Review Article

Role of epigenetic mechanisms in inflammatory bowel disease

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Abstract: Epigenetic mechanisms maintain gene expression states within a cell and through cellular generations and involve DNA methylation and chromatin changes, such as histone modifications. These mechanisms play roles in inflammatory processes. Here we review recent advances about what we know about their impact in inflammatory bowel disease (IBD). The incidence and prevalence of IBD have significantly increased in recent decades, establishing it as one of the most common gastrointestinal disorders. Lifestyle changes, including dietary factors, have been identified as potential contributors to this phenomenon. Although the heritability of IBD cannot be solely attributed to common genetic variants, their examination has shed light on the involvement of epigenetic and chromatin factors, such as DNMT3A and SP140, in the development of IBD. Studies focusing on SP140 have provided a paradigm by demonstrating the association between genetic alterations in this gene and changes in chromatin structure, gene expression, and the composition of the microbiome, ultimately resulting in abnormal inflammation. Genetic deletion coupled to experimental colitis studies in mice have highlighted roles of additional important factors linked to DNA methylation, MBD2 and UHRF1, and histone methylation, such as SETD2, in regulating the inflammatory processes in the gut. Further research is needed to investigate how environmental factors contribute to the predisposition of IBD through epigenetic mechanisms. This line of inquiry holds the potential to pave the way for new intervention strategies.

Keywords: Inflammatory bowel disease (IBD); epigenetics; DNA methylation; histone modification; SP140

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Introduction

Inflammatory bowel disease (IBD) affects millions of people worldwide (1). While the number of new cases in western countries has remained steady or decreased, there has been a rapid surge in IBD prevalence in newly developed regions, notably Asia, Africa, and South America. While the definite causes of this rise are not clear, it has been attributed to changes in life-style factors, such as the availability of highly processed foods and high-fat-high-sugar Western diets.

IBD presents in two primary forms. The first, known as ulcerative colitis, is characterized by inflammation occurring in the rectum and colon, while Crohn's disease is characterized by patchy inflammation affecting various segments of the gastrointestinal tract (2). In ulcerative colitis, the inflammation starts in the rectum and ascends the colon in a contiguous inflammation, only affecting the mucosa, submucosa, and crypts, leading to crypt abscesses (3-5). Conversely, Crohn's disease typically involves the terminal ileum, cecum, and colon, but it can manifest with patchy inflammation affecting any region of the gastrointestinal tract in a non-continuous pattern (3,4,6). The clinical presentation of IBD is characterized by recurring episodes of abdominal pain, diarrhea, and bloody stools, and can be recurrent over decades. These disorders can lead to chronic intestinal damage and an elevated risk of developing colorectal cancer (7,8). There is likely to be additional heterogeneity beyond these two IBD subtypes (9). This further underlines the complexity of IBD and its potential pathogenesis. At the cellular level, active IBD is characterized by pronounced infiltration of innate immunity cells (macrophages, dendritic, and natural killer cells) into the lamina propria, that drive the initial pathology. At later phase, there is infiltration of adaptive immune cells (B and T lymphocytes) including T regulatory cells (Treg), Th1, Th2, and Th17 T cells, reviewed in (10).

Although IBD has been extensively investigated, a definitive etiology for the disease remains elusive. Multiple factors have been identified to contribute to its development, including environmental influences, dietary factors, lifestyle choices, genetic predisposition, and epigenetic factors (11-16). One of the main hypotheses is that the disruption of the homeostasis between the intestinal epithelium, the microbiome and the mucosal immune system initiates the disease (17,18). Several genome wide association studies (GWAS) have identified more than 240 genetic polymorphisms that may be linked to IBD, highlighting the complexity of IBD. Many of these polymorphisms occur in noncoding regions, possibly affecting gene regulatory mechanisms (13,19). Only approximately 20% of IBD cases can be attributed to these genetic variants (11,12,20). While GWAS analysis of IBD has provided important insights into mechanisms of the disease, the majority of identified loci are still poorly understood, and genetics alone cannot account for most IBD cases. Yet, GWAS highlighted the link of several factors involved in epigenetic mechanisms to IBD. Given that epigenetic mechanisms play a crucial role in connecting external factors such as the environment, diet, and microbiome to changes in gene expression, these findings are not unexpected. Nevertheless, our understanding of the specific roles of epigenetic mechanisms in IBD is still limited. In this review, we summarize several recent key findings in this regard, showcasing how epigenetic factors are critically implicated in IBD.

Epigenetics and IBD

Environmental, microbial and genetic factors affect IBD

While genetic variation undoubtedly plays a very important role in the risk of developing IBD, the rapid increase of the incidence of IBD, especially in developing countries, highlights the role of the environment into the etiology of this disease (21). Several lifestyle factors, such as diet (22), obesity (23), smoking (24), may influence the risk of developing IBD. These lifestyle factors may have a significant impact on the natural history and clinical outcomes of patients diagnosed with IBD.

One important non-genetic factor is the gut microbiota, which, in turn, is strongly affected by diet. The prevailing hypothesis suggests that IBD arises from an abnormal immune response to the gut microbiota or pathogenic bacteria in individuals with a genetic predisposition (25). Alterations in the gut microbiota are widely recognized in IBD pathology; however, it remains uncertain whether these changes are the cause or consequence of intestinal inflammation, and the precise contributions of these bacteria to IBD pathogenesis remain unknown. The human gut microbial community is a dynamic and varied collection of commensal bacteria, fungi, and viruses, with bacteria accounting for the majority (around 1,000 distinct species) (26). In the healthy human gut, over 90% of bacterial species are classified into four major phyla: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*.

The gut microbiome establishes a mutually beneficial

symbiotic relationship with the human host, where the host provides a nutrient-rich environment for the microbiota, and in turn, the gut microbiota performs various physiological functions to maintain the host's health (27,28). Under normal physiological conditions, the gut microbiota acts as a homeostatic quasi organ, involved in the fermentation of complex undigested polysaccharide polymers, the production of short-chain fatty acids (SCFAs, such as butyrate, propionate, acetate), the synthesis of certain vitamins, the extraction of energy from diet, the integrity of the intestinal mucosa, and the exclusion of pathogenic microbes. Bacterial dysbiosis, defined as an imbalance of harmful and commensal bacteria, is characterized in IBD by a decrease in the phyla *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* and an increase in *Proteobacteria*. *Firmicutes* and *Bacteroidetes*, for example, are key providers of energy substrates for intestinal epithelial cells as well as anti-inflammatory metabolites such as butyrate and other SCFAs.

The incidence of IBD is higher in first-degree relatives of individuals with IBD compared to the general population, with Crohn's disease demonstrating a higher heritable risk compared to ulcerative colitis (29). To date, GWAS have identified over 240 genetic risk variants for IBD affecting several intra- and inter-cellular pathways (11). The analysis of the genes and genetic loci identified in IBD indicates that multiple pathways, such as epithelial barrier function (30), innate mucosal defense (31), immune regulation (32), cell migration, autophagy (33), adaptive immunity (31), and metabolic pathways associated with cellular homeostasis (33) play important roles in maintaining intestinal homeostasis. Pathways linked to IBD are involved in recognizing microbial products (e.g., *NOD2*), autophagy (e.g., *ATG16L1*), genes regulating epithelial barrier function (e.g., *ECM1*) and pathways regulating innate and adaptive immunity (e.g., *IL23R* and *IL10*) are also associated with IBD risk (30-33). Genetic variations account for approximately 8–13% of Crohn's disease cases and 4–7% of ulcerative colitis cases (34). Remarkably, GWAS identified genes coding for proteins playing key roles in epigenetic mechanisms as loci predisposing to IBD, in particular *DNMT3A* and *SP140*. Thus, understanding the contribution of epigenetic mechanisms in IBD is pivotal.

Epigenetic mechanisms

The interplay between extrinsic factors, such as the environment, and genes is governed by epigenetic factors that contribute to the formation of an organism's phenotype.

Epigenetics encompasses the study of heritable changes in gene expression and phenotype that do not stem from alterations in the underlying DNA sequence.

Given the broad definition of 'epigenetics', anything that modulates the function of the genome might be considered 'epigenetic', for example transcription factor networks or signalling pathways. However, a more narrow, contextual understanding of the term 'epigenetic' is widely accepted, whereby epigenetic mechanisms are regulatory functions involving DNA methylation, histone modifications, chromatin changes or noncoding RNA and this is how we interpret this concept here (*Figure 1*). What unites these mechanisms is that they support gene expression states over cellular or even organismal generations, imbuing cells with a memory function. Epigenetic mechanisms are also thought to be critical to allow cells to integrate environmental stimuli into a long-term gene expression response (35).

Of the listed epigenetic mechanism, RNA-based mechanisms are the most diverse, including small regulatory RNAs, such as microRNAs and interfering RNAs, as well as long noncoding RNAs, such as Xist, involved in X-inactivation in mammals.

DNA methylation is a process where epigenetic inheritance is best understood. It occurs in mammals as 5-methylcytosine in a CpG context whereby recognition of hemi-methylated DNA by the maintenance DNA methylation machinery perpetuates this modification through cellular replication (36). This latter step is primarily achieved by the enzyme DNA methyltransferase 1 (DNMT1), whereas the enzymes DNMT3A and DNMT3B are involved in *de novo* DNA methylation. DNA methylation is linked to transcriptional repression in repetitive elements, X-inactivation, imprinting and specific genomic loci, but the precise role of DNA methylation is context dependent (36).

DNA methylation and IBD

Given the important role of DNA methylation as epigenetic mechanism, is there are role of this process in IBD? The gut microbiome contributes a large fraction of circulating metabolites, such as short chain fatty acids, indole-3-acetate, bile acid derivatives and other compounds, which, in turn, are taken up by cells and affect the epigenome (37). Thus, it is not surprising that the gut microbiota affect DNA methylation of the intestinal epithelium and this

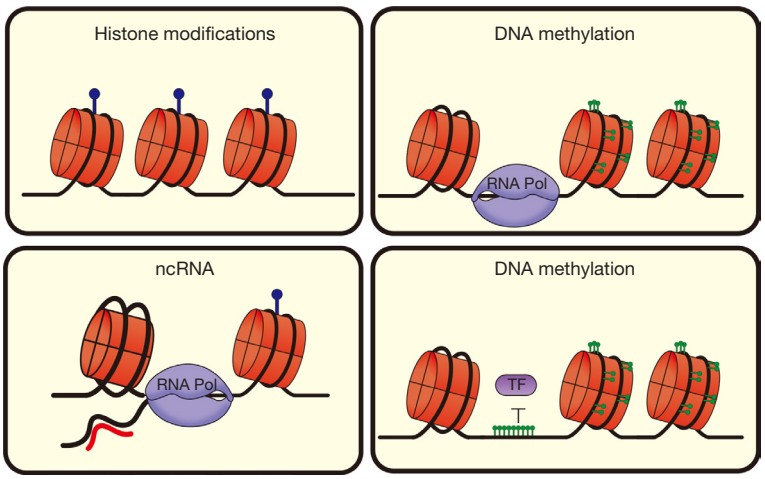

Figure 1 Graphical summary of key epigenetic mechanisms. Upper left quadrant: nucleosomes wrap DNA and are decorated by various histone modifications, that acts as chemical flags. Lower left quadrant: ncRNA such as interfering RNAs may bind mRNA and affect its stability. Upper right quadrant: DNA methylation collaborates with histone modification to condense chromatin, excluding transcription. Lower right quadrant: DNA methylation may also directly impede the binding of sequence specific DNA-binding TF. ncRNA, non-coding RNAs; TF, transcription factor.

correlates with gene expression (38). Several studies have identified disease-specific DNA methylation signatures in peripheral blood cells and intestinal biopsies of IBD patients (39-43). For instance, the epigenetic silencing of the tumor suppressor protein RASSF1A through DNA methylation changes has been linked not only to colorectal cancer but also to IBDs (44,45). Furthermore, GWAS flagged up an association between the *DNMT3A* locus and Crohn's disease (46). To test the link between the *DNMT3A* gene and IBD, Rosenstiel and co-workers studied the role of this gene in mice through tissue specific genetic deletion of DNMT3A in intestinal epithelial cells (30). They found that this leads to altered epithelial ultrastructure with shortened apical-junctional complexes, reduced Goblet cell numbers and increased intestinal permeability in the colon *in vivo*. These mice suffer from increased susceptibility to experimental colitis with reduced epithelial regeneration.

DNA methylation mechanistically regulates genome function in part by creating binding sites for methyl-CpG-binding proteins that, in turn, are part of protein complexes that change chromatin structure, affecting gene expression. A recent study by Jones *et al.* has shown that MBD2, a methyl-CpG-binding protein, regulates susceptibility to experimental colitis in the mouse by acting in dendritic cells and the intestinal epithelial cells (47). MBD2 deficiency leads to an enrichment of pro-inflammatory cell types, including IL-1β expressing monocytes and neutrophils, in the colon of mice with colitis. Restriction of MBD2 deficiency to CD11c+ dendritic cells and macrophages, or to intestinal epithelial cells resulted in increased dextran sodium sulfate colitis severity. In dendritic cells (cDC1) this is likely due to increased expression of the ETS2 transcription factor on *MBD2* deletion. ETS2 controls the induction of microRNA miR-155, a potent pro-inflammatory mediator that is found elevated in the mucosa of IBD patients. *MBD2*$^{-/-}$ colon epithelial cells displayed profound dysregulation of genes controlling major histocompatibility complex, resulting in elevated intestinal CD8+ T cell responses. Thus, MBD2 controls the inflammatory response by acting in the immune and the epithelial compartment, highlighting a key role of the epithelium in this response.

Another study focused on epigenetic regulator, UHRF1 [ubiquitin-like with plant homeodomain (PHD) and RING finger domains 1]. This factor acts as a mediator of DNA methylation by binding to hemi-methylated DNA during S-phase and recruiting the maintenance DNA methyltransferase protein DNMT1. The researchers demonstrated that UHRF1-deficient macrophages overexpress pro-inflammatory cytokine tumor necrosis factor-α (TNF-α), leading to the damage of intestinal epithelial cells and promoting the development of severe experimental colitis (48). This was due to hypomethylation of the promoter region of TNF-α in UHRF1-deficient

macrophages. Additionally, excess of TNF-α led to reduced levels of UHRF1, and consequently decreased methylation by DNMT1 on the promoter region. This led to an overexpression of TNF-α and a pro-inflammatory positive feedback loop. Together, these findings flag up core epigenetic mechanisms as important in IBD.

Histone modifications

Histone modifications are another important facet of epigenetic mechanisms and there is important crosstalk between histone modifications, DNA methylation and changes in chromatin structure (36,49). Histones assemble with almost 2 turns of DNA into micro-spools called nucleosomes to organize the eukaryotic genome into chromatin superstructures. Peptide domains called histone tails protrude from the nucleosome core body and are subject to a plethora of chemical modifications, many happening on lysine residues that are highly conserved. These chemical tags, in turn, often create binding sites for chromatin remodelling factors, regulating gene expression (50-52). Specific histone modifications are critical to maintain chromatin structures through cell generations, propagating gene expression states, such as repression. This is exemplified by histone H3 trimethylation at lysine 9 (H3K9me3) or 27 (H3K27me3), which are critical for the silencing of retrotransposons (H3K9me3) or genes during development (H3K27me3). Histone modifications include lysine acetylation and other acylations, lysine and arginine methylation, phosphorylation, ubiquitination, sumoylation and many others. These modifications are set by a plethora of enzymes, for example histone acetyltransferases, with critical roles in gene expression control and other genomic functions. These enzymes are sometime referred to as 'writers'. The modified histones are then recognized by 'readers' of the modification that affect chromatin structure and removed by 'erasers' (e.g., histone deacetylases). 'Readers' usually contain domains that recognize and bind specifically modified histones, such as bromodomains that bind acetylated histones (49). As the chemical 'flags' involved in epigenetic modifications are derived from precursors (e.g., acetyl coenzyme A for histone acetylation) that are directly connected to cellular metabolism, there is a strong coupling between metabolism and epigenetic regulation (53,54). This is illustrated by various histone acylations, such as acetylation, propionylation, butyrylation, crotonylation and others, which are also affected by the bacterially derived SCFAs, see accompanying review by Fernandes and Vinolo (55-59). Changes in histone acylations have been linked to IBD, this is reviewed in the accompanying paper from Fernandes and Vinolo (59).

SP140 an epigenetic reader implicated in IBD

Recently, a string of studies shed light onto the role of a specific histone "reader" protein called SP140 in IBD, connecting findings from GWAS to cell biological, genetic and microbiome analysis. SP140 is a bromodomain, PHD and SAND (SP100, AIRE-1, NucP41/75, DEAF-1) domain containing protein and is selectively expressed in cells of the immune system (60,61). Bromodomains usually bind acetylated (or acylated) histones while some PHD 'fingers' have been shown to interact with methylated histones. SAND domains interact with DNA. GWAS showed that single-nucleotide polymorphisms (SNPs) within the gene coding for SP140 are significantly associated with Crohn's disease (11,46). Macrophages are critically involved in the inflammatory process of mucosa, producing inflammatory cytokines, which promote the differentiation and activation of Th1 and Th17 cells. SP140 was identified as a key regulator of the macrophage transcriptional response to cytokines and microbes (60,61). It performs this task by binding chromatin predominantly at regulatory regions (60,61). There are currently somewhat divergent results to exactly the nature of where SP140 binds, with one study indicating gene promoter regions decorated with the repressive histone H3K27me3 mark in the macrophages (60), while another study indicating an interaction with active genes (61). These divergent results may be the result of the use of different antibodies for the chromatin immunoprecipitation studies (61). Crohn's disease-associated SNPs within the *SP140* gene lead to elevated expression of a SP140 isoform devoid of exons 7 and 11 and an overall reduction in total SP140 expression and this is linked to reduced expression of genes linked to innate immunity (60). Consistent with all these findings, hematopoietic knockdown of mouse SP140 exacerbates experimental colitis (60).

A further study by the Jeffrey laboratory offers an additional perspective on SP140 (62). The researchers discovered that SP140 binding to chromatin inhibits the activity of proteins related to DNA unwinding and chromatin organization, topoisomerases (TOPs) 1, 2A, and 2B. This interaction with TOPs prevents DNA accessibility and lineage-inappropriate gene expression. Therefore, unchecked activity by these TOPs due to the lack of SP140

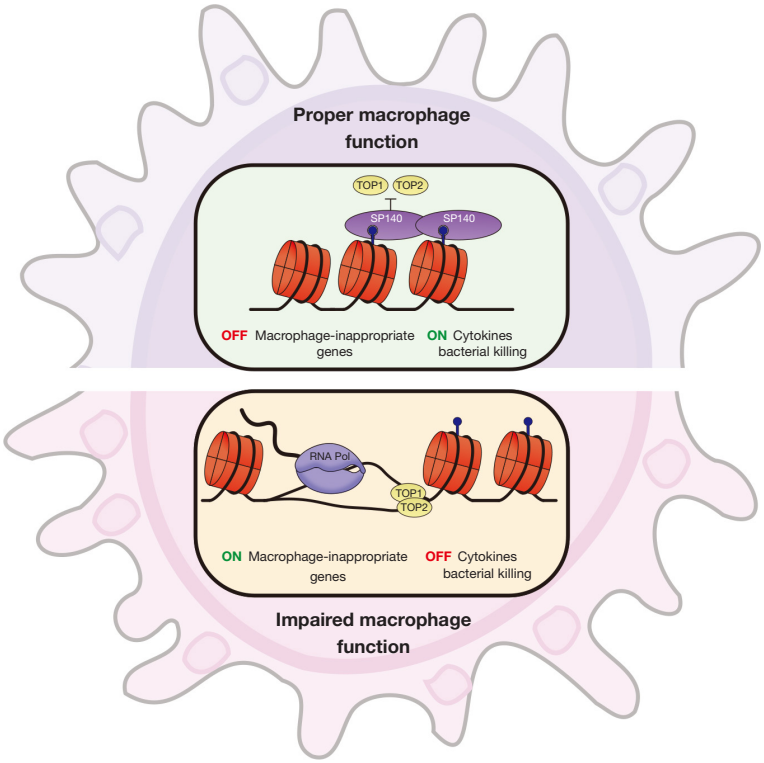

Figure 2 Upper panel: SP140 binds to chromatin, possibly recognizing specific histone modifications and limits the activity of topoisomerases, ensuring expression of lineage appropriate genes in macrophages, resulting in macrophages that express cytokines and kill pathogenic bacteria. Lower panel: in the absence of SP140, topoisomerase function is not properly regulated, leading to incorrect gene expression and impaired macrophage function.

inhibition triggers an inapt differentiation of macrophages and decreased production of cytokines (*Figure 2*).

Other studies illuminated the interaction between SP140 and the gut microbiota. It has been estimated that roughly 10% of the variation in the gut microbiota is explained by the host's genetic architecture (63). Remarkably, a microbiome genome-wide association study (mGWAS) probing human genetic variation influencing the diversity of gut microbial communities (genotype-beta-diversity association testing) identified the *SP140* gene among 4 loci, suggesting that SP140 affects the microbiome composition (64). In line with these findings, mutation or loss of SP140 in mice and human led to expansion of pathobionts in the gut, blooms of *Proteobacteria*, including Helicobacter in mice and Enterobacteriaceae in humans bearing the Crohn's disease-associated *SP140* loss-of-function variant (65). These pathogenic microbes could be transferred to non-mutant host mice via cohousing, inducing a severe colitis phenotype. In a separate study, *SP140*$^{-/-}$ mice were also shown to be susceptible to infection by *Legionella pneumophila* and *Mycobacterium tuberculosis* (66). Thus, a change of an epigenetic factor leads to a changed microbiome and infection, which, in turn, contributes to an exacerbated colitis phenotype.

Work of de Jong and colleagues added to this picture by showing that SP140 expression increases in response to bacterial stimuli, such as lipopolysaccharides, in human "M0" (non-activated) and "M1" (pro-inflammatory) macrophages *ex vivo*, leading to increased expression of pro-inflammatory cytokines such as IL-6 and TNF (61). The study further demonstrated that silencing the *SP140* gene through siRNA reduced expression of several key factors related to Crohn's diseases, including TNF signaling, IL-6, CXCL9, and JAK2, and unlike the studies from the Jeffrey lab, found binding of SP140 over promoters of actively expressed genes in M1 macrophages. Thus, this study indicates that SP140 promotes inflammation-linked gene activation by binding to promoters of many genes, such

as those with roles in immune defense against microbes. The researchers identified a molecule, GSK761, that could competitively bind SP140 and prevent its binding to chromatin, potentially serving as a treatment for Crohn's disease in combination with anti-TNF or for patients who do not respond well to anti-TNF therapy. In this light, it is noteworthy, that low SP140 expression levels in intestinal biopsies of Crohn's disease patients were found to correlate with better response to anti-TNF therapy (60), suggesting that treatment with the SP140 inhibitor may support anti-TNF therapy in non-responder Crohn's disease patients.

While there are clearly still issues about the nature of SP140 binding to chromatin that need to be clarified, the combined studies highlight the crucial role of epigenetic readers in regulating homeostasis and underscore the importance of understanding these regulatory mechanisms for advancing treatments for inflammatory diseases.

Interestingly, there is also a link between SP140 and multiple sclerosis (67,68), suggesting that there are parallels between these pathologies and, potentially, a microbiome link to multiple sclerosis.

Role of SETD2 in IBD

Numerous studies using tissue specific gene deletion in the mouse of have identified potential roles of epigenetic factors, histone modifying enzymes or chromatin remodelling enzymes in the colitis response and these studies also illustrate the roles of the various cell types involved in the pathology [reviewed in (69)]. A recent example in this respect is the analysis of the role of SETD2 in the intestinal epithelium in colitis (70). SETD2 is the only known histone H3 lysine 36 trimethyl-transferase, mediating H3K36me3, a modification usually found over actively transcribed regions and thought to promote transcription. *SETD2* mutations have been implicated in colorectal cancer (71), deletion of *SETD2* specifically in the intestinal epithelial epithelium worsened the pathological response in experimental colitis in the mouse (70). This exacerbation was found to be associated with dysregulated microRNAs (miRNAs) and genes involved in the response to oxidative stress, with these changes in gene expression linked to the loss of H3K36me3 over the affected genes (70,72). Pathology could be alleviated by treating the mice with anti-oxidant N-acetyl-L-cysteine. This is noteworthy, as oxidative stress contributes to the colitis pathology. SETD2 expression was down in IBD patients and mice subjected to experimental colitis (70) and SETD2 was found to be mutated in samples from ulcerative colitis patients with a high risk of developing colorectal carcinoma (73). Regulatory T (Treg) cell or group 3 innate lymphoid cells (ILC3s) specific deletion of *SETD2* showed that this factor also regulates the inflammatory response through these immune cells (74,75). Thus, these tissue-specific deletion studies allow dissection of the roles of epigenetic regulators in the various cell types that contribute to the inflammatory process.

Outlook and conclusions

GWAS identified additional loci associated with genes coding for epigenetic/chromatin factors, such as *DMNT3B* (13) and bromodomain factor *BAZ1A* (76,77) that have not been explored further in this context. The intestinal epithelium is a highly dynamic and proliferative tissue that constantly renews itself from stem cells. Thus, it is plausible that genetic defects in factors that are involved in cellular proliferation, including chromatin assembly and remodelling, may impinge on the intestinal barrier function and this may predispose to inflammation. It is likely that we will identify more components of the epigenetic machinery with a role in IBD in human. However, sometimes findings are counterintuitive, as illustrated with SMARCAD1, a chromatin remodelling factor that has been linked to chromatin replication and DNA repair (78,79). Remarkably, intestinal epithelium specific deletion of this factor rendered mice colitis resistant (80), the precise cause of this is not clear but may be the result of defective regulation of expression of genes involved in regulating inflammation.

An exciting challenge that has really not been taken is to test if epigenetic change *per se* predispose to IBD. Would, for example, the presence of specific bacterial taxa or metabolic products in the gut cause epigenetic changes in the immune compartment or intestinal stem cells that underlie pre-disposition to IBD? We have reached the point where we can address this question through the use of gnotobiotic mice and defined gut microbiota.

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Footnote

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