inactivation.[35] In addition, the microbial (*Bacteroides thetaiotaomicron)* metabolism of cruciferous vegetables results in the production of sulforaphane cysteine and sulforaphane *N*-acetyl-cysteine, and the metabolism of garlic produces allyl mercaptan and diallyl disulfide. These metabolites are all potent inhibitors of histone deacetyl transferase enzymes.[36] The in vitro anticancer and apoptopic properties of butyrate, a microbial product, and other HDAC inhibitors in different colon cell lines have also been reported.[37] Treatment of HCT116 colon

cells with butyrate acetylated the SP1 ubiquitous transcriptional factor, which negatively regulated the genes involved in cell cycle regulation and apoptosis. The acetylation of SP1 decreased the ability of SP1 to bind at promoters of the antitumor p21 gene and the proapoptotic BAK gene, which subsequently enhanced the expression of these genes, thus promoting apoptosis and arresting the progression of the cell cycle at the G2/M transition resulting in inhibition of cancer. SP1 is known to be associated with class I HDACs, and its decreased binding ability may provide an alternative way to enhance antitumor activity by utilizing the transcription activator molecule SP3.[37]

METHYLATION AND DEMETHYLATION

Another well-understood epigenetic modification involves DNA and histone methylation, and the enzymes responsible are the methyltranferases. DNA from most prokaryotes and eukaryotes contains the methylated bases 4-methylcytosine, 5-methylcytosine, and 6-methyladenine. The modifications due to methylation of bases take place after DNA replication by DNMTs. Methylation involves covalent attachment of a methyl group to cytosine and adenine as well as to histone proteins at several arginine and lysine residues in their N-termini. In animal, plant, and microbial cells, about 50 to 100 different DNMTs have been identified thus far, including more than 20 lysine and 10 arginine histone methyltransferases.[13] Mammalian DNMTs have been divided into 4 families on the basis of their function. The DNMT1 family has maintenance functions, copying pre-existing methylation patterns onto the new DNA strand during DNA replication; the DNMT3a and DNMT3b families are de novo methyltransferases; and the fourth family, DNMT2 methyltransferase, has little involvement in setting DNA methylation patterns.[24] On other hand, DNA demethylases, such as the dioxygenase TET (10–11 translocation) proteins and isocitrate dehydrogenase, mediate the removal of methyl groups. TET, by successively oxidizing 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine, helps in DNA demethylation, whereas as a mutated isocitrate dehydrogenase, instead of converting isocitrate into α-ketoglutarate (αKG), produces D-2-hydroxyglutarate. D-2-hydroxyglutarate, which competes directly with αKG, inhibits αKG-dependent dioxygenases involved in DNA demethylation.[25,26] Lysine histone demethylases (KDMs), numbered from KDM1 to KDM6, also demethylate specific lysine residues by involving $Fe2^{+}/2^{-}$ oxoglurate (2-OG) during their catatysis and can either activate or repress gene expression.[23] Methylation and demethylation are both reversible and are involved in gene suppression and activation, respectively, in a wide variety of prokaryotes and eukaryotes.

Commensal gut microbes are reported to change the methylation pattern of epithelial cells and thus may modulate cellular functioning. Takahashi et al.[38] showed that microbes are important for maintenance of *TLR4* gene methylation in mice intestinal cells. In the presence of bacteria, CpG motifs of *TLR4* had significantly higher methylation frequencies in intestinal cells than in $CD45^{+}$ splenic cells and thereby averted an excessive inflammatory response. Similar results were obtained in vitro using different human cell lines. This established the anti-inflammatory activity of gut microbes via increased methylation at the *TLR4* promoter. Interestingly, the frequency of methylation of CpG motifs in the 5′ region of the *TLR4* gene of large intestinal cells was significantly lower in germ-free mice than in conventional mice, whereas the frequency of methylation of CpG motifs in small intestinal cells was almost the same in germ-free and conventional mice, suggesting that this mechanism was more important in the large intestine than in the small intestine. On the other hand, signals derived from commensal gut microbes also help to mount an effective response against intracellular pathogens by inducing requisite inflammatory responses. The presence of commensal bacteria in the gut of mice promotes transcriptionally active H3 trimethylation of various inflammatory genes, including *IL-6* and *IFNb1*. This induces the priming of natural killer cells and thus augments immunity against intracellular pathogens in conventional mice compared with genetically modified mice.[39] These reports clearly suggest the critical association of commensal gut microbes and their metabolites with the epigenetic machinery of host cells. Moreover, the epigenetic machinery seems significantly influenced by drug and food intake as well as by microbially produced secondary metabolites/nutrients.

BIOTINYLATION

Biotin is consumed from wide range of food sources, although egg yolk, milk, and some vegetables are considered to be the richest dietary sources. Biotin deficiency and supplementation are both common in North American and African nations. About 50% of pregnant American women have biotin deficiency or overdose, which is likely to affect the gene expression and genome stability. Biotinylation is an epigenetic process in which gene expression is repressed by covalent attachment of biotin on histone H2A at K9, K13, K125, K127, and K12; on histone H3 at K4, K9 and K18; and on histone H4 at K8 and K12.[40] Mammalian cells cannot synthesize biotin, and thus normal histone

biotinylation is dependent on a constant supply of biotin from food and intestinal microbiota. Histone biotinylation in mammalian cells is catalyzed by holocarboxylase synthetase, biotinidase, and the microbial nonselective enzyme BirA ligase. BirA ligase plays an important role in biotin biosynthesis through cell signaling and chromatin remodeling in prokaryotes, but holocarboxylase synthetase has been reported to play the same role in eukaryotes.[27]

In human and mouse cell lines, the covalent binding of biotin catalyzed by holocarboxylase synthetase to lysine 12 in histone H4 (H4K12bio) and lysine 9 in histone H2A (H2AK9bio) represses transcription of retrotransposons. Exhaustion of H4K12bio and H2AK9bio in biotin-deficient cells was correlated with increased production of viral particles and a greater frequency of transposition events, leading to chromosomal instability.[40] Biotinylation of K16 in histone H4 has been found to repress gene expression through chromatin condensation and resulted in greater inhibition than biotinylation of the H4K12 residue.[41] Treatment with a combination of biotin and folate synergistically repressed the long-terminal repeats in human T-lymphoma Jurkat cells and monocytic myeloid U937 cells via an epigenetic mechanism mediated by holocarboxylase synthetase. However, the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-6 increased the response to biotin supplementation when U937 cells in folate-supplemented medium were cultured in media with defined concentrations of biotin.[42] Xue et al.[43] demonstrated the increased expression of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) via holocarboxylase synthetase–dependent biotinylation of extracellular heat-shock protein SP72 in human embryonic kidney 293 cells. In human embryonic kidney 293 cells, Bao et al.[44] also observed that biotin regulated the synthesis of holocarboxylase synthetase at its physiological concentration via increased expression of miR-539, suggesting a significant contribution of biotin to the regulation of cellular functioning.

PHOSPHORYLATION

Phosphorylation events catalyzed by different types of kinases and phosphoprotein phosphatases regulate gene expression at the epigenetic level. Colonization by the commensal gut microbe *Bacteroides vulgatus* within the rat gut induced phosphorylation and nuclear translocation of RelA of the nuclear factor kappa B(NF-κB) complex in intestinal epithelial cells. RelA, after nuclear transport, binds to the IL-6 gene promoter and increases both acetylation of H3K9 and phosphorylation of serine 10 on histone H3, which results in enhanced IL-6 expression.[28] The gut microbiota, via phosphorylation, may also change the expression of nonhistone cytosolic proteins such as tissue factor, as confirmed by in vivo trials. Colonization of germ-free mice by members of normal microbiota promoted phosphorylation of tissue factor cytoplasmic domain by activation of protease-activated receptor-1 and angiopoietin-1, which in turn activated the proangiogenic factor angiopoietin-1 to bring about vascular remodeling.[45] Increased vascularization of the small intestine led to greater oxygenation of the villi and thus promoted higher nutrient absorption, as confirmed by increased adiposity in conventionally raised mice.[46]

RNA INTERFERENCE

RNA interference is an epigenomic process in which genes are turned off post transcriptionally by a group of small, noncoding, endogenous RNA molecules called *miRNA* by binding at the 3′-untranslated regions (3′-UTRs) of target mRNA or to specific epigenetic modifiers. These small RNA molecules, termed *epi-miRNAs*, are themselves regulated at the epigenetic level and also modulate epigenetics of the host cell as well. For example, members of the miR-29 family downregulated the expression of DNMT3a and DNMT3b in lung cancer cells by targeting the 3′-UTR regions of these DNMTs.[29] In neural stem cells, the histone methyltransferase EZH2, which suppresses gene transcription via trimethylation of histone H3 at lysine 27, is also downregulated by miR-101 and miR-137 via complementary binding with the 3′-UTR region.[30] Similarly, HDAC1, which is frequently overexpressed in many types of cancers, is downregulated by miR-449a in prostate cancer cells. This occurs when miR-449a binds the complementary 3′-UTR region in HDAC1, thus diminishing its cancerous effects.[31]

Micro RNA can also directly modulate gene expression by targeting the promotor region of specific genes. As reported by Place et al.,[47] the transfection of PC-3 cells with miR-373 enhances the expression of E-cadherin and cold shock domain-containing protein C2 by enriching the RNA polymerase II within promoter sequences of both genes. In silico analysis further confirmed the presence of complementary promoter sequences for miR-373, which helps recruit various transcriptional factors like Ago1, dicer, and polycomb proteins to the promotor region of nuclear factor I-A.[48] miR-373 also controls expression of nuclear factor I-A, either by repression of H3K27 trimethylation or induction of H3K4 trimethylation, resulting in either granulopoiesis or erythropoiesis in humans. Huang et al.[32] further proved the binding of miR-744, miR-1186, and miR-466d-3p within the complementary promotor

sequences of the cyclin B1 gene. The binding of Ago1 and RNA polymerase II also induced cyclin B1 expression.

The expression of these small molecules, also called *biological microprocessors*, is heavily dependent on the gut microbial community.[49] The colonization of germ-free mice by members of a normal microbiota changes the expression of miRNA in both the ileum and the colon, which ultimately modulates host gene expression.[50] Among a large number of genes studied, *Abcc3*, a multidrug resistance gene, was identified as a highly likely target of mmu-miR-665 miRNA, which downregulated *Abcc3* mRNA and proteins levels by targeting the 3′-UTR region.[50] The same results were found in vitro when the murine macrophage RAW 264.7 cell line was used. Commensal gut microbes also regulate the expression of various miRNAs like miR-143, miR-148a, miR-200b, miR-200c, and miR-378, whose levels otherwise decrease upon infection with *Listeria monocytogenes*.[51] Commensal gut microbes like *Escherichia coli* and A4 bacteria suppress the expression of miR-10a in dendritic cells via toll-like receptors that activate an MyD88-dependent pathway. This ensures the expression of the IL-12/IL-23p40 molecule, which is important for inducing Th1 and Th17 cell differentiation. Micro RNA miR-10a, implicated in the development of various tumors, is negatively regulated by the gut microbes and could serve as an important target for treatment of inflammatory bowel disease.[52] Probiotics also seem to modulate the expression levels of various host miRNAs, thereby regulating gene expression. As confirmed by in vitro trials, treatment of T84 epithelial cells with probiotic *E. coli* Nissle 1917 decreases cellular levels of miR-203, miR-483-3p, and miR-595, which target tight-junction genes and bind to their 3′-UTR regions, promoting gut barrier disintegrity.[53] Approximately 30% of eukaryotic genes are regulated by miRNAs, and even prokaryotic genomes are reported to possess the sequences that code for miRNAs.[13] However, the mechanisms of miRNA action are yet to be fully established, and the association between miRNAs and cellular processes at the molecular level during health and disease has not been explored.

MICROBIAL METABOLITES: POTENT EPIGENETIC MODULATERS

The endogenous gut microbiota produces diet-dependent, low-molecular-weight compounds such as vitamins and SCFA polyamines (Figure 1), as well as diet-independent products such as LPS (gram-negative cell wall component) and peptidoglycan (gram-positive cell wall component), within the intestinal microenvironment.[54] These commensal bacterially derived by-products, especially butyrate, serve as the primary source of nourishment for colonocytes and are able to maintain their normal phenotype and cellular homeostasis.[55] These metabolites also modify the epigenome of host cells, and the epigenetic changes in turn alter the development and functions of the cell as well as regulate gene expression[56] throughout life of an individual (Table 2).[57–61]

SHORT-CHAIN FATTY ACIDS

Of the microbial metabolites, SCFAs have received considerable attention because of their predominantly beneficial effects. Short-chain fatty acids, like butyrate, acetate, propionate, succinate, and lactate, are produced by fermentation of nondigestible carbohydrates like dietary fibers and resistant starch by the gut microbiota. These metabolites can be incorporated by intestinal epithelial cells or can diffuse across the epithelium into the underlying intestinal lamina propria.[62] Short-chain fatty acids have been found to activate G protein–coupled receptors, such as GPR41 and GPR43, although G protein–coupled receptors can also be regulated independently of SCFAs.[63,64] Short-chain fatty acids are well known for their multiple roles in host cells. For example, butyrate regulates transepithelial fluid transport, reduces mucosal inflammation, strengthens the epithelial defense barrier, lowers total cholesterol, enhances fetal hemoglobin during hemoglobinopathic conditions, and prevents insulin resistance, diabetes, metabolic diseases, and ischemic injuries.[65] Moreover, SCFAs control key regulator molecules by inhibiting HDACs and their antimitogenic activities.

ACETATE

Acetate is a product of microbial fermentation of dietary fiber and is known to play an important role in maintaining homeostasis within the mammalian gut. Supplementation with acetate has been reported to attenuate neuroglial activation and cholinergic cell loss in a rat model of LPS-induced neuroinflammation by increasing the acetylation of brain histones H3 at lysine 9 and H4 at lysines 8 and 16.[66] In addition, supplementation with acetate resulted in significantly reduced expression levels of HDAC-2 but an insignificant change in HAT expression. However, compared with the extent of HDAC inhibition after supplementation with butyrate, the extent of HDAC inhibition was comparatively less.

Acetate also has a potent role in the modulation of inflammatory cytokines in primary astrocytes. In vitro treatment of primary astrocytes with acetate downregulated the expression of proinflammatory cytokines such

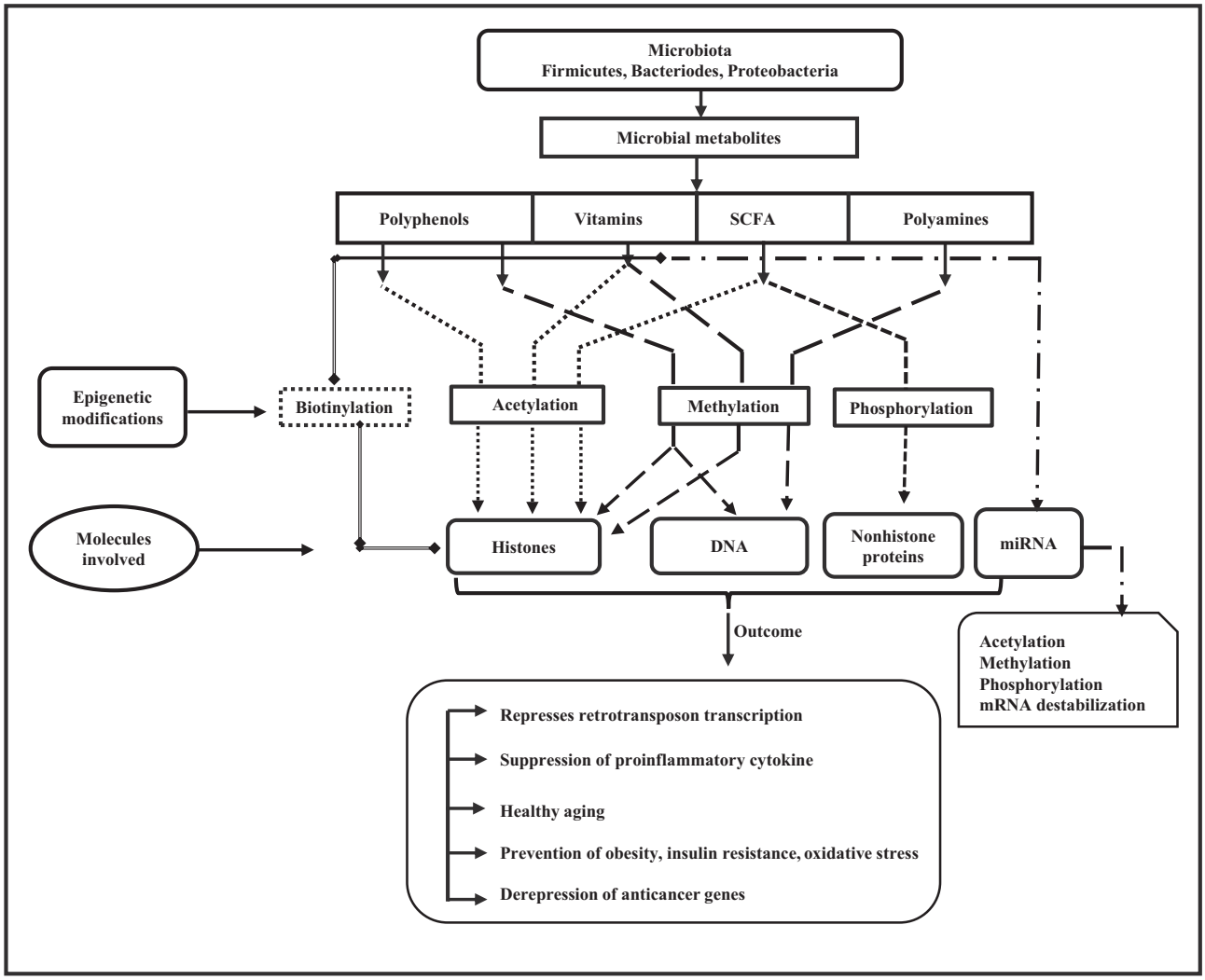

Figure 1 **Epigenetic regulation by gut microbial metabolites.**

Table 2 **Epigenetic modifications caused by intermediate metabolites produced by gut microbes**

Metabolite	Epigenetic modification	Consequence
2d (polyamine analogue)	Inhibition of LSDM	Activation of *e-cadherin* gene via H3K4 hypermethylation[57]
Acetate	Phosphorylation and acetylation	Reduction in proinflammatory cytokines IL-1β and TNF-α and increase in anti-inflammatory molecules like TGF-β1 and IL-4[58]
Butyrate	Inhibition of HDACs	Activation of oxidative resistance genes like *FOXO3A* and *MT2* via acetylation at their respective promoters[59]
EGCG	Inhibition of DNMTs and HDACs Increase in H3 and H4 acetylation	Rearticulation of tumor-suppressor genes like $p16^{INK4a}$ and *Cip1/p21*[60]
Fisetin	Activation of HDACs and suppression of HATs	Inhibition of inflammatory molecules NF-κB, IL-6, and TNF-α[61]

Abbreviations: 2d, 1,15-bis {N^5 [3,3-(diphenyl) propyl]–N^1 biguanido}4,12-diaza pentadecane); DNMTs, DNA methyltransferases; EGCG, epigallocatechin-3-gallate; HDAC, histone deacetylase; LSDM, lysine-specific histone demethylase.

as IL-1β and TNF-α, whereas expression of the anti-inflammatory cytokines transforming growth factor (TGF) β1 and IL-4 was upregulated.[58] The treatment was also effective in reducing the phosphorylation of extracellular signal–related kinases (ERKs) 1 and 2, the p65 subunit of NF-κB at serine 536, and mitogen-activated protein kinase (MAPK) p38. Acetate supplementation also increased H3K9 acetylation, which is

responsible for increased expression of anti-inflammatory molecules. Under in vitro microglia culture, acetate treatment reversed the LPS-induced H3K9 hypoacetylation and decreased the expression of proinflammatory cytokines IL-1β, IL-6, and TNF-α. Moreover, expression of the anti-inflammatory cytokines TGF-β1 and IL-4 increased upon acetate treatment, implying a gene-specific effect of microbial gut metabolites. Interestingly, coincubation with acetate also reduced the levels of LPS-induced nonhistone proteins such as total NF-κB and p65.[67] These results clearly suggest that the anti-inflammatory effect of acetate is largely associated with a disruption in MAPK and NF-κB signaling. In a rat model of Lyme neuroborreliosis, Brissette et al.[68] observed significant reduction in the activation of microglia and the expression of brain proinflammatory cytokine IL-1β upon supplementation with acetate, suggesting a possible treatment to reduce the injury phenotype and, possibly, the injury progression in Lyme neuroborreliosis.

BUTYRATE

Butyrate, another SFCA, has generated considerable interest for its beneficial effects in a range of tissues, from the intestinal tract to peripheral tissues. It has multiple mechanisms of action and belongs to the widely recognized group of epigenetic substances known as HDAC inhibitors. Butyrate has been found to activate epigenetically silenced genes like Bcl-2 homologous antagonist, a proapoptotic protein, and cell-cycle inhibitor p21 in cancer cells, which has important implications in the prevention of cancer.[69] A number of studies are confirming that an increased concentration of butyrate in the colon could be an important mediator in the prevention of colorectal cancer.[70] Modulation of the canonical Wnt signaling pathway, which is constitutively activated in most colorectal cancers, can be analyzed to determine the protective role of butyrate against colorectal cancer.[71] A number of HDAC inhibitors, alone or in combination with various other anticancer drugs, are being used to treat cancers at different developmental stages. However, the molecular mechanisms underlying the response to HDAC inhibitors are still not fully understood in cancer patients.

Butyrate, through its ability to inhibit HDAC activity, exerts anti-inflammatory activity by suppressing *NF-κB* activation, inhibiting interferon gamma production, and upregulating peroxisome proliferator-activated receptor-γ.[72] The anti-inflammatory properties of butyrate led to investigation of its possible therapeutic use in inflammatory bowel disease. Studies in patients with ulcerative colitis suggested that luminal administration of butyrate or stimulation of luminal butyrate production by the ingestion of dietary fiber results in an amelioration of inflammation.[73] Another study confirming the anti-inflammatory role of butyrate was carried out in neutrophils under both in vitro and in vivo conditions. Butyrate-mediated inhibition of HDAC activity and *NF-κB* activation was believed to decrease the LPS-induced production of nitric oxide and stimulation of inflammatory cytokines (TNF-α and CINC-2$\alpha\beta$).[74]

Butyrate is an essential secondary metabolite for the development and functioning of several immune cell lineages. Butyrate derived from commensal bacteria was observed to exert anti-inflammatory effects in the colon by stimulating histone acetylation of the *Foxp*3 (forkhead box P3) locus in naive $CD4^+$ T cells, leading to increased *Foxp3* expression that promoted the differentiation of T_{reg} cells.[75] Arpaia et al.[76] reported that changing the histone acetylation levels via HDAC inhibition promoted butyrate-dependent colonic T_{reg} differentiation in myeloid cells. Chang et al.[77] also reported that treatment of macrophages with *n*-butyrate led to downregulation of LPS-induced proinflammatory molecules like nitric oxide, IL-6, and IL-12. Butyrate seems to modulate immune cell function, but whether it directly affects enzymatic activity or expression of HDAC is yet not clear. On one hand, the loss of HDAC3 expression in intestinal epithelial cells ruins intestinal barrier function, but on other hand, HDAC inhibition by SCFAs in T_{reg} cells is associated with attenuation of inflammation.[54] Thus, further investigations are needed to fully elucidate the role of SCFAs in immune-related functions.

Butyrate is also known to have preventive effects against obesity, cardiovascular disease, and oxidative stress. As reported by Gao et al.,[78] the dietary intake of butyrate prevents diet-induced obesity and insulin resistance in C57BL/6 mice through increased expression of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), which results from inhibition of HDACs and activation of AMP-activated protein kinase (AMPK).[78] The treatment of human intestinal epithelial cell line Caco2/TC7 with propionate and butyrate was also effective in reducing the expression of 9 important genes associated with cholesterol synthesis, including the key gene *HMGCR* (3-hydroxy-3-methylglutaryl-coenzyme A reductase).[65] Hence, SCFAs may be very effective in the treatment of metabolic syndrome in humans. Butyrate can also be used to overcome oxidative stress, as the exogenous supplementation of β-hydroxy butyrate in mice enhances the promoter acetylation of oxidative stress genes like *FOXO3A* and *MT2* via selective depletion of HDAC1 and HDAC2.[59]

Butyrate may have a positive effect on stem cells. Butyrate treatment enhanced the efficiency of induced

pluripotent stem cells derived from human adult or fetal fibroblasts via epigenetic remodeling and the increased expression of pluripotency genes associated with increased histone H3 acetylation and promotor DNA methylation.[79] Sodium butyrate inhibits the propagation of endometrial cancer stem-like cells.[80] Thus, as a small molecule, butyrate can serve as a powerful tool to elucidate the molecular mechanisms of cellular reprogramming.

POLYPHENOLS

Polyphenols are widely distributed in fruits, vegetables, and plants but are also produced from microbial metabolism of dietary foods in the gut, where they are transformed into various derivatives of aromatic SCFAs such as phenylacetate or phenylbutyrate. These compounds are generated by gut microbes such as *Bacteroides* species, *Clostridium* species, *Eubacterium limosum*, and *Eggerthella lenta* and are reported to have wide range of activities in the prevention and alleviation of various diseases and conditions like cancer, neuroinflammation, diabetes, and aging.[81] Polyphenols inhibit HDAC activity, although the degree of inhibition varies from one compound to the next. For example, phenylbutyrate inhibits HDAC activity to a greater degree than phenylacetate. There are other intestinal metabolites of nutritional polyphenols like 3-OH-phenylacteate, 3-phenyl-propionate, and 3-(4-OH-phenyl)-propionate as well as a number of aromatic acids that bear an unsaturated side-chain. Such aromatic acids include *trans*-cinnamic acid, *p*-coumaric acid, and caffeic acid, all known to inhibit HDAC activity.

Treatment of human skin cancer A431 cells with polyphenol (−)-epigallocatechin-3-gallate (EGCG) increased the acetylation of respective lysine residues of H3 (K9 and K14) and H4 (K5, K12, and K16) histones.[60] The same treatment was effective in reactivating various tumor-suppressor genes like $p16^{INK4a}$ and *Cip1/p21* via inhibition of DNMT and HDAC activity. In the skin cancer cells, EGCG also decreased methylation at histone H3 lysine K9 via inhibition of methyltransferases, leading to transcriptional activation of various tumor-suppressor genes. (−)-Epigallocatechin-3-gallate was also effective in decreasing global DNA methylation in a concentration-dependent manner upon treatment on squamous cell carcinoma 13 cells. Polyphenols also seem to modulate immune functions, as human monocytic THP-1 cells, upon treatment with polyphenol fisetin in a high-glucose milieu, inhibited the expression of NF-κB genes, including IL-6 and TNF-α, via activation of HDACs and suppression of HATs.[61] Thus, polyphenols can also serve as an important tool in investigating epigenetic reprogramming.

POLYAMINES

Polyamines such as putrescine, spermidine, and spermine are low-molecular-weight aliphatic polycations present in all mammalians species. In recent decades, polyamines have been an active area of research because of their wide range of bioactivities, which include modulation of chromatin structure; synthesis and stabilization of DNA, RNA, and protein; cell growth and proliferation; and involvement in ion channel function and receptor-ligand interactions. Polyamines also exhibit strong anti-inflammatory properties and hence can be used to avert chronic inflammation. In addition, polyamines are closely associated with maintenance of intestinal mucosal barrier function, since they promote the secretion of mucous or secretory immunoglobulin A from intestinal epithelial cells, the recovery of damaged mucosal layers, and the upregulation of tight-junction proteins and E-cadherin.[82] Currently, various polyamine analogues are being used to reactivate epigenetically silenced tumor-suppressor genes (eg, E-cadherin) that are repressed by H3K4 hypomethylation during cancer development. The exposure of acute myeloid leukemia cells to the polyamine analogue 2d (1,15-bis{N5[3,3-(diphenyl) propyl]–N^1 biguanido}4,12-diazapentadecane) inhibits LSD1 (lysine-specific histone demethylase 1), which subsequently leads to activation of the E-cadherin gene via H3K4 hypermethylation. A decrease in proliferating cell nuclear antigen (PCNA) levels was also observed, suggesting a growth inhibitory or cytotoxic effect of the polyamine analogue 2d.[57] The feeding of mice with *Bifidobacterium lactis* LKM512-enriched chow increases intestinal polyamine concentrations, which inhibits colonic senescence and enhances longevity in mice.[83] Moreover, long-term oral administration of the same probiotic strain (LKM512) together with arginine suppressed inflammation, improved longevity, and protected from age-induced memory impairment in mice and thus may prevent aging-related deterioration in quality of life via the upregulation of polyamines.[82] Polyamines appear to be critical for maintenance of intestinal health and thus are essential target molecules whose mechanism of action, while thought to be via epigenetic reprogramming, is still being elucidated.

INTERDEPENDENCE BETWEEN KEY EPIGENETIC MODIFIERS AND COMPOSITION OF THE GUT MICROBIOTA

Not only does the microbiota influence the activities of intestinal HDACs, but in turn, the intestinal HDACs alter the composition of gut microbiota. $HDAC3^{\Delta IEC}$ mice (mice with deletion of the intestinal epithelial

cell–specific epigenome-modifying enzyme HDAC3) showed a significant alteration in intestinal commensal bacteria as well as extensive gene dysregulation, loss of Paneth cells, and, most importantly, impaired intestinal epithelial cell function. Deletion of HDAC3 made mice highly susceptible to intestinal damage and inflammation, indicating the central role of HDACs in maintaining intestinal homeostasis. However, rederivation of $HDAC3^{\Delta IEC}$ mice back to germ-free status restored the distorted intestinal function, indicating HDAC3 is critical for establishing normal diversity of the intestinal microbiota and maintaining intestinal homeostasis.[84]

Recently, it was reported that SCFAs derived from commensal bacteria play an important role in increasing naive $CD4^+$ T cells, T_{regs}, and other immune cell populations by inhibiting HDACs.[55] However, it is not clear how pathways downstream of SCFAs or exogenous HDAC inhibitors provide feedback for regulation of gut microbial diversity. Class I HDACs, such as HDAC1 and 2, have also been reported to play an essential role in maintaining intestinal homeostasis by attenuating the intestinal inflammatory response and regulating the proliferation and differentiation of intestinal epithelial cells.[85] However, in contrast to $HDAC3^{\Delta IEC}$ mice, $HDAC2^{\Delta IEC}$ mice were found to be resistant to colitis, reflecting a differential effect of HDACs on intestinal epithelial cells.[85] The interaction of HDACs with histones also depends upon the availability of acetyl-coenzyme A, and hence any alteration in the cellular metabolism of acetyl-coenzyme A may change the histone–HDAC interaction and could influence the response of HDAC to microbiota-derived signals. Further, it is reported that the acetylation of lysine residues on nonhistone proteins may occur upon introduction of conventional microbiota to germ-free mice.[86] Therefore, signals derived from commensal bacteria seem to regulate acetylation of nonhistone as well as normal substrates. Future research should focus on the role of HATs and HDACs in the regulation of microbiota-dependent acetylation of nonhistone proteins.

BEHAVIORAL SHIFTS CAUSED BY NEUROEPIGENETIC CHANGES RELATED TO THE GUT MICROBIOTA

The microbiota–gut–brain axis is an evolving concept in modern medicine, wherein brain and behavior are susceptible to alteration by the gut microbiota. The endogenous gut metabolites influence the epigenetic machinery within brain cells, leading to differential neuronal expressions that may cause changes in host behavior. A number of studies in animal models of stress, anxiety, and depression have shown a correlation between microbiota and mental illness.[87–90]

Epigenetic changes have been recently found to play a vital role in cognitive processes during health and disease by regulating gene expression in brain cells.[87] The study of these modifications is called *neuroepigenetics*. The neuronal nucleus, like all other nuclei, undergoes plastic changes, and these changes are used to understand the transcriptional regulation that occurs in brain cells upon stimuli in various conditions of disease and health. For example, epigenetic alterations, which are critical for fear memory consolidation and synaptic plasticity in the lateral amygdala of rats, may occur through histone acetylation and DNA methylation.[88] In addition, an increase in histone H3 acetylation and DNMT3A expression in the lateral amygdala caused auditory fear conditioning. Interestingly, however, fear memory consolidation was enhanced by intra–lateral amygdala infusion with the HDAC inhibitor trichostatin A but was impaired by infusion with the DNMT inhibitor 5-AZA.

Chemicals produced by gut microbes, such as spermidine, butyrate, and polyphenols, have been found to have neuromodulatory effects and thus may have potential in the prevention of various neurological disorders. Spermine produced by the gut microbes *B. thetaiotaomicron* and *Fusobacterium varium* has been found to have beneficial effects on aging and age-related memory loss, which is mediated through histone acetylation.[87,89] Butyrate, in the form of sodium butyrate, produced mainly by *Clostridium*, *Eubacterium* and *Butyrivibro* gut microbes, improved memory impairment and learning power in a mouse model of Alzheimer disease by increasing the acetylation and, thus, the expression of learning-associated genes. Sodium butyrate also protected neurons from death in various models of Parkinson disease via increased histone acetylation of nerve cells.[90] In a mouse model of Huntington disease, administration of phenylbutyrate significantly improved survival chances and attenuated neural atropy by reversing the decreased histone acetylation induced by mutant Huntington protein.[91] Polyphenols produced by gut microbial metabolism are also effective HDAC inhibitors and thus can be used to reverse the reduced histone acetylation associated with neurodegeneration.[87] Collectively, these findings seem to indicate that alterations in the microbiota could prove fatal for the host unless the microbiota is normalized by beneficial microbes such as probiotics. Thus, they also form the basis of an important correlation between mental illness and the gut microbiota. Although epigenetic machinery seems to be involved in this complex interaction, much more research is needed to understand this potential link.

Epigenetic reprogramming mediated by gut pathogens

Viruses and bacteria use epigenetic modifications to promote infection. Cancer-associated pathogens may also influence the epigenetic processing of host cells, which leads to epigenetic reprogramming and the development of cancer. For example, Epstein-Barr virus causes gastric cancer via excessive CpG hypermethylation at both the promotor and the nonpromotor CpG regions of the human genome, and the degree of this hypermethylation exceeds that in any type of tumor studied within The Cancer Genome Atlas Network.[92] Human papilloma virus (HPV), a common human pathogen that infects cutaneous or mucosal epithelia and causes about 5% of all cancers worldwide, also uses the epigenetic machinery of the host to cause various types of cancers. Transfection of primary human foreskin keratinocytes with 2 high-risk HPV strains, HPV16 and HPV18, was found to increase widespread methylation changes in cellular genes by augmenting the activity of DNMT1 and DNMT3B, respectively.[93] In addition, individually, these viruses were effective in enhancing the expression of both these methyltransferases in cervical intraepithelial neoplasia 3. HPV16 infection in a NIKS keratinocyte cell line was also reported to decrease the expression of cellular E-cadherin by increasing the expression and activity of DNMT1.[94] The oncoprotein E7 suppressed E-cadherin expression by directly binding with DNMT1 and increasing DNMT activity; however, the diminished expression of E-cadherin was reversible upon treatment with the DNMT inhibitor 5-aza-2′-deoxycytidine.

A number of pathogenic bacteria use this very complex but intriguing machinery to create disturbances within host cells. For example, uropathogenic *E. coli* infection in human uroepithelial cells upregulates the expression of DNMT1. This results in methylation of CpG islands in the *CDKN2A* gene, which codes for a G1-specific cell-cycle inhibitor and thus leads to uroepithelial cell proliferation.[95] *Helicobacter pylori*, a causative agent of gastric cancer, induces dephosphorylation of H3S10 as well as deacetylation of H3K23, which leads to gastric cell proliferation.[96] It also increases methylation at the promotor region of the DNA repair gene O^6-methylguanine DNA methyltransferase (*MGMT*) in patients with chronic gastritis. Interestingly, the *CagA*-positive strain of *H. pylori* was found to enhance methylation levels and hence was able to induce a more intense inflammatory response.[97] Furthermore, a significant reduction in *MGMT* promotor methylation was observed after eradication of *H pylori* infection, suggesting an extensive epigenetic alteration during *H. pylori*-induced gastritis.

Lipopolysaccharide, through epigenetic mechanisms, induces changes in cellular expression profiles. Recently, it was reported that LPS treatment of bovine peripheral blood mononuclear cells induced expression of various inflammatory molecules like TNF, IL-2, interferon G, IL-1β, IL-4, and IL-8. Subsequent treatment with trichostatin A, an HDAC inhibitor, showed a decrease in the expression of all these inflammatory molecules except IL-1β, which was nonsignificantly increased.[98] The increased expression of inflammatory molecules upon LPS treatment was believed to be caused by downregulation of the *HDAC6* and *HDAC7* and *DNMT3A* and *DNMT3B* genes. A similar type of study also showed that lung inflammation in mice is caused by an LPS-induced increase in IL-6 via acetylation of histones H3 and H4. The inflammatory response was potentiated following treatment with trischostatin A but was reversed upon treatment with anacardic acid, a HAT inhibitor.[99]

RELATIONSHIP BETWEEN THE EPIGENOME AND VITAMINS PRODUCED BY THE GUT MICROBIOTA

Besides diet, members of the gut microbiota, especially the *Bifidobacterium* and *Lactobacillus* genera, also supply host cells with vitamin K and most of the water-soluble vitamins like biotin, cobalamin, nicotinic acid, pantothenic acid, pyridoxine, folate, riboflavin, and thiamine from the nondigestible resistant starch of dietary fibers.[100] Some of these vitamins, such as folate, biotin, and vitamins B_2, B_6, and B_{12}, directly or indirectly cause epigenetic changes in the host and thus produce different effects.

Folate production

Folate, or folic acid, plays a role in various vital metabolic functions like DNA replication, DNA repair, and nucleotide synthesis.[101] It is also an important component of one-carbon metabolism, serving as a crucial methyl group donor for methylation reactions and thus maintaining DNA methylation.[102] Insufficient provision of dietary and/or microbial methyl donors or coenzymes alters one-carbon metabolism, leading to hypomethylation in various essential epigenomic-associated pathways and resulting in an increased risk of various hepatic, coronary, vascular, and cerebral disorders.[13] A diet moderately deplete of folate resulted in significantly decreased genomic DNA methylation of leukocytes in elderly women, which was reversed upon folate supplementation.[103] Steegers-Theunissen et al.[104] reported that methylation of the insulin-like growth factor 2 gene (*IGF2*) was enriched post partum in mothers supplemented periconceptionally with folic acid

compared with nonsupplemented mothers. In another study, decreased expression of *FMR1* (fragile X mental retardation 1 gene) due to methylation of existing CpG residues in the promoter region was observed in a human lymphoblastoid cell line when a surplus amount of folic acid was added to cells.[105] In a mouse model of neural tube defects, folate consumption by mothers rescued neural tube defects by decreasing H3K27 methylation marks in the *Hes1* and *Neurog2* gene promoters, associated with neural tube development.[106] Mice fed folic acid during gestation showed substantial alterations in the methylation patterns of CpG and non-CpG sites of key developmental genes, which altered gene expression in cerebral hemispheres and, thus, influenced overall development.[107] Long-term supplementation with folic acid and vitamin B_{12} in elderly individuals altered the genome-wide DNA methylation of several genes implicated in normal developmental processes and carcinogenesis.[108]

Folate, via miRNA alterations, also modulates the gene expression profile of cells. Liu et al.[109] reported that APP/PSI mice fed a folate-deficient diet showed decreased expression of miRNA and a subsequent upregulation of miRNA target genes. The upregulation of target genes further enhanced the deposition of amyloid-β peptides in the brain of mice with Alzheimer disease. Similar results were observed in vitro when a folate-deficient cell culture medium was added to a mouse neuroblastoma N2a cell line. Importantly, administration of folic acid to APP/PSI transgenic mice in a model of Alzheimer disease inhibited the formation of APP (amyloid precursor protein), PS1 (presenilin 1), and Aβ proteins, leading to recovery from neurodegeneration.[110] In another mouse model, Wang et al.[111] observed ethanol-induced teratogenesis caused by differential expression of miR-10a, which negatively regulates the *Hoxa1* gene, leading to improper embryonic development. However, the negative regulation of *Hoxa1* was reversed upon coincubation of mouse embryonic brain with folic acid, which decreased the expression of miR-10a.

Vitamins B_2, B_6, and B_{12}

Vitamins B_2, B_6, and B_{12} are involved in both folate metabolism and one-carbon metabolism, thereby aiding in the production of the universal methyl donor *S*-adenosyl methionine, which acts as a substrate for DNA methyl transferases. Vitamin B_{12} serves as a coenzyme of methionine synthase, which converts homocysteine to methionine, thereby producing *S*-adenosyl methionine. Vitamin B_6 is coenzyme of serine hydroxymethyltrasferase, which catalyzes the conversion of tetrahydrofolate to 5,10-methylene tetrahydrofolate during the folate cycle. Vitamin B_2 is used as precursor for synthesis of flavin-adenine dinucleotide (FAD), which acts as cofactor of methylenetetrahydrofolate reductase (MTHFR) to convert 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.[102]

Several studies have confirmed epigenetic modifications by vitamins B_2, B_6, and B_{12}. Adaikalakoteswari et al.[112] reported that vitamin B_{12}–deficient women of child-bearing age showed higher rates of cholesterol biosynthesis as a result of hypomethylation at promoters regions of 2 genes that are key in the regulation of cholesterol biosynthesis, *SREBF1* and *LDLR*.[112] Azzi et al.[113] found increased methylation of the *ZAC1* gene, which controls fetal growth and metabolism, in women with dietary intakes of vitamin B_2 during the 3 months prior to pregnancy and the last 3 months of pregnancy. The increased incidence of colorectal cancer in men with a high dietary intake of vitamin B_6 was due to hypermethylation of the mismatch repair gene MutL homolog 1 (*MLH1*).[114] Human breast adenocarcinoma cells (MCF-7) cultured in vitamin B_6-deficient medium showed reduced serine hydroxymethyltransferase activity and stability, which subsequently led to hypomethylation of the genome due to nonavailability of 5,10-methylenetetrahydrofolate.[115] Thus, because they can induce epigenetic changes in host cells, vitamins produced by the gut microbiota play a highly significant role in modulating the host gene expression profile.

PROBIOTICS AND EPIGENETIC CHANGES

Probiotics are live microorganisms that, when ingested, enhance the health of an individual. Species of *Lactobacillus* and *Bifidobacterium* are used most commonly as probiotics, but the yeast *Saccharomyces cerevisiae*, some *E. coli* strains, and some *Bacillus* species are also used. Importantly, probiotics have species- and strain-specific effects, and thus it is imperative that probiotic strains used for health maintenance or therapeutic purposes be selected on the basis of activity demonstrated in vivo and in vitro. As described above, imbalance of the gut microbiota is associated with many disorders, and probiotics have been shown to prevent and overcome these ailments. These beneficial bacteria have the capacity to alter the gut microflora, which in turn can influence the production of dietary metabolites, reported to modulate epigenetic-related processes in a manner similar to that of gut microbes (Table 3).[116–121]

As reported by Ghadimi et al.,[119] *Bifidobacterium breve* and *Lactobacillus rhamnosus* GG exerted anti-inflammatory effects in a 3D co-culture model composed of human intestinal HT-29/B6 or T84 cells and peripheral blood mononuclear cells by downregulating

Table 3 **Effects of probiotic administration on epigenetic regulation of gene expression**

Probiotic strain	Mechanism of chromatin molding	Consequence
Lactobacillus acidophilus	DNA methylation	Increased expression of genes suppressed in colorectal cancer: *Icam 5*, *Clstn2*, *Ppm1e*, *Runx3*, *Timp3*, *Rgl1*, and *Rassf1a*[116]
Akkermansia muciniphila	Upregulation of HDAC3 and HDAC5	Regulation of symbiotic balance between host and gut microbiota[117] Transcriptional regulation[117]
Lactobacillus rhamnosus GG (LGG)	Micro RNA interference	Downregulation of p38 MAPK expression via miR-155 and miR-146a[118]
Bifidobacterium breve and *Lactobacillus* rhamnosus	Global DNA methylation and acetylation	Inhibition of IBD-causing factors *IL-2*, *IL-17*, and *CD40*[119]
Saccharomyces cerevisiae	Phosphorylation	Decrease in expression of proinflammatory genes *IL-6*, *IL-8*, *CCL20*, *CXCL2*, and *CXCL10*, and increase in expression of PPAR-γ nuclear receptor[120]
Escherichia coli Nissle (EcN) 1917	Micro RNA interference	Upregulation of epithelial barrier function genes like *ZO-2*, *PAR-3*, and *PAR-6* via downregulation of miR-203, miR-483-3p, and miR-595[121]

Abbreviations: CCL, chemokine ligand; HDAC, histone deacetylase; IBD, inflammatory bowel syndrome; IL, interleukin; PAR, partitioning defective protein; PPAR-γ, peroxisome proliferator-activated receptor gamma; ZO, zonula occludens.

the expression of factors that cause inflammatory bowel disease (IL-23/IL-17/CD40). The inhibition of histone acetylation and the enhancement of DNA methylation resulted in limited access of NF-κB to gene promoters and reduced NF-κB–mediated transcriptional activation. Patients with symptoms of inflammatory bowel disease, when fed *F. prausnitzii*, showed a reduction in the inflammatory cytokine IL-2 but an increase in the anti-inflammatory cytokine IL-10.[122] This anti-inflammatory effect resulted from the inhibition of HDAC by microbial metabolites.

At present, probiotics are used to treat various types of cancers in different animal models. In mice, the severity of colorectal cancer was reduced and apoptosis of cancer cells enhanced upon oral inoculation with *Lactobacillus acidophilus*.[123] In another study, proliferation of colon cancer was inhibited by administration of *Lactobacillus reuteri*, which acted by suppressing TNF-induced NF-κB activation, leading in turn to downregulation of the NF-κB gene products that promote cell proliferation (Cox-2, cyclin D1) and suppress apoptosis (Bcl-2, Bcl-xL).[124]

Treatment of C57B/L6 mice with the protective lipoteichoic acid–deficient *L. acidophilus* strain (NCK2025) was more effective in increasing the expression of various tumor-suppressor genes like *Icam5*, *Clstn2 Ppm1e*, *Runx3*, *Timp3*, *Rgl1*, and *Rassf1a*, which, in colon cancer, are often silenced via DNA methylation, while treatment with the wild-type NCK56 strain did not increase expression.[116] Similarly, under in vitro conditions, when the same probiotic strains were incubated with HT-29 cells, the lipoteichoic acid–deficient strain NCK2025 was more effective than others. Moreover, in differentiated porcine intestinal epithelial IPEC-1 cells, the enterotoxigenic *E. coli* (ETEC)-induced expression of proinflammatory transcripts IL-6, IL-8, CCL20, CXCL2, and CXCL10 was halted upon treatment with *S. cerevisiae* (strain CNCM I-3856), which caused a decrease in ERK1/2 and p38 MAPK phosphorylation and an increase in the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), an anti-inflammatory nuclear receptor.[120] Two next-generation probiotics, *Akkermansia muciniphila* and *F. prausnitzii*, are also reported to increase the expression of HDAC3 and HDAC5, leading to different transcriptional responses in mice, although the former has a stronger effect on the host than the latter.[117] Various probiotic strains are also effective in modulating immune responses via RNA interference. Treatment of human dendritic cells with inactivated strains of *L. rhamnosus* GG induced a significant downregulation of miR-146a expression and a simultaneous upregulation of miR-155 expression, which led to downregulation of p38MAPK, thus preventing inflammation.[118] T84 intestinal epithelial cells, upon incubation with *E. coli* Nissle 1917, showed decreased expression of miRNAs such as miR-203, miR-483-3p, and miR-595. Intestinal barrier integrity was enhanced by the upregulation of tight-junction-associated genes like ZO-2, PAR-3, and PAR-6.[121]

Probiotics have also been shown to reduce the severity of enterotoxigenic *E. coli*-induced diarrhea,[125] to improve immune health,[126] to exert strain-specific antioxidative activity,[127] and to enhance differentiation and apoptosis in neoplastic cells.[128] Their correlation with epigenetics, however, is yet to be fully elucidated.

CONCLUSION

The composition of the gut micriobiota has a tremendous influence on host metabolism. Alterations in the composition of the microbiota can lead to various epigenetic modifications that change the host gene expression profile. Gut microbe metabolites, such as SCFAs, polyamines, and polyphenols, can change the host cellular levels of important epigenetic modifiers like HATs, HDACs, DNMTs, DNA demethylases, histone methyltranferases, histone demethylases kinases, and miRNA. Changes in these modifiers ultimately affect the brain–gut physiology of an individual, sometimes with lethal consequences. Although a significant association between the gut microbiota and the epigenetics of host cells seems to exist, far more research is needed. Namely, it is essential to know the exact cellular and molecular pathways through which the microbiota-produced metabolites interact with host epigenetic machinery. Findings thus far indicate that alterations in the epigenetic machinery could possibly serve as marks for therapeutic treatment of various ailments. Furthermore, modulation of the epigenetic machinery might lead to improvements in several pathogen-associated disorders. The potential ability of probiotics to alter the intestinal microbiota and act as epigenetic modifiers could be another area of research, as global chromatin modifications can now be mapped out using methods such as chromatin immunoprecipitation followed by DNA microarray (ChIP–chip) and chromatin immunoprecipitation followed by sequencing (ChIP seq).

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