Effects of DNA methylation and its application in inflammatory bowel disease (Review)

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Abstract. Inflammatory bowel disease (IBD) is marked by persistent inflammation, and its development and progression are linked to environmental, genetic, immune system and gut microbial factors. DNA methylation (DNAm), as one of the protein modifications, is a crucial epigenetic process used by cells to control gene transcription. DNAm is one of the most common areas that has drawn increasing attention recently, with studies revealing that the interleukin (IL)-23/IL-12, wingless-related integration site, IL-6-associated signal transducer and activator of transcription 3, suppressor of cytokine signaling 3 and apoptosis signaling pathways are involved in DNAm and in the pathogenesis of IBD. It has emerged that DNAm-associated genes are involved in perpetuating the persistent inflammation that characterizes a number of diseases, including IBD, providing a novel therapeutic strategy for exploring their treatment. The present review discusses DNAm-associated genes in the pathogenesis of IBD and summarizes their application as possible diagnostic, prognostic and therapeutic biomarkers in IBD. This may provide a reference for the particular form of IBD and its related methylation genes, aiding in clinical decision-making and encouraging therapeutic alternatives.

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Contents

1. Introduction

Inflammatory bowel disease (IBD) is a chronic, recurrent inflammatory condition of the intestine associated with an increased risk of developing colon cancer, and it includes Crohn's disease (CD) and ulcerative colitis (UC) (1,2). Increased intestinal epithelial cell (IEC) mortality is a defining feature of IBD, as it weakens the gut barrier, stimulates immune cells and leads to further IEC death (3). IBD can cause abdominal pain, fever, diarrhea, anemia and weight loss (4,5). Population-based studies have discovered an increase in IBD cases in recently industrialized nations in South America, Asia and Africa; the highest percentages are found in developed countries in North America, Oceania and Europe, possibly indicating the influence of the environment on IBD (6). IBD is influenced by environmental (7), genetic (8), immune (8) and gut microbial factors (9). During IBD, there is a release of pro-inflammatory cytokines, such as interleukin (IL)-1β (10), IL-18 (11), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), IL-17F, IL-1α and IL-25 (12).

DNA methylation (DNAm) is an epigenetic process used to control gene transcription, and the functions of DNAm include cell differentiation and gene expression, which are essential for the immune response (13). Genes linked to IBD may have their levels of gene expression considerably altered by changes in the methylation status, which would affect the development and progression of the illness (14). The genes identified in multigene DNAm research in IBD may be highly variable. Identical pathways have been found to include the IL-12/IL-23 pathway (15) and genes involved with inflammation (4). In addition, genetic and epigenetic connections in the IL-23R/IL-17 axis have been shown to be linked to an increased expression of IL-17 and the pathophysiology of IBD (16). According to the study by Bae *et al* (17), the methylated transcription elongation regulator 1-like gene *(TCERG1L)*, that is implicated as a biomarker in colorectal cancer (CRC), is the same gene observed in CD, suggesting that methylated genes observed in IBD may provide insight into the diagnostic, prognostic and therapeutic markers for IBD. Additionally, the methylated gene suppressor of cytokine signaling 3 *(SOCS3)* has been found to participate in the onset of the inflammatory process and the development of CD (18).

DNAm is involved in the etiopathogenesis of IBD and affects immune, inflammatory and genetic pathways, which may be similar to IBD development and progression. Although the specific pathway of DNAm in IBD is not yet well defined, DNAm is an assayable, dynamic, yet generally stable epigenetic mechanism, thus rendering it an appealing target for creating diagnostic and prognostic biomarkers (19,20). Hence, the present review discusses and summarizes the potential applications and roles of DNAm-associated genes and categorizes them as diagnostic, prognostic, and therapeutic markers of IBD.

2. General applications of DNAm in diseases

Statistics on DNAm have grown to be a crucial source of knowledge for the creation of biomarkers (19); therefore, DNAm states have been implicated for diagnostic and prognostic purposes for therapeutic relevance (20) due to their ability to advance precision medicine (21).

DNAm alterations and gene expression changes in tissues from patients with heart failure (HF) have been identified, offering potential as diagnostic and therapeutic targets in HF (22). Additionally, collagen type XII alpha 1 chain has been acknowledged as a potential druggable site for the treatment of intrahepatic cholangiocarcinoma epigenetic (23). Again, DNAm alterations in the transcription factor 7-like 2 promoter region have been identified as a possible biomarker that can predict the diagnosis of type 2 diabetes (24). Furthermore, the IFN-induced protein 44-like, forkhead box *(FOX)P3* and MX dynamin-like GTPase 1 genes have been identified as potential biomarkers for systemic lupus erythematosus (25). IBD researchers have identified *zinc* finger and BTB domain-containing protein 7B *(ZBTB7B)* as a possible biomarker for the detection and management of UC (26). These are only a few of the numerous DNAm biomarkers discovered under various circumstances. In light of this, potential biomarkers with crucial diagnostic and prognostic roles may offer precision medicine benchmarks for tailored treatments (27).

3. Enzymes and proteins involved in DNAm and IBD

Enzymes. A methyl group is covalently transferred to the cytosine ring's C-5 position on a DNA strand during the DNAm process (28). In mammals, DNA methyltransferases (DNMTs) are the main enzymes responsible for cytosine methylation at CpG sites of epigenetic gene control (29). DNMT1 contributes to maintenance methylation, while DNMT3A, DNMT3B and DNMT3L primarily execute *de novo* methylation (30).

During UC-associated neoplasia development, DNMT1 is significantly expressed, according to Fuji *et al* (31). Similarly, Saito *et al* (32) found that in patients with UC with active mucosal inflammation, DNMT 1 and 3B were significantly manifested in colon epithelial cells. Moreover, it is documented that the expression of DNMT1 and DNMT3A is considerably higher in UC-related carcinogenesis when compared with non-inflammatory colorectal carcinogenesis (33). Additionally, Ueda *et al* (34) found that the neoplastic rectal epithelium exhibited a higher expression of DNMT3B than the non-neoplastic epithelium, and superior results in differentiating UC-associated neoplastic lesions have been linked to an immunohistochemical examination of DNMT3b expression. Furthermore, it has been discovered that alpinetin reduces colitis in mice and that the ameliorative activity of this flavonoid compound prevents DNMT1 from being expressed (35). Hence, this may imply that the expression of DNMT1 is increased in colitis. These suggest that the pathogenesis of IBD and associated complications (cancer) may involve DNMTs.

It has been shown that inflammatory colon tumors have a higher expression of DNMT1 and that there is a positive connection between tumor DNMT1 and CD68 (36). Through *DNMT1*, IL-6 causes DNA cytosine methylation in colon cancer cells (36). The signal transducer and activator of transcription (STAT)3 activates DNMT1 expression in malignant T-cells by interacting with the *DNMT1* gene promoter and enhancer 1, and DNMT1 expression in these cells has the effect of preserving the ongoing activation of STAT3 (37). This may explain the role of DNMT1 in preserving the activation of STAT3 to cause malignant cell changes. This provides evidence of a direct association between oncogenic, abnormal cell signaling and epigenetic gene silencing, which often impacts tumor-suppressor genes (37). Moreover, with DNMT1, STAT3 may induce the epigenetic silencing of Src homology region 2 domain-containing phosphatase 1, which could partially transform cells (38). This evidence further corroborates the role of DNMT1 and STAT3 in the pathogenesis of IBD-associated cancer. In other studies, 28.6% of type 1 cases and all type 2 cases of intraductal papillary neoplasms of the bile duct have high expression levels of the DNMT1 protein (39). However, it has been revealed that the epithelial cells of patients with IBD have lower levels of DNMT3A, and IECs that lack functional DNMT3A are more vulnerable to experimental colitis (40). This further corroborates other findings by Li *et al* (41), who found that DNMT3A protein expression was reduced in the prefrontal cortex, hippocampus and cerebellum of rats with valproic acid-induced autism spectrum disorder.

Ten-eleven translocation (TET) enzymes, which regulate methylation patterns, have the ability to reverse DNAm in active genomic areas (42). The TET family of DNA dioxygenases oxidizes 5-methylcytosine (5mC) to 5-hydroxymethylcytosine

(5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) in that order (43), promoting demethylation both passively and actively (42). DNA base-excision repair components can remove 5fC and 5caC, resulting in unaltered cytosines (43).

TET3 plays a crucial role in maintaining tissue homeostasis by controlling the transcriptome and DNA methylome of the gut epithelium, particularly in response to luminal stresses (43). Recent research has suggested epigenetic mechanisms modulating DNAm in the pathophysiology of various inflammatory and cancerous diseases, and the TET-2 enzyme has been shown to catalyze the demethylation, thereby controlling the activity of numerous genes that promote and repress tumors (44). Additionally, comparing normal colon tissues to those with IBD and colon cancer, the expression of TET2 and its epigenetic mark 5hmC is significantly downregulated in the latter two conditions (45). This may suggest that the upregulation of TET2 may prevent IBD and colon cancer. In human tissues, aberrant methylation induction is caused by DNMT activation owing to nitric oxide generation and *TET* inhibition due to nuclear factor κB activation (46). The expression of a protein called connexin 43 (Cx43), which is involved in gap junction complexes and intercellular communication, is altered in pathological diseases, such as cancer and IBD (44). The levels of Cx43 and the demethylating enzyme TET-2 are increased in inflammatory situations. However, in sporadic colon adenocarcinomas, Cx43 expression is downregulated when TET-2 levels are low, which suggests that TET-2 functions to inhibit Cx43 and its potential to reduce tumors (44). In a different study, *in vitro* analyses revealed that TET2 overexpression and DNMT3A knockdown prevented oral squamous cell cancer from proliferating and migrating (47). The TET2/3 deletion also affects the microbiota, making the intestine more susceptible to inflammation during homeostasis and acute inflammation-induced mortality (48). These findings may provide evidence of the role of TET enzymes in preventing inflammation and tumors.

Proteins. Methyl-CpG-binding domain (MBD)-containing proteins bind at 5 mC and translate the information about methylation patterns into the proper functional cellular states (49). Ludwig *et al* (50) reported that methyl-CpG binding protein 2 and MBD2 play a crucial physiological function as keepers of the epigenome. The expression of MBD2 and DNMT3B is suppressed by black raspberries in dextran sodium sulfate (DSS)-induced UC (51), and this may imply that MBD2 expression is increased during IBD. However, there are only a limited number of studies available on this topic; hence, further studies are required to explore the interactions between DNMT, TET and MBD2 in DNAm and IBD pathogenesis. This may provide additional therapeutic strategies for the treatment of IBD.

4. Common signaling pathways involved in DNAm and IBD

IL-12/IL-23 pathway. Of particular interest is the discovery of a number of methylation loci connected to IBD that are part of the IL-12/IL-23 pathway, as this is a crucial regulator in the emergence of intestinal inflammation and the pathogenesis of IBD (15,52). IL-12 and IL-23 are produced by macrophages, which share the p40 subunit encrypted by the *IL-12B* gene as a heteromer partner to promote T helper (Th)1 and Th17 specialization, which mediates the development of IBD; hence, blocking p40 can be an effective treatment for IBD (53). Moreover, it is noteworthy that the disease-associated methylated gene *p40* is jointly mediated by both IL-12 and IL-23 and that BCL3 transcription coactivator, *STAT3*, oncostatin M and *STAT5* are a few of the methylation loci that are implicated in the control or downstream signaling of the IL-23 pathway (14) (Fig. 1). To add to the premise that the *IL23R* gene is involved in IBD, Duerr *et al* (54) discovered an extremely significant correlation between CD and the *IL23R* gene on chromosome 1p31, which codes for a portion of the receptor for the proinflammatory cytokine IL-23, and this further increases the evidence that the IL-23 pathway is harmful in human illnesses, as suggested by the genome-wide association studies (GWAS) findings (55). The *IL23R* gene is significantly associated with psoriasis and IBD using GWAS in several populations, indicating that the pathophysiology of these diseases may be affected by disruption of the IL-23 signaling pathway (55). These findings support the premise that the methylation genes may use the IL-12/IL-23 pathway to exacerbate or control IBD.

Wnt signaling axis. Wnt signaling gene methylation increases in IBD and IBD-associated neoplasia (56). Dhir *et al* (56) found that there was a frequency of Wnt signaling genes, such as adenomatous polyposis coli (APC) regulator of WNT signaling pathway (*APC*)*1A*, *APC2*, secreted frizzled-related protein (*SFRP*)*1* and *SFRP2* being methylated in IBD and IBD-associated neoplasia. The Wnt signaling pathway, which is necessary for epithelial formation, is controlled by the tumor suppressor gene (TSG) *APC* (57). *APC* inactivation or silencing promotes CRC (57). Additionally, according to reports, *SFRP* regulates Wnt signaling, which is considered to have a crucial function in the development of tumors (58). There is hypermethylation of all SFRP (1, 2, 3, 4 and 5) genes during malignancy, causing gene silencing (59). Therefore, the increased methylation of *SFRP1, SFRP2* and *SFRP5* in patients with CD, as discovered by Kim *et al* (60), may be involved in the Wnt signaling pathway.

IL-6/STAT3/SOCS3 signaling. The initiation of the expression of DNMT1 in persistent colonic inflammation may unleash IL-6 signaling towards STAT3 from suppression through *SOCS3*, increasing the likelihood of a cancerous transformation (61). Additionally, *SOCS3* discovered in intestinal tissues increases the vulnerability to CD, according to Sanati *et al* (18). They stated that the inflammatory state of the mucosa was connected to the abnormal methylation of the CpG islands within the promoter zones of the *SOCS3* gene in the colonic mucosa in CD. This helps provide knowledge of the mechanisms through which methylation affects the inflammatory process and the formation of CD (18). The higher methylation of the *SOCS3* gene (18) may have led to the inability of the gene to suppress cytokines, leading to CD. These findings suggest the function of IL-6, STAT3 and *SOCS3* signaling in the progression of IBD and IBD-associated neoplasia.

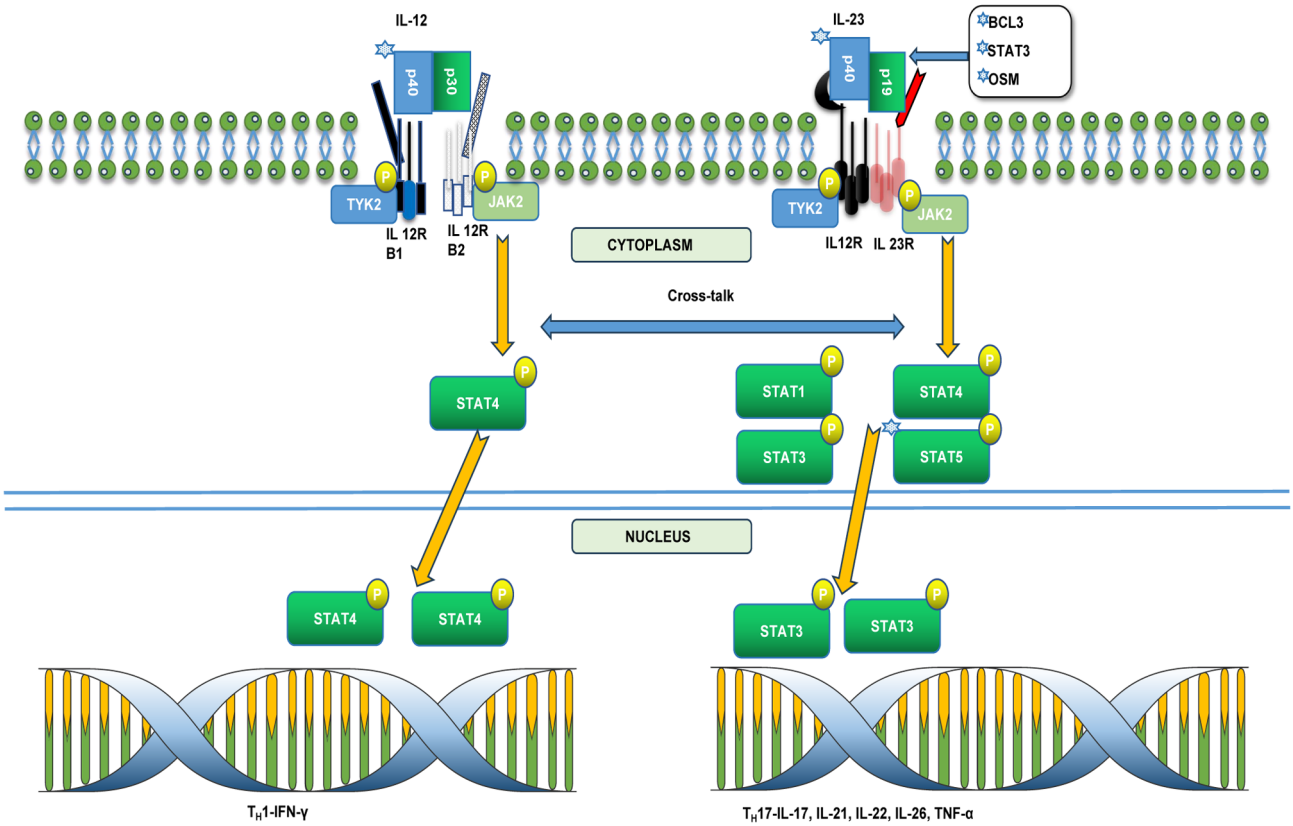

Figure 1. Disease-related DNAm in B cells from patients with IBD. The blue star symbol indicates genes with DNAm loci connected to IBD. IBD, inflammatory bowel disease; DNAm, DNA methylation; *BCL3*, BCL3 transcription coactivator; IFN-γ, interferon-γ; IL, interleukin; JAK2, Janus kinase 2; OSM, oncostatin M; R, receptor; *STAT3*, signal transducer and activator of transcription 3; TH1, T-helper 1; TNF-α, tumor necrosis factor-α; TYK2, tyrosine kinase 2.

Apoptosis signaling pathway. Another study revealed that the *BAD* gene (downregulated and hypermethylated) was involved in the apoptosis signaling pathway in DNAm, providing a novel therapeutic approach for managing colitis-associated cancer (CAC) (62). *BAD* engages in cell cycle arrest and apoptosis (63); thus, the hypermethylation and downregulation of the *BAD* gene may hinder the apoptotic activity of the gene, thereby increasing the proliferation of tumors in IBD, leading to CAC. This supports the research by Huang *et al* (63), who found that *BAD* upregulation suppressed cell growth in CRC tissues when Ras-related C3 botulinum toxin substrate 1 was silenced, suggesting that *BAD* downregulation may promote CRC.

5. Samples and tools for detecting the DNAm status in IBD

The most prevalent samples used to study DNAm include whole blood, serum, colon biopsies and stool. However, it has been demonstrated that DNAm patterns related to CD observed in blood samples are a consequence of the inflammatory characteristics of the disease and are less likely to contribute to disease genesis or progression (64). Although a fraction of blood-derived methylation quantitative trait loci may be implicated in CD-related activities, the vast majority are common across individuals (65). Methylation trends observed in blood specimens from patients with CD accompany acute inflammation, and after receiving therapy, they shift to methylation patterns similar to those seen in individuals without intestinal inflammation (64). Since IBD is a chronic, recurrent disease, this could signal that a blood sample may not be sufficient as a DNAm specimen. Therefore, further research using immunological and epithelial samples obtained from mucosal biopsies is needed to confirm these findings (64). As a result, using both blood and tissue from biopsies may suffice for a DNAm specimen.

Although tissue samples from a mucosal biopsy are ideal for DNA analysis, their clinical utility as indicators may be limited. It is challenging to recover high-quality DNA from formalin-fixed and paraffin-embedded tissue (FFPET) due to the DNA degradation caused by formalin and the impediment of paraffin for DNA extraction (66). As a result, the low quality of genetic material recovered from FFPE samples may have an impact on the feasibility and reliability of sequencing data (67) in the clinical setting. However, other alternatives may be used to improve the quality of the DNA. Glyoxal acid-free and acid-deprived formalin fixation), two acid-deprived fixatives, provide the optimal DNA preservation and sequencing results, enabling more intricate molecular profiling of tissue samples (67). Despite research demonstrating that tissues can be used for DNAm testing in IBD, other studies have shown that the use of tissue biopsies can predict the occurrence of cancer, and these studies have reported that they could be used for the early detection of CRC in patients with IBD who are at an increased risk (68,69). For patients with IBD with tiny adenomas and serrated lesions, specific DNA markers associated with progressive IBD neoplasia can also be found in their tissues and feces (70). Fecal samples are also emerging as DNA

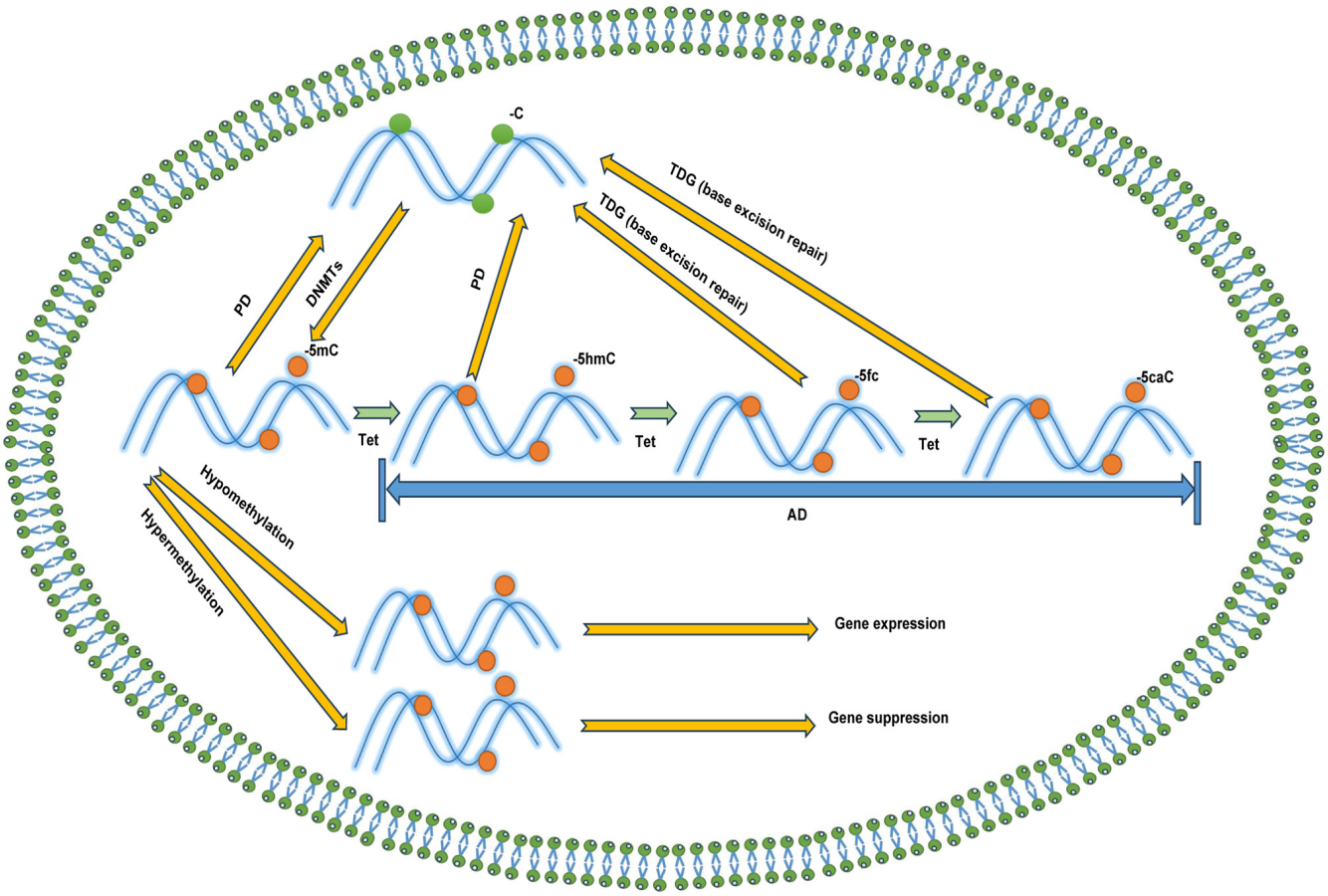

Figure 2. Association between methylation status and gene expression or suppression in IBD. An unmethylated gene becomes methylated by DNMTs, affecting gene function and leading to IBD. TET enzymes help to reverse aberrant DNAm. Methylation status and gene expression or suppression may be used as biomarkers for IBD and associated neoplasia. IBD, inflammatory bowel disease; DNAm, DNA methylation; AD, active demethylation; c, cytosine; caC, carboxylcytosine; DNMTs, DNA methyltransferases; fc, formylcytosine; hmC, hydroxymethylcytosine; mC, methylcytosine; PD, passive demethylation; TDG, thymine DNA glycosylase; TET, ten-eleven translocation.

specimens. Therefore, it will be possible to use fecal DNAm as a marker for IBD and CRC (71). Kisiel *et al* (72) demonstrated the testing viability of stool DNA for the non-invasive identification of colorectal neoplasia linked to IBD.

In addition, DNAm data utilization for new clinical applications, IBD diagnosis and treatments may have resulted from the study by Kang *et al* (73), who employed tissue samples for DNAm in IBD. Gene expression profiles from tissue samples from patients with IBD and drug-treated IBD cell cultures can be correlated, potentially leading to the identification of novel molecular target genes for IBD treatment and drug development (74). These findings suggest that tissues and stool may be used to identify CRC in patients with IBD and for drug development and treatment.

Some of the current tools for assessing changes in or the status of DNAm include the Illumina HumanMethylation EPIC BeadChip array (75), Illumina arrays (76), Illumina MethylationEPIC and Illumina Multi-Ethnic arrays (65), Illumina EPIC Beadchip (v1.0) (77) and the Infinium MethylationEPIC BeadChip (Illumina) (78).

6. Roles of DNAm in IBD

As a diverse condition with a complex etiology, only a tiny fraction of the IBD disease variance can be attributed to genetic variation, according to quantitative genetic studies, suggesting that differential epigenetic regulation may be associated with the etiology of IBD (79). A growing body of research suggests that alterations in the methylation status of IBD-related genes may impact gene expression levels and may be involved in the development and progression of the illness (18). Researchers have identified several genes for promising diagnostic and prognostic uses in this complex disease. These genes are methylated (hypermethylation or hypomethylation) and specific to IBD, leading to reduced or overexpressed genes. The reduced or increased methylation of the genes stimulates or represses their functions, leading to IBD pathogenesis (Fig. 2). Additionally, a few of these genes are potential biomarkers for IBD and IBD-neoplasia.

Contribution to the etiology, development and disease activity. McDermott *et al* (79) identified that the tripartite motif-containing 39-ribonuclease P/MRP subunit p21 (TRIM39-RPP21) promoter region was markedly hypomethylated. In addition, TNF receptor-associated factor 6 (TRAF6) was hypermethylated, and a decrease in TRAF6 gene expression in pediatric patients with IBD was also found in peripheral blood mononuclear cells, indicating their contribution to the pathological process and activity of IBD (79). Surprisingly, even though the precise mechanism is unknown, this was the first

study to link abnormal DNAm and TRAF6 gene expression to the pathogenesis or pathophysiology of IBD (79). According to a different study, animals lacking TRAF6 in IECs have more pronounced DSS-induced inflammatory reactions, causing chronic intestinal inflammation to develop (80). This further corroborates the notion that the *TRAF6* gene may contribute to the pathological activity of IBD. Also, a recognized modulator of the interferon response, the TRIM39/RPP21 read-through transcript, is an essential pathway in the pathophysiology of systemic lupus erythematosus and cutaneous lupus erythematosus (81). Additionally, Arabian foals with juvenile idiopathic epilepsy have been discovered to have genetic variations within TRIM39-RPP21 (82). Thus, the mechanisms through which TRIM39-RPP21 contributes to IBD are unknown. However, based on the aforemtioned findings, it is likely that TRIM39-RPP21 may contribute to the etiology and activity of IBD when genetically expressed (i.e., hypomethylated).

Moreover, Nimmo *et al* (83) also investigated the methylation level among patients with CD and controls. It was found that there are substantial methylation changes in genes involved in immune activation, such as *IL-21R*, in patients with CD. This offers crucial information about the role of epigenetic pathways in the etiology of CD (83). In addition, the cellular environment of giant cell arteritis arteries exhibits hypomethylation of *IL21* (84). It is well known that patients with IBD have higher serum levels of IL-21, indicating that the etiology of IBD may be impacted by IL-21/IL-21R signaling (85). Therefore, the hypomethylation of *IL-21R* may lead to an increased gene expression of the IL-21/IL-21R pathway, leading to the increased discharge of IL-21 in CD. This supports the research of Holm *et al* (86), who discovered that IL-21 and IL-21R were significantly expressed in the guts of patients with CD and that neutralizing IL-21 in experimental T-cell-driven colitis resulted in a decline in pathological and clinical manifestations. Recently, another study also revealed an elevated expression of IL-21 in the CD and UC categories (87). Another study discovered that IL-21/IL-21R enhances macrophage pro-inflammatory responses during a respiratory *Chlamydia muridarum* infection (88). Previously, Wang *et al* (85) demonstrated that Th1 suppression and Th2, Th17 and Treg activation in mice were two mechanisms through which IL-21/IL-21R signaling protected against DSS-induced acute colitis. However, a recent study by Holm *et al* (86) demonstrated that IL-21 and IL-21R expression may contribute to the pathogenesis of IBD.

In the study by Sanati *et al* (18), the DNAm status in the promoter zone of the human *SOCS3* gene of intestinal samples from 15 patients with CD and 15 age- and sex-matched healthy controls was analyzed. The *SOCS3* gene promoter region was found to be more methylated in patients with CD than in the healthy controls, indicating that the abnormal methylation of the CpG islands within the promoter area of the *SOCS3* gene in CD colonic mucosa is linked to a mucosal inflammatory status (18). This sheds light on the mechanisms through which methylation may be involved at the beginning of the inflammatory process and in the development of CD (18). The primary physiological regulator of cytokine-mediated STAT3 signaling is the SOCS3 protein (89). It has been documented that *SOCS3* deficiency in myeloid cells aggravates colitis caused by DSS, and it also keeps the immune system from being overtly activated in patients with IBD (90). The loss of *SOCS3* in myeloid cells leads to an enhanced infiltration of monocytes and neutrophils in the colon and higher numbers of monocytes and neutrophils in the spleen (90). This suggests that *SOCS3* suppresses cytokines when in abundance or activated. Therefore, the loss of *SOCS3* activity due to hypermethylation may have increased the release of inflammatory cytokines that led to CD. The physiological processes by which long-term inflammatory reactions in the colon may encourage CRC remain unclear; they may involve the decreased negative control of IL-6 signaling toward STAT3 activations through the loss of SOCS3 expression, releasing the full toxic potential of this transcription factor (61). It has also been discovered that hepatocellular carcinoma (HCC) linked to the hepatitis B virus is associated with *SOCS3* hypermethylation (91).

IFNG DNAm in the mucosal compartment has been examined in both normal and IBD populations and compared with its peripheral counterparts. Lamina propria T-cells had considerably lower overall *IFNG* methylation (across eight CpG sites) than peripheral blood T-cells (92). According to this research, *IFNG* expression roughly triples in response to a 5% reduction in promoter methylation status. Hence, cytokine production in the mucosa may be modulated by *IFNG* in a mechanistic manner (92). *IFNG* has been well-established to be associated with IBD; however, its allele is yet to be understood. However, Gonsky *et al* (93) found that improved IFN-γ release in IBD is related to the *IFNG* rs1861494 T allele. In CD, it is associated with a complex disease, and this gene may indicate the severity of IBD (93). In other studies, CpG site methylation levels and functional polymorphisms in the *IFNG* gene have been linked to the pathophysiology and prognosis of autoimmune thyroid diseases, particularly Graves' disease intractability (94). Furthermore, genetic variations in *IFNG* could act as hereditary determinants for chronic prostatitis/chronic pelvic pain syndrome vulnerability (95). These findings indicate that *IFNG* may contribute to the pathogenesis of a number of diseases, including IBD.

Surveillance or monitoring of the progression of IBD to cancer. Specific genes are isolated during IBD progression to neoplasms and at the early stage of the disease. Hence, these markers may predict the outcome of an initial IBD diagnosis, the progression to cancer, and the need for further intervention. Of note, although *FOXE1,* spectrin repeat-containing nuclear envelope protein 1 (*SYNE1*), *TCERG1L* and other genes have been identified as promising biomarkers of neoplasia in IBD, they may be used as markers for cancer surveillance and monitoring in IBD patients.

Papadia *et al* (96) examined the methylation status of *FOXE1* and *SYNE1* in 93 patients with chronic IBD undergoing a cancer monitoring program and 30 healthy controls. It was discovered that there was an increase in methylation of *FOXE1* or *SYNE1*, leading to the epigenetic silencing of these genes in a region of dysplasia in patients with IBD compared with controls; hence, these genes can predict neoplasia in chronic IBD (96). At least one of the *FOXE1* and *SYNE1* genes may experience epigenetic suppression or an epigenetic alteration, such as methylation before aberrant new growth or cancer is visible (96). *SYNE1* has also been discovered to be regularly methylated in the majority of premalignant adenomas, and this

makes it a helpful biomarker in the early identification of these adenomas and CRCs (97). Therefore, under colonoscopic surveillance, a patient at a higher risk than the average may be identified through the CAC recording of hypermethylation of *SYNE1* and *FOXE1* (96). The increased DNAm of *FOXE1* has also been observed in breast cancer (98) and cutaneous squamous cell carcinoma (99). A higher methylation rate of the *SYNE1* promoter region has also been observed in gastric cancer (GC) tissues compared to adjacent normal tissues (100).

Additionally, patients with CD have elevated methylation levels in the *TCERG1L* gene (17). It was also implicated previously as being frequently methylated (hypermethylated) and silenced in colon tumors during the initial identification of colon cancer patients and selected as a biomarker for colon cancers (17,101). Hence, the methylation status of the *TCERG1L* gene may have the capability of developing into a risk indicator for the progression of severe disease. It is proposed that routine colonoscopic monitoring with sensitive DNAm markers in serum samples may identify patients with CD, which may help reduce the risk or prevent the advancement of severe stages of the illness (17). Furthermore, although the frequent methylation of *TCERG1L* has been observed in UC, it has been shown that *TCERG1L* is substantially methylated more prominently in patients with CRC than in patients with UC (102). Regular colonoscopic surveillance may help combat the development of severe disease in patients with UC by evaluating their *TCERG1L* gene methylation (102). This further corroborates the findings of *TCERG1L* as an indicator of CD and UC progression to cancer or for cancer monitoring.

According to Scarpa *et al* (33), APC, h-cadherin (CDH13), O^6-methylguanine-DNA methyltransferase (MGMT), mutL homolog 1 and the runt-related transcription factor 3 (RUNX3) methylation status in the non-neoplastic mucosa may be utilized as a marker of CRC. These may enable the modification of a patient's surveillance interval and the identification of patients with UC who require close monitoring (33). According to Alafaria and Jalal (103), MGMT may be a predictive biomarker for non-invasive oral cancer screening and early tongue squamous cell carcinoma diagnosis in the clinical setting. Nonetheless, APC gene promoter methylation, identified as a possible marker in lung cancer, may not be appropriate for screening individuals for lung cancer in general due to its low sensitivity (104). Other methylated genes, such as RUNX3 (105) and CDH13 (106), have been found to play a role in gallbladder cancer and colorectal tumorigenesis, respectively.

Again, Kim *et al* (107) found the hypermethylation of the fragile histidine triad (*FHIT*) gene in samples from patients with CD compared with normal controls. It is noteworthy that the *FHIT* gene also functions as a TSG. It is frequently methylated in tumor growth, although its function as a TSG is lost due to epigenetic alterations (108). Hence, the hypermethylation of the gene in CD may lead to gene suppression, leading to a loss of function in its TSG ability to suppress tumors. This may increase the risk of tumor formation in CD. In a different study, the risk of liver cancer was substantially correlated with hypermethylation of the *FHIT* gene (109). Additionally, *FHIT* hypermethylation and inactivation of the *FHIT* gene contribute significantly to the development of cancer and might act as a possible sign for diagnosis (110). Therefore, the *FHIT* gene may be helpful for the clinical monitoring of the progression to cancer in patients with CD (107).

It has been documented that the tissue factor pathway inhibitor *(TFPI)2* gene has an age-dependent increase in methylation frequency with IBD progression. Again, IBD, precancerous and malignant tissues have also been found to have increased integrin subunit alpha (*ITGA)4* methylation frequencies (111). However, *ITGA4* and *TFPI2* are completely unmethylated in controls (111). Moreover, methylated ITGA4 exhibits incredible potential as a marker gene for the timely identification of colonic cancers (112). Additionally, it has been discovered that TFPI2 is a potent inhibitor of extracellular matrix breakdown, preventing the growth of metastases and tumor cell invasion (113). Hence, the methylation markers *ITGA4* and *TFPI2* are transcriptionally repressed after increasing methylation, and reduced expression may lead to the loss of function of these genes. These make them acceptable risk factors for inflammation-associated colon cancer and could be helpful in the future for CRC screening (111).

Function as biomarkers in IBD

Potential diagnostic markers in UC. It has been well-documented that the model of DSS-induced colitis has been used to investigate the molecular mechanisms underlying the development of UC as regards *ZBTB7B*. DNA alterations have also been found by comparing UC tissues with those from healthy volunteers as controls. It has been shown that the epigenetic DNA hypomethylation and overexpression of *ZBTB7B* drive $CD4^+$ T-cell maturation and suppress $CD4^+CD8^+$ T-cell differentiation, leading to the generation of inflammatory cytokines and colonic inflammation in UC. Therefore, *ZBTB7B* may serve as a biomarker for both the diagnosis and treatment of UC (26). ZBTB7B plays a crucial role in regulating the growth of natural killer T (NKT)-cell subsets identified by the expression of CD4 and CD8 on their cell surfaces (114). ZBTB7B closely regulates CD4 expression, to an extent that even a slight decrease in the expression of this factor reduces $CD4^+$ NKT-cells; CD8+ NKT-cells only appear when ZBTB7B is absent (114). ZBTB7B inhibits RUNX-mediated CD4 repression to increase CD4 expression (115), and this may imply why the increased expression of *ZBTB7B* drives the maturation of $CD4^+$ T-cells, which leads to the production of cytokines and hence, its use as a diagnostic marker of UC.

Furthermore, Kang *et al* (73) examined the DNAm changes in 79 patients with UC, and found that patients with UC had hypermethylated versions of the genes for the family with sequence similarity 217 member B *(FAM217B)*, *KIAA1614* and *RIB43A* domain with coiled-coils 2 *(RIBC2)* compared to the controls. These genes were found to be transcriptionally repressed in specimens from patients with UC using reverse transcription-quantitative PCR, suggesting that their silencing corresponds with promoter hypermethylation. Therefore, it was proposed that these genes can provide fresh clinical data that can be applied to the diagnosis and effective therapy of IBD (73). Although the mechanisms through these genes cause disease are unclear, FAM217B has been identified as a protein biomarker for syndromic CLN3-Batten (116). In addition, differentially expressed *RIBC2* is a prognostic biomarker in cervical cancer (117). The progression of CD appears to be

associated with nine single-nucleotide polymorphisms close to *KIAA1614* (118). This implies that these genes may be involved in the pathogenesis of IBD and have been suggested as biomarkers for the disease.

In a previous study, the DNAm status in untreated, left-sided colonic biopsy specimens from 22 controls, 15 untreated CD clients and 9 untreated UC clients was examined. It was found that the interferon-induced transmembrane protein 1 *(IFITM1)*, integrin subunit beta 2 *(ITGB2)*, S100 calcium-binding protein A9, secretory leukocyte peptidase inhibitor, serum amyloid A1 *(SAA1)* and *STAT3* genes had an altered epigenetic gene expression associated with immunological and defensive reactions in the colonic mucosa, indicating their use as etiologic, diagnostic and therapeutic markers for UC (119). Similarly, other studies have validated the inflammation-associated increased gene expression of *SAA1* (120), *ITGB2* and *IFITM1* (121) in UC. In a previous study, DNAm analysis performed on 240 newly diagnosed IBD cases and 190 healthy controls also found deferentially methylated regions linked to IBD as *ITGB2* (122). That study was performed on a larger sample. In other studies, *ITGB2* methylation in the blood (123) and ferroptosis- and immune-related-differentially expressed genes (*IFITM1)* (124) were documented as possible biomarkers for coronary heart disease detection and kidney renal clear cell carcinoma diagnosis and prognosis, respectively.

Potential diagnostic markers in CD. The promoter methylation status of TSGs [*SFRP1, SFRP2, SFRP5, TFPI2*, SRY-box transcription factor 17 *(SOX17)* and GATA binding protein 4 (*GATA4*)] in patients with CD has also been examined. It was discovered that the TSGs were strongly hypermethylated, which suggests their probable clinical uses for the non-invasive diagnosis and prognosis of patients with IBD (60). *TFPI2* methylation has been detected in upper gastrointestinal cancer (UGC) (125) and in HCC (126) and is suggested as a biomarker for early detection of UGC and the prognosis of HCC. Similarly, methylation of *SOX-17* has been found in advanced GC (127) and HCC (128). *SOX-17* has also been identified as a marker for the non-invasive detection of pancreatic precursor neoplasms (129). It is known that *GATA-4* promoter hypermethylation and transcriptional suppression are common in CRC and GC (130). GATA-4 upregulates genes that could be anticancer targets (130), implying that its silencing may lead to tumor progression in CRC and GC. Moreover, all five *SFRP* genes, namely 1, 2, 3, 4 and 5, are hypermethylated during tumor formation, which results in transcriptional suppression (59). The frequent hypermethylation of TSGs, which silences genes, has already been documented. The loss of TSG function may promote the development of CD into neoplasia, suggesting that it may be useful as a diagnostic and prognostic marker in CD.

According to the findings from the study by Bae *et al* (17), colon cancer cell lines had a substantially greater *TCERG1L* methylation level than CD patient blood and tissue samples. Furthermore, they revealed that *TCERG1L* was sufficiently sensitive for detecting inflammatory illness in tissue and blood samples from patients with CD (17). The promoter DNA hypermethylation of the *TCERG1L* gene and its silencing in CRC tumors may be a superior biomarker for the early detection of CRC (101). Therefore, the *TCERG1L* gene may be used to diagnose CD progression to cancer.

Potential diagnostic markers in IBD (CD and UC). Previously, Karatzas *et al* (131) found five hypermethylated genes [C-X-C motif chemokine ligand *(CXCL)14, CXCL5, GATA-3, IL17* and *IL4R]* in patients with UC; however, patients with CD did not have hypermethylated genes when compared to healthy controls. Hence, these genes have been suggested for use as a non-invasive marker for the diagnosis and prognosis of IBD patients due to the specific DNAm signatures CD and UC exhibit (131). However, Taman *et al* (132) examined the genome-wide DNAm and gene expression of patients with UC who had not yet received any medication and controls. They found the hypomethylation of genes involved in the immune response, such as chemokines and interleukins (132). These differences in chemokine methylation patterns may require further investigation to confirm their methylation statuses and allow treatment strategies in larger cohorts. Epithelial cells of the intestinal mucosa of patients with CD, UC and acute appendicitis have been shown to express CXCL5, a significant CXC chemokine (133). Furthermore, Friedrich *et al* (134) demonstrated that elevated levels of IL17C played a role in the pathophysiology of IBD. These findings further affirm those from the study by Taman *et al* (132), since hypomethylation leads to increased gene expression. In IBD, a decreased production of IL-4 may lead to compromised immunosuppressive and anti-inflammatory processes, which could promote the pathophysiology of the disease (135). Notably, the degree of CXCL14 expression could be a helpful adjuvant parameter in CRC prognosis prediction in GC (136). Tumor site, clinicopathological stage and lymph metastasis have all been linked to CXCL14 expression downregulation (136). It has been documented that hypermethylation leads to decreased gene expression. Hence, the downregulation of CXCL14 in gastric tumors and its association with metastasis further confirm the findings of the study by Karatzas *et al* (131). Furthermore, the CpGs of CXCL5 and CXCL14 have been shown to be associated with the prognosis of patients with pancreatic adenocarcinoma (137). These differences may imply that DNAm may have methodological differences contributing to the challenges of the study of DNAm.

Furthermore, the diagnostic utility of transforming growth factor beta 1 *(TGF-β1)* in differentiating UC and CD among patients who have never received treatment has been evaluated. Patients with CD and controls were differentiated by 14 TGF-β1 CpG locations; UC and control patients were also distinguished by nine TGF-β1 CpG locations; three TGF-β1 CpG locations distinguished between CD and UC; and six TGF-β1 CpG locations distinguished colonic CD from UC. According to reports, it has been suggested that CpG methylation in the *TGF-β1* gene's promoter zone can effectively distinguish between CD and UC and may serve as an essential diagnostic marker in kids with IBD (138). IBD and CAC have both been linked to TGF-β signaling abnormalities (139). In patients with IBD, there is deficient TGF-β1/Smad signaling due to elevated Smad7, a suppressor of TGF-β1 activity (140). In isolated lamina propria mononuclear cells from individuals with CD, TGF-β1 cannot block the generation of pro-inflammatory cytokines; however, the inhibition of Smad7 restores TGF-β1 signaling and allows TGF-β1 to inhibit cytokine production (141). Although the methylation status is not specified, Smad7 expression may be elevated, inhibiting TGF-β1

signaling activity during DNAm. Therefore, further studies may be required to discover the mechanisms involved. In other research, the increased expression of TGF-β1 was shown to be a potential marker of early cardiac fibrosis, observed even before hypertrophy, as it indicates that the chronic inflammatory state contributes to myocardial fibrosis in patients with Fabry disease (FD) (142). Therefore, in patients with FD, TGF-β1 biomarkers may be useful as predictive markers for unfavorable cardiovascular outcomes (142). Similarly, urine TGF-β1 has been identified as a possible marker of early kidney damage in sickle cell disease (143). Additionally, TGF-β1 is a potential biomarker for bone non-union (144). These findings provide further evidence of the use of TGF-β1 a biomarker for several conditions, including IBD.

Azuara *et al* (68) also found that patients with IBD with an increased likelihood of dysplasia or cancer have higher methylation of slit guidance ligand 2 *(SLIT2)* and transmembrane protein with EGF-like and two follistatin-like domains 2 (*TMEFF2)* than those with low risk. Therefore, when high-risk patients with IBD have colorectal dysplasia or cancer, methylation markers may help detect these conditions early (68). In chronic myeloid leukemia, the hypermethylation of the *SLIT2* promoter is associated with the advancement of the illness (145). In addition, *TMEFF2* DNAm is a useful predictive marker for adult diffuse gliomas and may be linked to the progression of glioma tumors (146). During the hypermethylation of these genes (TSGs), there may be a suppression of their capability to regulate cell growth (loss of TSG function), hence increased tumor growth in these conditions. Therefore, *SLIT2* and *TMEFF2* may be potential diagnostic biomarkers in IBD-associated CRC.

The methylation markers ITGA4 and TFPI2 (111) have been discussed above as markers for CRC screening in patients with IBD; nonetheless, it has been documented that the methylation of *ITGA4* and *TFPI2* is a viable epigenetic marker for the early detection of cancer associated with IBD (111). In a related study, *ITGA4* demonstrated potential as a pancreatic cancer diagnostic marker (147). In addition, one potential diagnostic biomarker for GC and CRC may be *TFPI2* hypermethylation (148). Therefore, these genes may be helpful as diagnostic biomarkers for IBD-associated neoplasia.

Cell specificity of DNAm in IBD. DNAm cell specificity has also been found to play a critical role in the pathophysiology of IBD and has been identified as a biomarker of IBD. For instance, it has been discovered that clinical outcomes in adults with IBD correspond with the cell-specific DNAm of the $CD8^+$ T-cell gene; however, only the initial cohort of adult patients with IBD had the previously described $CD8^+$ T-cell prognostic expression and exhaustion markers (149). Recently, Gasparetto *et al* (149) found that children with IBD and a second cohort of adults with IBD did not exhibit $CD8^+$ T-cell prognostic expression. At the time of diagnosis and during the illness, $CD8^+$ T-cells were extracted for genome-wide transcription and DNAm profiling (149). This suggests that the gene may only be applied to the diagnoses of children, and not prognoses. This further affirms the findings of the study by Venkateswaran *et al* (150) that revealed that at the time of diagnosis, the UC rectal mucosa displays a higher number of immune cells than fibroblasts and epithelial cells, along with variations in the DNAm pattern (150). Additionally, the severity and prognosis of UC are associated with cell-specific epigenetic alterations in the rectal mucosa (150). UC-associated $CD8^+$ effector T-lymphocytes can cause tissue lysis and release TNF-α, while post-effector cells develop inherent markers to take on regulatory roles that could reduce excessive inflammation (151). Furthermore, Li Yim *et al* (152) demonstrated that the illness status and activity differences of patients with CD can be revealed by whole-genome DNAm profiling for $CD14^+$ monocytes. Patients with active CD have larger subsets of peripheral monocytes with a more mature phenotype (153). Monocytes enter the irritated mucosa during inflammation and eventually mature into pro-inflammatory macrophages (152). A distinguishing feature of CD-associated intestinal $CD4^+$ cells is the hypomethylation of Th17-related genes linked to open chromatin regions and CCCTC-binding factor (CTCF) binding sites (154). Moreover, Howell *et al* (155) also discovered specific variations in the DNAm and transcriptome patterns of IECs from pediatric patients newly diagnosed with IBD compared to the controls. Only IECs from patients with CD had an altered transcription and DNAm patterns in the terminal ileum epithelium (155).

Of note, the majority of research on DNAm in IBD (60,119,131,138,149) used smaller sample sizes; however, large samples may also be required to further validate the results from these cohorts. However, some whole genome analyses with larger sample sizes have been conducted. Further substantial investigations need to be conducted to verify these biomarkers, even if only a limited number of whole genome studies have been performed in this context of methylation status and associated biomarkers in IBD.

7. DNAm as a potential therapeutic marker (strategy) in IBD

Since epigenetic mechanisms play a role in the pathogenesis of IBD and associated neoplasia, therapies have been created, and moderate doses of the DNMT-inhibiting drugs decitabine (DAC) and azacitidine (AZA) have been demonstrated to have potent anticancer effects in CAC (62). Several methylated genes have been identified in the pathogenesis of IBD and IBD-associated neoplasia for therapeutic relevance; however, only a limited number of genes have been reprogrammed using genetic editing. Examining the mechanisms underlying gene regulation can be advanced through targeted modification of gene expression (156). It can be used therapeutically to selectively alter a disease-causing gene's aberrant expression or to give the target cells a new purpose (156). Notably, adaptive immunity against viruses and plasmids is provided by the clustered regularly interspaced short palindromic repeats (CRISPR) or CRISPR-associated (Cas) systems, which use CRISPR RNAs to direct the silence of invasive nucleic acids (157). Transcription activator-like effectors (TALEs) are organically or synthetically created proteins that regulate the transcription of genes, and they were first discovered in *Xanthomonas* bacteria (158).

Human genes have been effectively targeted using synthetic TALEs (158). Consequently, the CRISPR/Cas9 and TALE systems have been successfully applied to gene editing (53,159). Therefore, it is simple to build transcription

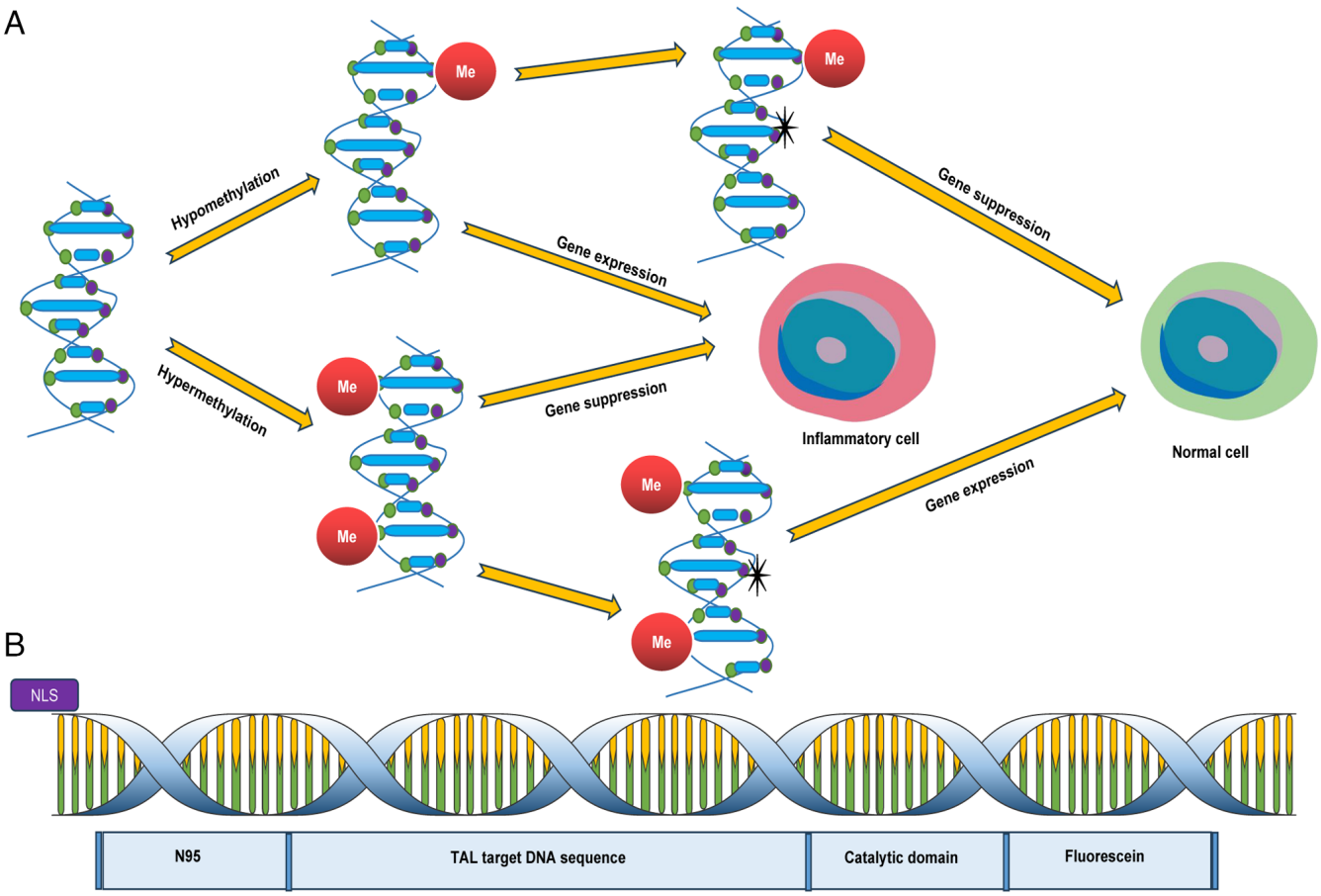

Figure 3. A model for the therapeutic strategy for IBD and its associated neoplasia. (A) Hypo- and hypermethylated genes that have been expressed and suppressed/silenced respectively may undergo epigenetic editing to reverse their altered expression levels that will reduce or prevent IBD. The star-shaped symbols indicate genetic editing tools. (B) Therapeutic strategy of *IL12B* gene in IBD. Active catalytic domains of epigenetic enzymes are combined with the synthesized TALE DNA-binding domain, and the *IL-12B* promoter is targeted by the expressed engineered enzymes (TALE-DNMT3A). IBD, inflammatory bowel disease; NLS, N-terminal with a nuclear localization signal; IL, interleukin; TALE, transcription activator-like effectors; DNMT3A, DNA methyltransferase 3A.

activator-like effector nucleases (TALENs) to bind to a particular genomic location, allowing for the insertion of precise genetic changes such as gene knockouts and additions (160). Recently, designer epigenome modifiers (DEMs) have been developed by Mlambo *et al* (156) to create a novel platform for precise epigenome editing. It has been discovered that DEMs may be utilized successfully and with astonishingly high specificity to mute target gene expression in essential human cells, opening the door for the emergence of a possible new class of medicines (156). DEMs merge a DNA binding domain based on highly specialized TALEs with several effector domains that can locally change the chromatin structure and induce DNAm to suppress the expression of a target gene (156).

The epigenetic silencing of TSGs during carcinogenesis is reversed by engineering sequence-specific epigenome editing tools (161). Garcia-Bloj *et al* (161) found that CRISPR/dCas9 VP64 with synergistic activation mediator (SAM) upregulated the TP53-dependent G2 arrest mediator candidate, reprimo, leading to phenotypic reprogramming in GC and improving the activation of highly silenced TSGs to their tumor suppressive function. Hence, editing techniques to revive heavily methylated TSGs can serve as a promising treatment for conditions like cancer and others (161).

Chen *et al* revealed that methylating the DNA on the *IL-12B* promoter can be used as an epigenetic editing technique that can be used to cure IBD. It was further revealed that the functioning catalytic domains of epigenetic enzymes were combined with the synthesized TALE DNA-binding domain, and the *IL-12B* promoter is targeted by the expressed engineered enzymes (TALE-DNMT3A), thereby inducing locus-specific DNA and downregulating *IL-12B* expression (53) (Fig. 3). Hence, this may prevent or reduce the proinflammatory cytokine release by *IL-12B*. This implies that genetic engineering of genes may reduce IBD, thus its usefulness as a therapeutic marker.

Although there are a limited number of DNAm genes in digestive organs that have gone through these gene editing technologies, these technologies (CRISPR/Cas9 and TALEs) have been employed in other genes for effective outcomes. As demonstrated in the study by Bernstein *et al* (162), cyclin-dependent kinase inhibitor 2A *(CDKN2A)* expression was decreased and primary human fibroblast replication was increased when directed DNAm with a TALE-DNMT was used to target the *CDKN2A* locus, which encodes the cyclin-dependent kinase inhibitor p16. Additionally, the Cynomolgus monkey (*Macaca fascicularis*), which has biallelic microcephalin 1 mutations, recapitulates the majority

Table I. Potential diagnostic, prognostic and therapeutic markers for IBD and related complications.

Marker	Type of IBD	Type of tissue/specimen	Diagnostic potential	Prognostic potential	Potential in therapeutics	(Refs.)
TCERG1L	CD	Serum	√			(17)
SFRP1, SFRP2, SFRP5, TFPI2, SOX17, GATA4	CD	Colon	√	√		(60)
IFITM1, ITGB2, S100A9, SLPI, SAA1, STAT3	UC	Colon	√			(119)
FAM217B, KIAA1614, RIBC2	UC	Colon	√			(73)
ZBTB7B	UC	Colon, serum	√			(26)
CD8^{+}*T-cell*	CD, UC	Blood	√			(149)
CXCL14, CXCL5, GATA3, IL17C, IL4R	CD, UC	Blood, tissue biopsy	√	√		(131)
TGFβ1	CD, UC	Blood	√			(138)
IL12B[a]	IBD	Plasmid, cells			√	(53)
SLIT2, TMEFF2	CRC in IBD	Colon, stool	√			(68)
ITGA4, TFPI2	CAC	Colon	√			(111)

[a]Genetically engineered with transcription activator-like effectors. IBD, inflammatory bowel disease; CAC, colitis-associated cancer; CD, Crohn's disease; UC, ulcerative colitis; CRC, colorectal cancer; *CXCL*, C-X-C motif chemokine ligand; *FAM*, family with sequence similarity; *IFITM*, interferon induced transmembrane protein; *IL*, interleukin; *ITGA*, integrin subunit alpha; *RIBC2*, RIB43A domain with coiled-coils 2; *S100*, S100 calcium binding protein; *TCERG1L*, transcription elongation regulator 1 like; *SAA*, serum amyloid A; *SFRP*, secreted frizzled related protein; *SLIT*, slit guidance ligand; *SLPI*, secretory leukocyte peptidase inhibitor; *SOX17*, sry-box transcription factor 17; *STAT3*, signal transducer and activator of transcription 3; *TGFβ1*, transforming growth factor β1; *TMEFF*, transmembrane protein with EGF like and two follistatin like domains; *ZBTB7B*, zinc finger and BTB domain containing 7b.

of the significant clinical traits seen in humans with microcephaly using TALEN (163). Hence, further studies on the epigenetic editing of IBD and IBD-associated neoplasia genes are warranted to further enhance therapeutic options. The potential applications of DNAm markers in IBD and related complications are summarized in Table I.

8. Challenges of DNAm

DNAm in immune-mediated disorders is underexplored due to the technical difficulties of the methylation typing methods, statistical concerns and experimental design constraints. Due to these reasons, research on disease-related alterations in DNAm has been limited, with few conclusive results, and it has become challenging to compare studies for the same illness (164). DNAm in IBD and cancer is one of the areas that has recently drawn attention, and extensive research is being carried out to identify more reliable and sensitive markers for DNAm in these diseases. Despite recent successes, there are still some challenges encountered during DNAm.

It has been documented that patterns of methylation observed in blood specimens in IBD may be due to the acute phase of inflammation; hence, these methylation patterns resemble those of a typical patient after treatment, implying that blood as a specimen may not be a reliable sample for DNAm in IBD. Therefore, Somineni *et al* (64) proposed that further research using immunological and epithelial samples obtained from mucosal biopsies is necessary to confirm these findings. Although tissue samples from a mucosal biopsy are ideal for DNA analysis, the quality of the genetic material due to DNA degradation caused by formalin and the impediment of paraffin for DNA extraction may impact the feasibility and reliability of sequencing data (67). A biopsy is an invasive test that has limitations due to trauma, accessibility issues, complications and ethical issues (165). In addition to being uncomfortable, expensive, time-consuming, and risky for the patient, tissue biopsies may not accurately reflect the heterogeneity of the tumor (166,167). Notably, in cancer, blood biopsies using circulating cell-free DNA (cfDNA) offer serial samples for the real-time longitudinal tracking of tumor genomic evolution, surpassing the limitations of tissue biopsies (166). Physicians can efficiently match patients to the appropriate treatment for the proper target by using blood biopsy-based testing to determine drug resistance, metastasis and recurrence, and guarantee treatment efficacy (166). This may be helpful for IBD-associated cancer.

In addition, despite decades of research on methylation patterns in IBD, no standardized database of methylated genes in IBD yet exists (165), and there is no unified database of methylation genes in IBD, even though genes from numerous biochemical pathways have been examined (168).

Another challenge is the use of small sample sizes for DNAm in IBD. It may be challenging to develop diagnostic and prognostic biomarkers for patients with IBD and cancer due to smaller sample sizes; hence, several studies recommend the validation of these biomarkers in large, independent cohorts

before clinical application (138,149). This may be the reason why there are fewer concrete biomarkers for DNAm in IBD, although some studies have suggested potential biomarkers for IBD and its associated neoplasia.

Using bisulfite treatment, PCR amplification, restriction enzyme digestion, or sequencing, the methylation analysis of genomic DNA cytosines can be quantitatively evaluated. Nevertheless, after bisulfite conversion, the sequences of methylated and unmethylated molecules diverge. This may cause bias in some sequences during PCR amplification, resulting in an incorrect methylation estimate (169). Additionally, it is challenging to prepare libraries for whole genome bisulfite sequencing, since the bisulfite procedure causes significant DNA damage as a side-effect (170).

9. Conclusion and future perspectives

IBD, which includes CD and UC, is a complex disease whose mechanism remains unknown. DNAm has been linked to the pathogenesis of IBD and is reversed by TET enzymes. DNAm is one of the most common areas that has recently drawn increasing attention, with studies revealing that the IL-12/IL-23, Wnt, IL-6-associated STAT3/SOCS3, and apoptosis signaling pathways are involved in DNAm and IBD pathogenesis. Stool, colon biopsies, serum, and whole blood are often utilized as samples for DNAm research. However, it has been demonstrated that using blood as a specimen for DNAm testing may not be appropriate. As a result, tissue biopsies have been suggested to corroborate the findings of research that used blood samples. Surprisingly, tissue biopsies may pose challenges such as ethical concerns, injuries, discomforts, dangers and complications, costs, and inaccuracies in tumor heterogeneity measurements in the clinical setting. In addition, formalin-induced DNA degradation and paraffin extraction hindrance may degrade genetic material quality and compromise the viability and accuracy of sequencing results. Several methylated genes are involved in perpetuating IBD, and these genes are being explored as potential diagnostic, prognostic, and therapeutic markers of IBD. This may aid clinical decision-making and provide direct therapeutic alternatives.

Moving forward, further studies are required to focus on identifying discriminatory genes that can be used to differentiate between UC and CD and as prognostic and therapeutic markers for IBD. Further research with larger sample sizes, independent cohorts, and whole genome-wide studies should be conducted to advance the validation of these genes as biomarkers for clinical use. The multi-omics profiling methodology also used by Howell *et al* (155) may be used to collect large sample sizes for DNAm. Immunological and epithelial samples obtained from mucosal biopsies and blood samples may be used to study DNAm, with the tissue samples validating the outcomes of the blood samples. Stool samples are also emerging as specimens for DNAm and can be employed to study DNAm in IBD clinically. In addition, employing acid-deprived fixatives would provide the best DNA preservation and sequencing results for more intricate molecular profiling of tissue samples. This may aid clinicians with the correct diagnosis and therapeutic response of IBD patients. Moreover, enzymatic techniques in place of bisulfite conversion in new procedures may address some of the issues brought on by significant DNA degradation and enable the measurement of DNA methylation at lower input levels (171). Blood biopsies using cfDNA may also be employed in IBD-associated neoplasia. Additionally, epigenome modifiers that directly and selectively alter the aberrant expression of a disease-causing gene or may provide target cells with a new purpose can be explored clinically in addition to IBD drugs for effective outcomes.

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Availability of data and materials

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Authors' contributions

FM and FAA were involved in the conceptualization of the study. FM and ANF were involved in funding acquisition and project administration. DKWO and AW provided the software used in the present review [Reference manager software (EndNote), grammar editing software (QuillBot/Grammarly), and software for drawing figures (Adobe Illustrator)]. YX was involved in visualization. FAA and YZ were involved in the writing of the original draft of the manuscript. DKWO was involved in the writing, reviewing and editing of the manuscript. All authors have read and agreed to the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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