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Mini Review

Commensal microbiota-derived signals regulate host immune system through epigenetic modifications

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Commensal microbiota colonizing the digestive tract is a symbiotic partner of its host, as it plays a critical role in nutrient metabolism as well as the development and maturation of the host immune system. Although it is clear that regulation of the host-commensal relationship is crucial to mammalian health, the underlying mechanisms regulating gut homeostasis are yet to be elucidated. Epigenetic modifications, including DNA methylation and histone methylation/acetylation, alter the structure of chromatin to regulate the transcriptional program of eukaryotic cells. At the whole genome level, these modifications possibly play a key role in regulating the mutually beneficial relationship between the host and the gut microbiota. In this review, we describe how the commensal microbiota and its metabolic by-products modify the epigenome of host cells, and in turn, change their development and functional behavior.

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Introduction

In humans, the intestine is home to more than a thousand commensal bacterial species. Gut microbes outnumber host cells by more than 10-fold. As reviewed in this issue and elsewhere, mammalian gut microbiota play a critical role in regulating the host immune system and nutrient metabolism[1, 2)]. Extensive studies have recently demonstrated significant links between commensal bacteria-derived signals and epigenetic pathways in host cells. The term epigenetics is often used to describe the heritability of associated gene expression changes without alteration of the nucleotide sequence. DNA

methylation and histone modifications represent epigenetic phenomena. Accumulating studies have demonstrated broader epigenetic events such as the combination of these modifications and chromatin remodeling, most of which are involved in dynamic transcriptional regulation and replication of cells. These studies also defined proteins associated with particular DNA/histone modifications[3]. It is now evident that a wide range of chronic, immune-mediated human disorders, including asthma, allergies, diabetes, cancer, and inflammatory bowel disease (IBD), are a result of complex genetic and environmental interactions. The commensal microbiota has emerged as a crucial source of environmental stimuli that impact these diseases[4]. IBD pathogenesis, in particular, appears to be driven by changes in commensal bacteria composition and by host immune responses to these altered commensals. Thus, the regulation of a symbiotic, host-commensal relationship is essential to mammalian homeostasis. Researchers have set out to reveal a central role for epigenetic pathways in orchestrating in this relationship.

The host-microbiota relationship and gut homeostasis

The mammalian intestinal tract is one of the most complex ecosystems known on Earth. The microbial communities in the gut are comprised of bacteria, viruses, fungi, and Archaea[5-7]. In humans, the intestine is home to 100 trillion bacteria from more than 1,000 species. The gut microbiota in mammals is known to be commensal. The commensal microbiota performs a variety of complex functions and confers many health benefits to the host. Much of our knowledge about the roles played by commensal bacteria in mammalian physiology has been derived from studies conducted using germ-free (GF) animals. For example, commensal bacteria aid in food digestion, extraction and biosynthesis of nutrients and other metabolites, detoxification of harmful xenobiotics, and growth limitation of potential pathogens. In addition, commensal bacteria and their metabolic by-products are implicated in the development, function, and homeostasis of both the innate and adaptive immune systems at the mucosal surface of the intestinal tract[2, 8, 9]. Recent advances revealed that the influence of the commensal microbiota extends beyond the intestinal mucosa and affects the systemic immune system[10, 11].

Intestinal epithelial cells (IECs) reside at the interface between the host and commensal microbiota. IECs function as fundamental non-hematopoietic cellular mediators of the innate immune response, which integrates microbiota-derived signals to maintain the host-commensal microbial relationship and gut immune cell homeostasis[12]. IEC recognition of commensal-derived pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), flagellin, and nucleic acids through pattern recognition receptors (PRRs), leads to secretion of antimicrobial proteins, cytokines, and chemokines. This epithelial immune response confines commensal microbiota to the lumen and regulates immune cells in the lamina propria[12, 13]. The commensal microbiota itself influences the development, homeostasis, and function of immune cells. For example, certain species of commensal bacteria in the ileum and colon drive differentiation of $CD4^+$ T cells to distinct helper cell lineages[14, 15].

Overview of microbe-induced epigenetic modifications

In eukaryotic cells, DNA is packaged with histone proteins into chromatin, allowing DNA to be confined to the microscopic nucleus. The structure of chromatin has several levels of organization. The basic structural and functional unit of chromatin is the nucleosome, which consists histone protein octamers (two each of the histone H2A, H2B, H3, and H4) wrapped in approximately 146 base pairs of DNA. Nucleosomes line up along the DNA in nucleosomal arrays. They are associated with histone H1 proteins that function as a linker between nucleosomes, non-histone proteins, and RNAs, forming the higher-order condensed chromatin structure[16, 17]. The highly condensed chromatin structure, known as heterochromatin, is considered to be transcriptionally silent, as this condensed state limits access of the transcriptional machinery to the genome. Although all cells in the human body have the same DNA and therefore the same genes, cells found within different tissues and organs maintain the unique physical characteristics and biological functions. The unique properties of various cell types are caused by heritable differences in DNA and chromatin packaging, enabling cells to execute distinct gene expression programs. Epigenetics is the study of molecular processes that influence gene expression without changes in the underlying DNA sequence. Several molecular processes contribute to epigenetic gene regulation, including DNA methylation, related modifications of the DNA base cytosine, covalent modifications of histone proteins, non-coding RNAs, and ATP-dependent chromatin remodeling[18].

Well-characterized epigenetic modifications known to regulate chromatin structure are cytosine methylation and histone modifications. In particular, N-terminal histone tails, protruding from the nucleosome, provide a template for covalent modifications, such as acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, and ADP-ribosylation[19]. The epigenome refers to the complete profile of these potentially heritable epigenetic DNA and histone protein modifications that define the genome-wide transcriptional program[3, 18]. Epigenetic modifications are established and maintained by the balanced action of several enzymes, including DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), and histone demethylases (HDMs). Methylation of the DNA nucleotide cytosine is associated with a repressed chromatin state and inhibition of gene expression[20]. Three DNMTs (DNMT1, DNMT3a, and DNMT3b) are required for establishment and maintenance of DNA methylation patterns. DNMT1 appears to maintain established DNA methylation patterns, whereas DNMT3a and 3b mediate the establishment of *de novo* DNA methylation patterns[21]. Unlike DNA methylation, histone modifications have varied effects on gene expression and are either associated with active transcription or repression, providing an additional dimension to epigenetic regulation. While histone acetylation is generally associated with transcriptional activation, methylation can promote either gene activation or repression, depending on the level of methylation (mono-, di-, or tri-methylation) and the specific methylation pattern of lysine residues[22, 23]. Acetylation of the lysine residues by HATs, within the histone N-terminal tails, reduces DNA-histone interactions by neutralizing the cationic charge of the histone, leading to local relaxation of the chromatin structure and allowing transcription machinery access to DNA. Conversely, histone deacetylation by HDACs, leads to transcriptional repression by causing histones to interact more strongly with the DNA. To date, eighteen mammalian HDACs have been identified. They are grouped into four classes (Class I, II, III, and IV) based on their homology to prototypical HDACs found in yeast[24, 25].

A number of dietary components were shown to interfere with epigenetic gene regulation[26-28], and diet is a major factor driving the composition and metabolism of the microorganisms in the intestine. Recent studies highlight the ways in which bacterial and viral pathogens affect host epigenetic status by influencing DNA methylation and histone modification[17, 29]. The most well documented example is infection with *Helicobacter pylori*, which induces aberrant DNA methylation and deacetylation of Histone H3 in human gastric epithelial cells[30, 31]. Commensal bacteria-derived signals also influence host epigenetic pathways, particularly through DNA and histone methylation[32-36] and histone acetylation and deacetylation[37-41]. The commensal microbiota produces multiple low-molecular-weight by-products that modify the host cell epigenome and may alter the functional behavior of host cells. For example, short chain fatty acids (SCFAs) are generated in the mouse cecum and human colon by commensal bacteria, through fermentation of nondigestible carbohydrates, producing acetate, propionate and buturate at a ratio of 3:1:1[42, 43]. Acetate enters systemic circulation and is used in lipogenesis. Propionate is transported to the liver where it has a role in gluconeogenesis. Butyrate is the major energy source for colon epithelial cells, and it also functions as an HDAC inhibitor[42, 43]. We further discuss how commensal microbiota-derived signals regulate host epigenomics in later sections.

Microbiota-mediated epigenetic regulation of intestinal epithelial cells

As discussed in an earlier section, IECs play a crucial role in integrating signals from the gut intestinal environment to regulate intestinal homeostasis. IECs express PRRs, including Toll-like receptors (TLRs), enabling them to act as dynamic PAMP sensors to direct intestinal immune responses. Despite this ability, normal commensal bacteria do not elicit a functional response from IECs. This hypo-responsiveness is partly attributed to DNA methylation-dependent silencing of *Tlr4* in IECs, as observed in conventionally housed (CV) mice. In this study, the *Tlr4* gene promoter was hypomethylated under GF conditions, raising the possibility that commensal bacteria may induce hyporesponsiveness to LPS in IECs by repressing *Tlr4* through DNA methylation (Fig. 1)[34]. Intestinal absorptive cells (also called enterocytes) along with enteroendocrine cells, goblet cells, and Paneth cells, represent the principal IEC types in the small intestine. All IEC types contribute to the intestinal barrier between the internal cellular milieu and luminal microbiota. This barrier is established in several ways: 1) physical protection by tight junctions, and 2) biochemical barriers consisting of a mucus layer produced by goblet cells and antimicrobial proteins secreted by Paneth cells and other subsets of IECs. DNMT1 is highly

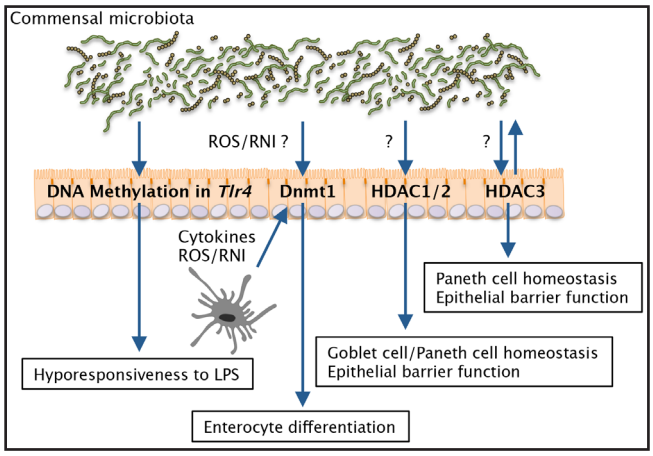

Fig. 1 Epigenomic regulation of commensal-bacteria-dependent IEC homeostasis

IECs play a critical role in regulating intestinal homeostasis by integrating signals from commensal bacteria. TLR4 senses the presence of LPS from commensal bacteria. TLR4 transcription is epigenetically repressed (through DNA methylation) in IECs to prevent excessive inflammatory responses to commensal bacteria. Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) derived from immune cells, as well as commensal bacteria, trigger oxidative stress in IECs, leading to increased DNMT1 expression and activity. Proinflammatory cytokines such as IL-1β from immune cells also stimulate DNMT1 activity in a nitric oxide (NO)-dependent manner through upregulation of inducible nitric oxide synthase (iNOS) in IECs. The expression of HDACs in IECs is regulated by both endogenous and exogenous factors. HDACs are essential in regulation of IEC homeostasis, development, and epithelial barrier function. In turn, host HDACs are important for regulating dynamic communication between the host and commensal microbiota.

expressed in proliferating IECs and stem cells within the crypts of the small intestine[44]. IEC-specific deletion of DNMT1 leads to increases in proliferating progenitor cells and a reciprocal decrease in differentiated enterocytes (Fig. 1)[45]. Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) trigger oxidative stress in IECs, leading to increased DNMT1 expression and activity[23, 46]. The proinflammatory cytokine Interluekin-1β (IL-1β), secreted from immune cells within the lamina propria, also stimulates DNMT1 activity in a nitric oxide (NO)-dependent manner through upregulation of inducible nitric oxide synthase (iNOS) in IECs (Fig. 1)[23, 46]. In addition, ROS and RNI species are released by immune cells upon stimulation with inflammatory cytokines, including IL-1β and IL-6, as well as by commensal bacteria in the lumen[23]. *Lactobacilli* and *Bifidobacteria* can generate large amounts of nitric oxide from nitrite, and dietary supplementation with nitrate may increase nitric oxide production[47, 48]. Together, these data support the concept that commensal bacteria-derived stimuli control host gene expression by regulating DNA methylation.

The class I HDACs, namely, HDAC1, HDAC2, HDAC3, and HDAC8, and the class II HDAC, HDAC4, are expressed by IECs in both the small intestine and colon[49, 50]. These HDACs have a role in maintaining cell proliferation, survival, and differentiation. HDACs are regulated by both endogenous and exogenous factors, and they are important in orchestrating host-microbiota interactions. Transient overexpression of HDAC1 and HDAC2 in fetal mouse intestinal explants reduces expression of some enterocyte differentiation makers, including *Apoa1, Fabp1, Fabp2, Prap1,* and *Mt2*[51]. In turn, IEC-specific deletion of HDAC1 and HDAC2 resulted in the loss of goblet cells in both the small intestine and colon, and of Paneth cells in the small intestine, resulting in disruption of epithelial barrier function and homeostasis (Fig. 1)[38]. Loss of IEC-specific HDAC3 expression leads to impaired Paneth cell survival, decreased intestinal barrier function, and changes in commensal microbiota composition[37]. Interestingly, HDAC3 expression is induced after commensal bacteria colonization. Under GF conditions, IEC-specific HDAC3-deficient mice maintain Paneth cell homeostasis and intact epithelial barrier function (Fig. 1)[37]. Although the underlying microbiota-dependent mechanism regulating HDAC3 function needs to be further defined, these data suggest that HDAC3 expression in IECs mediates functional crosstalk between the commensal microbiota and its host.

Microbiota-mediated epigenetic regulation of innate and adaptive immunity

Similar to IECs, emerging evidence supports a significant role for epigenetic machinery in modulating both the innate and adaptive immune systems. Direct links between commensal bacteria and epigenetic modifications were found in host immune cells, and they are implicated in regulating their development and function. Mononuclear phagocytes, including dendritic cells and macrophages, engulf microorganisms and gain bactericidal activity. PRR-initiated signals activate downstream transcription factors in mononuclear phagocytes from GF mice. However, their ability to bind to the promoter regions of various inflammatory response genes, including *Ifnb1, Tnf,* and *Il6*, was diminished. Therefore, these phagocytes fail to induce

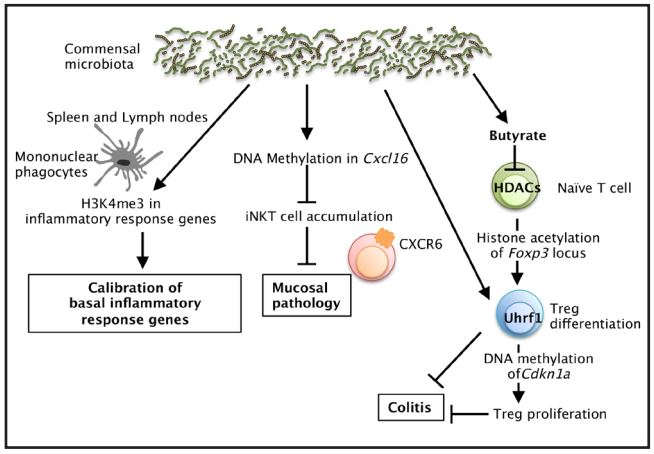

Fig. 2 Commensal bacteria-mediated epigenetic regulation of immune cell development and function

Commensal-derived stimuli increase trimethylation of lysine 4 of histone protein 3 (H3K4me3), a modification characteristic of transcriptional activation, in the promoter region of inflammatory response genes in mononuclear phagocytes at nonmucosal sites. This leads to inflammatory gene expression, thereby calibrating mononuclear phagocytes to respond promptly to pathogens. Colonization of commensal microbiota decreased DNA methylation levels of the chemokine, *Cxcl16*, in the colon and lung tissues. This corresponded with downregulation of *Cxcl16* expression and decreased accumulation iNKT cells in these tissues. This protects the host from pathology in models of ulcerative colitis (UC) and asthma. The commensal microbiota upregulates Uhrf1 expression in Treg cells, and Uhrf1-dependent DNA methylation of *Cdkn1a* is required for Treg cell proliferation. Butyrate, which has HDAC-inhibitory activity, is derived from gut commensal bacteria. It promotes the differentiation of Treg cells in the colon, in part, by stimulating histone acetylation of the *Foxp3* locus in naïve $CD4^+$ T cells. Thus, commensal bacteria-derived signals regulate the differentiation and proliferation of Treg cells, which is important for the prevention of colitis.

expression of proinflammatory cytokines[32]. Commensal-derived stimuli increase trimethylation of lysine 4 of histone protein 3 (H3K4me3), a modification characteristic of transcriptional activation, at the promoter region of inflammatory response genes (Fig. 2). Furthermore, treatment of CV mice with antibiotics can erase this H3K4me3 mark from inflammatory response genes. These data suggest that the commensal microbiota calibrates basal inflammatory gene expression in mononuclear phagocytes at non-mucosal sites, which is required for potent immune response to infections, by introduction of permissive chromatin modifications[32].

Invariant natural killer T (iNKT) cells are a specialized subset of T cells that use their T cell receptors to recognize endogenous and exogenous lipid antigens presented by CD1d. iNKT cells likely play a pathological role in the development of ulcerative colitis (UC), a form of IBD, and allergic asthma. The chemokine, CXCL16, is responsible for iNKT cell recruitment to the colon and lung tissues during inflammation. In GF mice, iNKT cells accumulate in the colonic lamina propria and lung. In CV mice, they migrate from these mucosal tissues early in life. Transfer of neonatal, but not adult (5-weeks-old), GF mice to specific pathogen-free (SPF) environment decreased DNA methylation levels of chemokine *Cxcl16* gene in the colon and lung tissues, leading to downregulation of *Cxcl16* expression and decreased accumulation of iNKT cells in these tissues (Fig. 2)[35]. Alternatively, treating newborn GF mice with the DNA methyltransferase inhibitor 5-Azacytidine, normalized *Cxcl16* promoter hypermethylaion to levels typically observed in adult SPF mice and inhibited *Cxcl16* mRNA expression and iNKT cell recruitment in these tissues. Further, exposure to the SPF environment during neonatal stage ameliorates development of T helper 2 (T_H2)-mediated oxazolone-induced colitis model as well as an ovalbumin-driven allergic-asthma model, suggesting that early-life microbial exposure may be critical for protection from iNKT-mediated diseases via DNA methylation pathway[35].

DNA methylation is also critical for $Foxp3^+$ regulatory T cell (Treg) homeostasis in the colon. Uhrf1 [ubiquitin-like, containing plant homeo domain (PHD) and really interesting new gene (RING) finger domains 1] plays a non-redundant role in the maintenance of DNA methylation by recognizing and recruiting Dnmt1 to hemi-methylated DNA in proliferating cells[52]. Colonization of GF mice with commensal microbiota upregulates Uhrf1 expression in colonic Treg cells and promotes Uhrf1-dependnent Treg cell proliferation in the colon[36]. Uhrf1 deficiency in T cells causes hypomethylation of the distal promoter of the *Cdkn1a* gene that encodes the cyclin-dependent kinase inhibitor p21. This hypomethylation eventually de-represses this gene, resulting in cell-cycle arrest of colonic Treg cells. As a consequence, T-cell specific Uhrf1-deficient mice spontaneously developed severe colitis (Fig. 2)[36]. Several recent studies proved that commensal bacteria-derived SCFAs, particularly butyrate, facilitate the differentiation of Treg cells in the colon. This mechanism is, in part, explained by histone hyperacetylation at the regulatory regions of the *Foxp3* locus in naïve $CD4^+$ T cells[41, 53, 54]. Butyrate, which has the most potent HDAC-inhibitory activity among SCFAs,

efficiently augments Treg cell differentiation. Propionate, a moderate HDAC inhibitor, promotes Treg differentiation to a lesser extent. Whereas acetate, lacking HDAC-inhibitory activity, shows little, if any, effect. Butyrate induces up-regulation of histone H3 acetylation at the promoter and CNS1/CNS3 enhancer regions of the *Foxp3* gene locus. This positively correlated with *Foxp3* expression during Treg cell differentiation (Fig. 2)[41]. In addition to histone chemical modification, butyrate also enhances acetylation of the Foxp3 protein, which is more stable and effective than the intact protein[54]. Butyrate-dependent HDAC inhibition in DCs may lead to indirect promotion of Treg cell differentiation in the colon by inducing Raldh1 expression[54]. Exposure to butyrate also confers anti-inflammatory properties to macrophages and DCs, most likely through activation of Gpr109a-dependent signaling[55]. Notably, the frequency of butyrate-producing bacteria is reduced in patients with IBD[56]. Collectively, commensal bacteria-derived butyrate may be a symbiotic factor that dampens excessive immune responses to colonizing bacteria, thus preventing inflammatory disorders such as IBD.

Conclusion

An increasing amount of evidence offers paths toward understanding the importance of epigenetics in the mutually beneficial relationship between the commensal microbiota and its host. The commensal microbiota and its by-products modulate host epigenomics through multiple mechanisms, and these epigenetic modifications may be crucial to mammalian immune homeostasis. Further investigation of the molecular mechanisms by which commensal microbiota exert their effects on host physiology would provide new insight into host-microbe interactions. The potential of these epigenetic mechanisms may represent innovative targets for new drugs, such as DNMT and HDAC inhibitors, for a number of immune-mediated diseases.

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Conflict of interests

None

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