DNA methylation changes in inflammatory bowel disease

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Abstract

The cause of inflammatory bowel disease, encompassing Crohn’s disease and ulcerative colitis, remains a mystery but evidence is accumulating that complex interactions between the genetic background and the gut microbiota of the host and environmental factors associated with rapid industrialization and westernized life styles may underlie its pathogenesis. Recent epigenetic studies have suggested that interactions between environment and host DNA may play a leading role in the phenotypical expression of both diseases, explaining amongst others the differences in disease expression in monozygotic twins. DNA methylation is the most studied epigenetic modification and during the last decade its correlation to IBD pathogenesis has been well established. Genes from different molecular pathways have been studied but till now there is no standardized database of methylated genes in IBD. Thus, a thorough and in-depth study of DNA methylation, its potential relation to IBD and its interaction with the available pharmaceutical armamentarium is of great interest.

Keywords Methylation, inflammatory bowel disease, inflammation, epigenetics

Introduction

Inflammatory bowel diseases (IBD), encompassing Crohn’s disease (CD) and ulcerative colitis (UC) are chronic, relapsing-remitting or continuously active diseases of the gastrointestinal tract and are occasionally associated with extra-intestinal manifestations [1]. The incidence of IBD has increased during the last decades in the developing world whereas it appears to be rather stable in northern Europe and North America [2,3]. Although the exact etiology of IBD remains unknown, current data converge on a dysregulation of the immune response against the intestinal flora in a genetically susceptible individual [1,4]. More specifically, IBD could be accounted as the result of interactions between the host and the environment, which encompass the intestinal microbiota, the immune system, the genetic composition of the host and specific environmental factors, such as the effect of smoking, breastfeeding, drugs, dietary products, etc. Regarding the interaction between environment and genome, epigenetic mechanisms and more specifically DNA methylation seem to be of great importance [5]. Epigenetic modifications include global DNA methylation, gene-specific DNA methylation, histone modifications, chromatin immunoprecipitation, non-coding RNA and microRNA hybridization. However, the two most widely studied forms of epigenetic modifications are the methylation of DNA [6-8] and the posttranslational modification of histone proteins [9,10], both of which have been functionally implicated in the regulation of gene expression in a variety of organisms. DNA methylation results from the transfer of a methyl group (CH₃) in the C5 site of cytosine, creating the 5-methyl-cytosine (5mC), by DNA methyltransferase (DNMT) enzymes. Methylation of the CpG dinucleotide is so frequent that 5mC has been referred to as the fifth nucleotide. Therefore, global DNA methylation is the analysis of the total 5mC content, whereas gene-specific DNA methylation refers to measurement of methylated CpG islands on a specific gene of interest. The attachment of methyl groups in the promoter region of a gene (hypermethylation) is associated with so-called “silencing” or inactivation of that gene. On the contrary, lower levels of methylation (hypomethylation) in the promoter region of a gene makes it transcriptionally active [11]. The role of DNA methylation is regulation of gene transcription, either by activating proteins which interfere with the suppression of gene transcription, or by inhibiting transcription factors binding to DNA. It is important to note that epigenetic silencing causes no structural alterations to DNA. Methylation and de-methylation occur normally in human cells in order to create a stable regulation pattern of gene transcription. The proper DNA methylation is essential for cell differentiation and embryonic development. Moreover, in some cases, methylation has been observed to play a key role in mediating gene expression. A number of studies have shown that methylation near gene promoters
varies considerably depending on cell type. Moreover higher methylation of gene promoters has been correlated to low or no transcription [12]. The functional consequences of promoter methylation on chromatin configuration and transcriptional regulation are well documented. However the role of gene body methylation remains largely unresolved and recent data suggest that gene body methylation is a widespread, conserved form of DNA methylation, the ancestral role of which may have been the reduction of transcriptional noise [13]. Undoubtedly, the high-throughput sequencing is likely to uncover many genomic regions that exhibit disease-specific epigenetic alterations [14]. However, large scale genome analysis of DNA methylation is still a costly approach and large scale epigenetic biomarker development may become more feasible only when several methods with very different trade-offs between genomic coverage and per-samples costs are combined. These requirements are met by bisulfite sequencing of region-specifically enriched DNA, and by array-based epigenotyping assays [15]. Until present time, a lot of evidence had associated DNA methylation with cancer development and dysplasia, but only recently has DNA methylation been related to IBD pathogenesis per se. We searched PubMed using the terms: "DNA methylation", "Crohn's disease", "Ulcerative colitis" and "inflammatory bowel disease". The aim of the present review was to highlight all the existing data on DNA methylation and IBD, focusing only on the relationship of methylated genes to the pathogenesis of IBD and its clinical aspects. The majority of the studies focus on UC patients and mainly on methylation in tissue biopsy samples.

**DNA methylation studies on intestinal tissue samples**

**DNA methylation changes and UC**

UC represents one major sub-phenotype of human IBD. In past decades, UC displayed a remarkably steep rise in incidence, which cannot be explained only by genetic variants. Beyond germline DNA variants, epigenetic variants e.g. DNA methylation (DNAm) and histone modifications, could modulate disease-relevant gene function [16]. Methylation studies extend to different kinds of genes and genetic loci (cadherins, genes related to transcription, kinase inhibitors etc), trying to detect the possible correlation of their methylation to IBD. Indeed, according to the current literature, epigenetic modifications represent promising candidates for elucidating processes of disease manifestation beyond the identified risk loci.

The first evidence that DNA methylation, as an epigenetic mechanism, is related to UC pathogenesis and was reported in 1996 by Gloria et al [17], who found that incorporation of the 3'-methyl groups into DNA was 10-fold higher in UC patients than controls and significantly higher in histologically active than inactive disease. Two years later, in a study conducted by Hsieh et al [18] 89 tissue samples from UC patients who underwent colectomy were collected and p16INK4a (a cyclin-dependent kinase inhibitor) methylation was observed in regions negative for dysplasia, dysplastic lesions and carcinomatous lesions. A progressive increase in methylation status (12.07%, 70%, and 100% respectively) was detected, evidencing the positive relation between DNA methylation and inflammation, as well as neoplastic progression in UC.

Another study on a different kind of gene, *E-cadherin* (a specific calcium ion-dependent cell adhesion molecule), proved that its promoter methylation was detected in 93% of dysplastic biopsy samples from long-standing UC in contrast to 6% of non-dysplastic biopsies. The result was confirmed by testing the level of *E-cadherin* synthesis, found to be reduced in samples with dysplasia and normal in samples without dysplasia, suggesting that long standing inflammation is related to hypermethylation of the gene promoter and that DNA methylation may be used as a biomarker for detecting high risk patients for developing colorectal cancer [19].

Estrogen receptor (ER), MYOD, GSPG2 and p16 gene exon 1, which are genetic loci all related to transcription, were evaluated in 33 samples from 23 patients; 12 UC patients with high-grade dysplasia or cancer, 6 UC patients without dysplasia or cancer and 5 non-UC controls. ER, MYOD and p16 were found to be methylated higher in dysplastic than normal appearing epithelium, indicating that methylation precedes dysplasia development [20]. This result was also confirmative of the increased methylation of p16 mentioned in the study by Hsieh et al [18]. Moreover a positive association between methylation of the aforementioned genes (ER, MYOD, GSPG2, p16) and increasing age was revealed. Saito et al [21] compared the methylation levels of four genes (*CDH1, GDNF, HPP1* and *MYOD1*) between tissue samples from actively inflamed mucosa and quiescent colon mucosa. The results showed that especially for *CDH1* (a calcium ion-dependent cell adhesion molecule) and *GDNF* (Glial cell Derived Neurotrophic Factor - a gene related to survival and differentiation of cells), active inflammation was independently related to higher DNA methylation. Moreover, longer disease duration and the total amount of steroids (>10 g) were important factors also related to higher DNA methylation of *GDNF*.

It is known that changes in *MDR1* function and/or expression contribute to the pathogenesis of inflammatory disorders of the gastrointestinal tract, including UC. *MDR1* promoter methylation has been studied in UC patients and found to be methylated in 61.4% of rectal inflammatory mucosal specimens. Also, it was methylated higher in mucosal biopsies from the rectum than from normal terminal ileum. *MDR1* was related to clinical phenotypes of UC; chronic continuously active colitis, extensive colitis, younger age of disease onset (<20 years old), higher number of hospitalizations, and severe disease phenotype were all independently related to *MDR1* hypermethylation [22]. An analogous study on protease-activated receptor (PAR2), which is a member of the large family of 7-transmembrane-region receptors that couple to guanine nucleotide-binding proteins, correlated higher PAR2 methylation levels in rectal mucosa with steroid-dependent or steroid-refractory colitis. Moreover,
PAR2 methylation levels were higher in rectal biopsies from patients with extensive colitis than patients with proctitis [23]. Another study, conducted in 2008 also showed different levels of methylation between rectum and terminal ileum in ulcerative colitis. p14 and p16, two promoters of ER gene, were methylated higher in rectal than in ileal mucosa but were also related to the type of the disease; ER methylation was higher in patients with relapsing-remitting disease than a single attack disease. The higher methylation was also found to be correlated to longer than 7 years of disease [24]. ER-1 and tumor suppressor candidate 3 (N-33) were also found to be higher methylated even in macroscopically normal mucosa of patients with UC compared to controls (7.9% vs. 5.9%; P=0.015 and 66% vs. 9.3%; P<0.001 respectively) [25].

In this regard, the study by Moriyama et al [26] investigated the possible relation of hypermethylation of p14 with high risk of dysplasia. Apart from the verification of the aforementioned hypothesis, hypermethylation of p14 (ARF) was also found to be related to longer duration of UC. Three years later Garrity-Park et al [27] proved that p14 shows no methylation in normal mucosa from control patients and increased methylation in tissue samples from inflamed mucosa and colorectal cancer complicating UC. The p14, p16 and CDH1 promoters’ methylation has also been studied in rectal mucosal biopsies from UC patients who were in clinical remission at endoscopy and displayed no dysplasia or neoplasia during the histopathological examination. Moreover CIHM (CpG Island Hyper Methylation) was detected in 45.2% for p16, 34.5% for p14 and 53.6% for CDH1, showing higher frequency in non-neoplastic colonic mucosa in UC patients and higher than that in the study of Garrity-Park et al [27,28].

Death-associated protein kinase (DAPK) is the translational product of a tumor suppressor gene and its inactivation through methylation has been related to many different types of cancer, including inflammation associated tumors and their pre-malignant lesions such as chronic gastritis - gastric cancer and Barrett’s esophagus - Barrett’s adenocarcinoma [29-31]. Hypermethylation of DAPK was found to be associated to the inflammation of mucosa in UC patients and a gradient from lower to higher methylation was similar to the gradient from mild to severe inflammation. Moreover in unmethylated mucosa DAPK protein expression was proportional to the severity of inflammation, probably representing a protective role of DAPK during chronic inflammation in UC [32]. Another similar finding was shown by Cooke et al [33]. A large number of genes were tested for methylation (Table 1) in UC and CD and hypermethylation was detected in UC samples compared to controls. But the most important result was that in UC the hypermethylation is present only in inflamed mucosa and not in the normal appearing (non-inflamed) mucosa.

Finally, in the most recent methylation study, by Haesler et al [34], a three-layer epigenome-wide association study was conducted on biopsies from 10 monozygotic twin pairs with discordant manifestation of UC. After the identification and validation of Methylation Variable Positions (MVPs, which represent individual methylation events within the proximal promoter region of transcription start sites) and differentially methylated regions (DMRs, which represent group effects of linked MVPs), 61 disease-associated loci with different DNA methylation were detected. Ten of them showed the closest proximity between MVP/DMR and transcript location (Table 1).

All the above studies indicate that DNA methylation is a really common phenomenon in UC, especially in mucosal biopsies from inflamed tissue. Moreover, DNA methylation has been related to many different clinical aspects, such as disease severity, disease duration, disease phenotype, disease extent, steroid use, steroid dependence or refractoriness, age of onset, number of hospitalizations and finally active inflammation and dysplasia.

**UC-associated neoplasia**

There is an established increased relative risk for the development of colorectal neoplasia in UC patients [35]. DNA methylation has already been studied in sporadic adenomas and cancer but seems to be also related to dysplasia and cancer development in UC patients [36]. Tahara et al [37] have shown that CIHM status may be influenced by CD14-159, IL-1β-31, p22PHOX242 and MBL2 codon54 single nucleotide polymorphisms in the rectal mucosa of UC patients and may therefore be substantially involved in UC-associated carcinogenesis. More recently, four genes (TGFBR2, SLITZ2, H33ST2 and TMEFF2) were evaluated for DNA methylation status in biopsies of four patient-groups: 60 patients with sporadic CRC, 32 patients with IBD-associated neoplasm, 85 patients with IBD without neoplasia and 28 healthy controls. The results showed a trend of increasing prevalence of DNA methylation from mucosal biopsies from healthy controls to mucosal biopsies from IBD patients without neoplasia, to adjacent nonneoplastic mucosa and finally to biopsies from IBD-associated neoplastic regions. Especially SLIT2 and TMEFF2 appeared to be more frequently methylated in the mucosa of IBD patients (15/20) at high risk of dysplasia or cancer than patients at low risk (32/63) [38]. Therefore, higher levels of DNA methylation could be related to an increased risk for cancer development. The most recent data from the same group of investigators, have added evidence for two more genes (AGTR1, WNT2) and confirmed the data for SLIT2 gene. All three genes were methylated higher in neoplastic tissue from IBD-colorectal samples (50% for AGTR1, 58% for WNT2 and 86% for SLIT2) than in adjacent non-neoplastic tissue (33%, 33% and 47% respectively) and tissue from healthy controls (5%, 3% and 5% respectively) [39].

**DNA methylation and CD**

On the contrary, limited data exists concerning the contribution of DNA methylation status in CD pathogenesis. Recently, Lin et al [40] compared normal to inflamed tissue from CD and UC patients and found significant differences in DNA methylation of seven CpG loci (BGN_P333_R,
Peripheral blood DNA methylation studies

One of the first studies comparing mucosal (lamina propria - LP) to peripheral blood (PB) DNA methylation, showed that IFNG DNA methylation in PB T cells from healthy individuals or IBD patients is higher compared to LP T-cells from healthy individuals or IBD patients respectively (35% and 38%, P<0.001 and P<0.001-0.03 respectively). Moreover no relationship was found between DNA methylation level and patient gender or age, anatomic location of disease, or concomitant therapy. When the DNA methylation index of IFNG was examined in three different regions of the gene (CNS-22, proximal promoter, transcribed region), the CNS-22 region was inversely correlated to DNA methylation of IFNG promoter and the transcribed region positively correlated. Regardless of disease status, IFNG CpG DNA methylation differs in LP T cells compared to peripheral blood (PB) T cells across all the aforementioned regions [41].

As a continuum of this study, Gonsky et al [42] showed that INF-γ secretion differs between mucosal specimens and PB T cells. Whereas DNA methylation of interferon (IFN)-γ gene is decreased in mucosal compartments and thus IFN-γ expression increased, PB T cells from IBD patients show decreased IFN-γ production as a result of increased IFN-γ gene DNA methylation.

Lin et al [43] complemented their previous study [40] by comparing the DNA methylation status of many different genes in B cells from PB. IBD-associated changes in DNA methylation were detected in 11 B cell line CpG sites from 18 IBD patients. Disease subtype-specific changes were found in 14 CpG sites from CD patients and 24 UC patients. Important notices are: a) several of the methylated loci, such as BCL3, STAT3, OSM and STAT5, are involved in the regulation or downstream signaling in the IL-23 pathway and b) both IL-12 and IL-23 share the same disease-associated methylated gene, p40, as a common subunit.

The DNA methylation status of 27,578 CpG sites, extracted from 21 CD patients, was analyzed by Nimmo et al [44]. Two groups were created: patients with well defined phenotype (adult-onset female nonsmokers with inactive ileal disease, not receiving immunomodulatory therapy at the time of investigation) and a sex-matched cohort of healthy controls. The CpG sites that showed the most significant differential DNA methylation include those at the genes MAPK13, FASLG, PRR1, S100A13, RIPK3, IL-21R; all plausible candidates in CD pathogenesis. Notably, the data suggests that also IL-27, IL-19, tumor necrosis factor (TNF)-α and NOD2 are subject to DNA methylation changes.

A recent research study by Harris et al [45] on peripheral blood DNA samples, examined 6 genomic loci (chr1:20771319-20771319, chr6: 31803832-31803832, chr2: 34719565-34719565, chr1: 44466601-44466601, chr10: 126360669-126360669). These loci were studied in two different groups: a) concordant (CD=4, UC=3) and discordant (CD=4, UC=7) monozygotic twin pairs; and b) discordant monozygotic twin pairs (CD=3, UC=3), treatment-naïve IBD pediatric cases (CD=14, UC=8) and controls (n=14). No IBD-specific peripheral blood leukocyte DNA methylation association was found, possibly indicating that microarray proofing of DNA methylation from peripheral blood leukocyte (PBL) is less likely to yield positive results in IBD. Despite the inconclusive results, a further investigation was made on DNA extracted from peripheral blood mononuclear cells (PBMCs) after the exclusion of neutrophil granulocytes. TEPP (testis prostate placenta) gene showed a CD-associated increase in DNA methylation in this specific subpopulation of peripheral cells, which was absent after treatment.

Balasa et al [46] conducted a study on the IRF5 promoter DNA methylation in PBL DNA samples: 87 samples of PBL DNA obtained from 49 healthy controls, 18 CD and 20 UC patients were assessed for IRF5 promoter DNA methylation and results showed insignificant differences in the average DNA methylation, indicating that the dysregulation of IRF5 promoter is unlikely to be associated to IBD.

A recent Korean study investigated the DNA methylation of signal transducer and activator of transcription 4 (STAT4) in colonic mucosa and PBMCs of IBD patients and its relation to specific polymorphisms. In both specimens, IBD patients showed higher expression of STAT4 than did the healthy controls and a correlation of DNA methylation status at -172 of the STAT4 promoter and risk alleles was observed [47].

SERPINA5_P156_F, TNFRSF1A_P678_F, AATK_P709_R, GABRA5_P862_R, MAPK10_E26_F, STAT5A_P704_R. Additionally, subtype specific DNA methylation changes were detected linking 25 CpG sites to CD and 13 CpG sites to UC. The aforementioned publication by Cooke et al [33] added evidence on the different level of DNA methylation between inflamed mucosa from CD/UC patients and controls for a large number of genes. It is important that the genes studied and found differentially methylated are involved in different pathways of IBD pathogenesis. DOK2, for example, acts as a scaffolding protein for the assembly of multi-molecular signaling complexes such as modulate the cellular proliferation induced by interleukin (IL)-4. Tap 1 is involved in the transport of antigens from the cytoplasm to the endoplasmic reticulum for association with MHC class I molecules. CD28 is a co-stimulatory molecule essential for CD4+ T cell proliferation, Th2 development and IL-2 production. ICAM3 encodes an intercellular adhesion molecule that plays a key role in leukocyte adhesion and migration as well as signaling. Extremely interesting is also the evidence of increased DNA methylation of CDH1. This gene encodes E-cadherin, which plays a central role in epithelial cell-cell adhesion. As mentioned earlier in this review, CDH1 has been reported to be down-regulated in areas of UC inflammation. It must be noticed that some of the genes tested, such as THRAP2, FANCC and GBGTT1 have been found methylated in inflamed mucosa of both UC and CD patients whereas others were only CD-specific (Table 1). Moreover, as stated earlier for UC, in CD the non-inflamed tissue shows no significant difference in DNA methylation levels when compared to controls.
Table 1: All known genes whose methylation has been related to inflammatory bowel disease (IBD), Crohn's disease (CD) or ulcerative colitis (UC)

| Disease type | Gene or polymorphism | Ref. |
|--------------|----------------------|------|
| IBD          | BGN_P333_R (Biglycan) [40] |      |
|              | TNFRSF1A_P678_F (Tumor necrosis factor ligand superfamily) [40] |      |
|              | GABRA5_P662_R (Gamma-aminobutyric acid receptor) [40] |      |
|              | STAT5A_P704_R (Signal transducer and activator of transcription) [40] |      |
|              | BCL3_E71_F (B-cell leukemia) |      |
|              | PPARG_P693_F (Peroxisome proliferator activated receptor Gamma) [43] |      |
|              | LMTK2_P1034_F (Lemur tyrosine kinase 2) [43] |      |
|              | CASP2_P192_F (Caspase 2, apoptosis related cysteine protease) [43] |      |
|              | COL18A1_P494_R (Collagen, type XVIII, alpha-1) [43] |      |
|              | STAT4 (Signal transducer and activator of transcription) [47] |      |
| CD           | IL 18BP (Interleukin 18) [40] |      |
|              | PDGFRB (Platelet derived growth factor receptor) [40] |      |
|              | S 100A4 (S100 Calcium binding protein) [40] |      |
|              | LAT (Linker for activation of T cells) [40] |      |
|              | AFF3 (AF4/FMR2 family, member 3) [40] |      |
|              | TJP2 (Tight junction protein 2) [40] |      |
|              | NOS3 (Nitric oxide synthase 3) |      |
|              | MEG3 (Maternally expressed gene 3) [40] |      |
|              | APOC2 (Apolipoprotein C-II) [40] |      |
|              | MPL (Myeloproliferative leukemia virus oncogene) [40] |      |
|              | SP11 (Spleen focus forming virus proviral integration oncogene) [40] |      |
|              | FANCC (Fanconi anemia complementation group C) [33] |      |
|              | B3GALT2 (Beta-1,3-N-acetylgalactosaminyltransferase 2) [33] |      |
|              | ULK1 (UNC51-like Kinase 1) [33] |      |
|              | GPI1 (Glutaminic Fructose-6-phosphate aminotransferase 1) |      |
|              | G15orf48 (Normal mucosa of esophagus-specific gene 1) [33] |      |
|              | HSPB6 (Heat-shock 27-KD Protein 6) [33] |      |
|              | GBGT1 (Globoside alpha-1,3-N-acetylgalactosaminyltransferase) [33] |      |
|              | DOK2 (Docking protein 2) [33] |      |
|              | FLJ32065 [33] |      |
|              | TNFSF4 (Tumor necrosis factor ligand superfamily member 4) [33] |      |
|              | MYOM2 (Myomesin 2) [33] |      |
|              | SLC2A9 (Solute carrier family 2, member 9) [33] |      |
|              | IL9 (Interleukin 9) [33] |      |
|              | CDC5L (Cell division cycle 5) [33] |      |
|              | U2AF1L3 (U2 small nuclear RNA auxiliary factor 1) [33] |      |
|              | GUCY2F P255 F (Guanylate cyclase 2F, retinal) [43] |      |
|              | SEPPANE1_P519_F (Serpin peptidase inhibitor, Clade D, Member 1) [43] |      |
|              | MST1R_P87_R (Macrophage stimulating 1) [43] |      |
|              | MXI1_P1269_F (Max interacting protein 1) [43] |      |
|              | GADD45A_P373_R (Growth arrest and DNA damage inducible gene) [43] |      |
|              | RHOH_P933_R (Rho homolog gene family) [43] |      |
|              | IL10_P348_F (Interleukin 10) [43] |      |
|              | FASLG (FAS ligand) [44] |      |
|              | S100A13 (S100 Calcium-binding protein) [44] |      |
|              | IL-21R (Interleukin 21) [44] |      |
|              | IL-19 (Interleukin 19) [44] |      |
|              | MST1 (Macrophage stimulating 1) [44] |      |
|              | TEPP (Testis prostate placenta expressed protein) [45] |      |
|              | NOTCH4 (NOTCH drosophila, homolog of, 4) [40] |      |
|              | MPO (Myeloperoxidase) [40] |      |
|              | THRAP2 (Thyroid hormone receptor-associated protein 2) [33] |      |
|              | TCOF1 (Treacher Collins syndrome) [33] |      |
|              | PTF1 (RNA polymerase 1 and transcript release factor) [33] |      |
|              | HK2 (Hexokinase 2) [33] |      |
|              | FOLR1 (Folate receptor 1) [33] |      |
|              | PHYH (Phytanoyl-CoA hydroxylase) [33] |      |
|              | MLY5 (Myosin light chain 5) [33] |      |
|              | COG8 (Component of oligomer cgo1 golgi complex 8) [33] |      |
|              | TAP1 (Transporter, ATP-binding cassette) [33] |      |
|              | POR (cytochrome P450 oxidoreductase) [33] |      |
|              | ZP (Zona pellucida glycoprotein) [33] |      |
|              | PSMB8 (Proteasome subunit beta-type 8) [33] |      |
|              | BMX (Bone Marrow Kinase, X-linked) [33] |      |
|              | GCET2 (Germin al center-expressed transcript 2) [33] |      |
|              | PIPOX (Prolactin-Inducible Protein) [33] |      |
|              | SEPT5_P464_R (Septin 5) [43] |      |
|              | CLDN4_P1120_R (Claudin 4) [43] |      |
|              | LMTK2_P1034_F (Lemur tyrosine kinase 2) [43] |      |
|              | BGN_P333_R (Biglycan) [43] |      |
|              | IL6_P93_R (Interleukin 16) [43] |      |
|              | EPHA2_P203_R (Ephrin receptor 2) [43] |      |
|              | LIF_P383_R (Leukemia inhibitory factor) [43] |      |
|              | MAPK3 (Mitogen activated protein kinase) [44] |      |
|              | PRF1 (Perforin) [44] |      |
|              | RIPK3 (Receptor interacting serine/threonine kinase 3) [44] |      |
|              | IL-27 (Interleukin 27) [44] |      |
|              | TNF (Tumor necrosis factor) [44] |      |
|              | NOD2 (Nucleotide binding oligomerization) [44] |      |
| Disease type | Gene or polymorphism [Ref.] |
|-------------|-----------------------------|
| UC          | TNFSF8 (TNF ligand superfamily) [40] |
|             | PADH4 (Peptidylarginine deiminase) [40] |
|             | CDH17 (Cadherin 17) [40] |
|             | HOXB2 (Homeobox B2) [40] |
|             | AATK (Apoptosis associated tyrosine kinase) [40] |
|             | EPHX1 (Epoxydehydrotyrosine 1) [40] |
|             | FMR1 (Fragile X mental retardation protein) [40] |
|             | FANCC (Fanconi anemia complementation group C) [33] |
|             | GFTPI (Glutamin:Fructose-6-phosphate amidotransferase) [33] |
|             | ANKR9 (Ankyrin repeat domain-containing protein 2) [33] |
|             | TNFSF12-TNFSF13 (TNF ligand superfamily) [33] |
|             | C18orf14 (Chromosome 18 open reading frame 14) [33] |
|             | TMEM116 (Transmembrane protein 116) [33] |
|             | CD28 [33] |
|             | CYP2D6 (Cytochrome P450, subfamily IID, polypeptide 6) [33] |
|             | CHML (CHM-like) [33] |
|             | TNFSF4 (TNF ligand superfamily) [33] |
|             | KCNK4 (Potassium channel, subfamily K, member 4) [33] |
|             | CD28 [33] |
|             | SPI1 (Spleen focus forming virus proviral Integration oncogene) [33] |
|             | MMRN2 (Multimerin 2) [33] |
|             | RBM13 (RNA-binding motif protein) [33] |
|             | DHCR24_P652_R (24-Dehydrocholesterol Reductase) [43] |
|             | TXB1_P520_F(TB-Box) [43] |
|             | PPT_E170_R (Phosphoribosylpyrophosphate aminotransferase) [43] |
|             | SEPT9_P85_R (Septin 9) [43] |
|             | SMARC81_P220_R (SWI/SNF-related, matrix associated, actin dependent regulator of chromatic, Subfamily C, Member 2) [43] |
|             | CD81_P272_R [43] |
|             | MUC1_E18_R (Mucin 1 transmembrane) [43] |
|             | HLA-DO8_E432_R [43] |
|             | ERN1_P809_R (Endoplasmic reticulum to nucleus signaling 1) [43] |
|             | LIG3_P622_R (Ligase III, DNA, ATP-dependent) [43] |
|             | IL16_P226_F (Interleukin 16) [43] |
|             | PECAM1_P135_F (Platelet endothelial cell adhesion molecule 1) [43] |
|             | FLNA (Filamin A) [34] |
|             | IGHG1 (IgG Heavy chain locus) [34] |
|             | P15 (P15) [34] |
|             | SPINK4 (Serine protease inhibitor) [34] |
|             | TK1 (Thymidine Kinase) [34] |
|             | E-cadherin [18] |
|             | MYOD (Myogenic differentiation antigen) [20] |
|             | CHD1 (Chromodomain helicase DNA-binding protein 1) [21] |
|             | HP1 (Hyperplastic polyposis gene 1) [21] |
|             | MDR1 (Multidrug resistance 1) [22] |
|             | ER (ER) [24] |
|             | ESR-1 (Estrogen receptor 1) [25] |
|             | p14 (ARF) [26] |
|             | P16 [28] |
|             | DAPK (Death associated protein kinase) [32] |
|             | PIK3R1 (Phosphatidylinositol 3 kinase regulatory subunit 1) [40] |
|             | CLDN4 (Claudin 4) [40] |
|             | HLA-DQA2 [40] |
|             | FRK (Fyn-Related Kinase) [40] |
|             | NOTCH3 (Notch, Drosophila, Homolog of, 3) [40] |
|             | TCOF1 [33] |
|             | THRAP2 (Thyroid hormone receptor-associated protein 2) [33] |
|             | GBGT1 (Globoside Alpha-1,3-N-Acetylgalactosaminyltransferase) [33] |
|             | PAQR6 (Progesterone Receptor Family) [33] |
|             | DOK2 (Docking protein) [33] |
|             | FUT7 (Fucosyltransferase 7) [33] |
|             | SPATA22 (Spermatogenesis associated protein) [33] |
|             | M4A44 (Membrane spanning 4 domains, subfamily A, member 4a) [33] |
|             | COG8 (Component of oligomeric Golgi complex 8) [33] |
|             | VWF (Von willebrand factor) [33] |
|             | TNFSF4 (TNF ligand superfamily) [33] |
|             | FANCC (Fanconi anemia complementation group C) [33] |
|             | FOLH1 (Folate Hydrolase) [33] |
|             | CLDN4 (Claudin 4) [33] |
|             | CDH17 (Cadherin 17) [33] |
|             | ΤΗΟΧΒ2 (Homeobox B2) [33] |
|             | ΜΕΣΤ (Mesoderm-specific transcript) [33] |
|             | ΝΟΤΧ3 (Notch, Drosophila, Homolog of, 3) [33] |
|             | ΤΗΟΧΒ2 (Homeobox B2) [33] |
|             | ΜΕΣΤ (Mesoderm-specific transcript) [33] |
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Concluding remarks

DNA methylation constitutes a well understood epigenetic mechanism which regulates the expression of human genes. Though some biochemical tests, such as CRP and calprotectin, and clinical scoring systems – Crohn’s disease activity index, Harvey-Bradshaw – help the clinician estimate the activity of IBD, none of them is adequate for the efficient clinical management of these patients [48,49]. In the struggle for developing a new 'ideal marker', which should be specific, clinically useful, non-interventional and able of detecting high-risk patients, the field of epigenetics and specially DNA methylation is really promising.

Until now and according to the aforementioned articles, DNA methylation has been well correlated to IBD and more specifically to some special clinical characteristics of the patients, such as the duration of disease, the severity of inflammation, the phenotype of disease (single or rare flares vs. chronic active vs. relapse-remitting disease, steroid-dependent/refractory disease, etc), the extent of colitis, the age of onset, the number of hospitalizations, the risk of dysplasia and neoplasia, and finally the applied therapies, including the total dose of steroids. Moreover it has been clearly shown that DNA methylation status can effectively distinguish the inflamed mucosa in IBD patients from non-inflamed mucosal specimens. It is also important to note that DNA hypermethylation is one of the biggest epigenetic changes, and gene hypermethylation has been reported to be related with colorectal carcinogenesis. Therefore, the evaluation of hypermethylation has the potential to contribute to early diagnosis of IBD-related colorectal cancer [50]. All the above are important clinical parameters and their relation to DNA methylation of specific genes promises a possible use for DNA methylation as a non-invasive biomarker.

Another important clinical use is the relation between DNA methylation, disease prognosis and treatment. Is the total DNA methylation status altered after therapy? Can the induction of remission reduce the hypermethylation of specific loci? Is the level of DNA methylation or the DNA methylation itself related to the severity and prognosis of the disease? According to the current literature, DNA methylation has been correlated only to the total dose of steroids and the steroid-dependent colitis. Apart from steroids all other treatments used, such as anti-TNF, immunosuppressive and methotrexate remain to be studied. Especially methotrexate, which inhibits dihydrofolate reductase and therefore reduces folic acid level (an important mediator of biological methylation, including DNA methylation), should be anticipated to reduce DNA methylation. For example, treatment with methotrexate has already been shown to increase the level of DNA methylation in PBMCs of patients suffering from arthritis [51].

Recent advances in our understanding of IBD-associated DNA methylation underlie many promising clinical applications such as molecular biomarkers for diagnosis and prognosis of the disease as well as prediction of treatment outcomes. Even if the results from different studies are encouraging, limitations have thus far prevented wide-spread clinical application. One possible reason could be that DNA methylation frequency of many candidate genes is not high enough to achieve the sensitivity needed for clinical use. Technical advantages in the near future are expected to reduce these problems, and DNA methylome is expected to play a key role in the development of personalized medicine.

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