Chapter 9
Epigenetics of Inflammatory Bowel Disease

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Abstract The term epigenome refers to the tissue- and cell-type-specific collection of DNA methylation, histone modifications, and chromatin accessibility and the set of coding and noncoding RNA molecules (Bernstein et al., Cell 125:315–326, 2006) that are dynamically modulated throughout the lifetime of an individual. Epigenetic modifications are critical for regular developmental processes in the intestine, but variation in the epigenome has also been associated with the development of intestinal diseases, including inflammatory bowel disease (Vavricka et al., Inflammatory Bowel Diseases 17:1530–1539, 2011). We hypothesize that plasticity of the epigenome in different cellular compartments links genetic susceptibility and environmental influences and may determine "decision points" in the progression towards disease onset (i.e., manifestation) and/or progression of IBD. This chapter reviews selected aspects of IBD research with the aim to link the current knowledge of genetic, epigenetic, and functional studies into an integrated view on the role of epigenetic variation in intestinal inflammation.

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Introduction

The term epigenome refers to the tissue- and cell-type-specific collection of DNA methylation, histone modifications, and chromatin accessibility and the set of coding and noncoding RNA molecules [1] that are dynamically modulated throughout the lifetime of an individual. Epigenetic modifications are critical for regular developmental processes in the intestine, but variation in the epigenome has also been associated with the development of intestinal diseases, including inflammatory bowel disease (IBD). We hypothesize that plasticity of the epigenome in different cellular compartments links genetic susceptibility and environmental influences and may determine "decision points" in the progression towards disease onset (i.e., manifestation) and/or progression of IBD. This chapter reviews selected aspects of IBD research with the aim to link the current knowledge of genetic, epigenetic, and functional studies into an integrated view on the role of epigenetic variation in intestinal inflammation.

Epigenetics: Background, Technology, and Potential for Common Disease Research

Epigenetics can be viewed as paradigm for phenotypic plasticity and was introduced as a separate field to complement genetics by Conrad Waddington in the early 1940s when studying how the genotype relates to different phenotypes. Although the underlying mechanisms were unknown at the time, Waddington envisioned the existence of an "epigenotype" to explain the phenotypic plasticity observed during normal development [2]. Since then, many of the mechanisms have been worked out in the context of a wide range of biological processes, such as X-chromosome inactivation in female mammals [3], parent-of-origin-specific gene expression (imprinting) [4], and developmental [5] and cellular [6] reprogramming to name but a few. Furthermore, altered epigenetic mechanisms have been linked to cancer as early as 1983 [7] and more recently also to other common diseases [8, 9]. Based on these findings, our perception of epigenetics has changed over the years and was recently redefined as "structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" [10].

Great progress has also been made in elucidating the types of epigenetic marks that register, signal, and perpetuate the activity states and the enzymes that read, write, and erase these marks which, in concert with other modifiers, bring about the structural adaptation of chromosomal regions. There is ongoing debate on what constitutes a true epigenetic mark but also agreement that all marks under consideration at least modulate the epigenome and hence are here referred to as chromatin or epigenome modulators of which there are three main categories. The best studied is DNA methylation in the context of CpG dinucleotides. Low methylation at promoters and high methylation at gene bodies are usually associated with gene

expression and, conversely, high promoter and low gene body methylation are associated with gene silencing [11]. This simple on/off concept has recently become more complex following the discovery of non-CpG methylation and other cytosine modifications [12]. Based on current knowledge, genomic cytosine bases can exist in at least six states (unmethylated, C5-methylated, N3-methylated, C5-hydroxymethylated, C5-formylated, C5-carboxylated) and more modifications may exist and at other bases as well. On the protein level, histone tails are the target for an ever-increasing number of posttranslational modifications (that form the second category), including acetylation, methylation, phosphorylation, ribosylation, ubiquitylation, sumoylation, citrullination, and some even more exotic modifications [13]. With respect to function, they can loosely be grouped into activating, repressive, or bivalent modifications. The latter define a combination of activating (e.g., H3K4Me3) and repressive (H3K427Me3) modifications that have been shown to mark poised chromatin which is typical for developmental genes [1]. The third and final category comprises all the remaining modulators, including the enzymes that lay down the modifications (the "writers"), the proteins that recognize them (the "readers"), and the enzymes that remove them (the "erasers") as well as nucleosomes, chromatin-remodeling complexes, and noncoding RNAs. Collectively, these chromatin modifiers provide function to the genome and define the epigenome.

The main bottleneck that has hampered epigenetic analysis of common diseases in the past has been technology. While genome-wide association studies (GWAS) [14] using single-nucleotide polymorphisms (SNPs) uncovered well over 1,000 new disease loci across all investigated human diseases with a tally of over 160 loci in IBD and significantly advanced the genetic analysis [15], no comparable technology was available for epigenetic analysis. This has changed with the emergence of genome-wide methods [16, 17] for the analysis of DNA methylation which is the most informative and accessible epigenetic modification in a clinical context. The currently most promising platform with respect to accuracy, coverage, throughput, and cost is the Illumina 450k Infinium BeadChip which is essentially the epigenetic equivalent of the 500k SNP chip that proved highly successful for GWAS. An obvious next step was to adapt GWAS to epigenetic analysis to enable epigenome-wide association studies (EWAS). Although both analyses have much in common, EWAS also presents new challenges. As the epigenotype is cell-type specific, special care must be taken to select the correct study material. In other words, blood-derived DNA (which is suitable for all GWAS) is not necessarily suitable for all EWAS. Another problem is a phenomenon known as reverse causality. While GWAS associations are usually linked to the underlying causal variation by linkage disequilibrium, EWAS associations can also be the consequence (rather than the cause) of the phenotype under investigation. This problem can be overcome by inclusion of prospectively sampled individuals in the study design as demonstrated in the first EWAS for type 1 diabetes [18]. As the genotype and epigenotype are inherently linked, the need to distinguish genetic from epigenetic effects adds further complexity but can be addressed, e.g., by using monozygotic twins that are disease discordant for the discovery phase [19]. These advances have paved the way to apply epigenetic analysis to common diseases, and the first wave of EWAS is now well underway, including for inflammatory bowel diseases.

Clinical Relevance of Epigenetic Events in Inflammatory Bowel Diseases

Inflammatory bowel diseases are complex disorders, which are known to be strongly influenced by the genetic background [20]. The high familiar concordance observed in IBD initially introduced this concept [21]. Further studies identifying various disease-associated variants supported this hypothesis [22]. Several identified variants additionally provide insight into potential disease relevant molecular mechanisms. A prominent example is *NOD2*, which was the first disease gene identified for Crohn's disease [23, 24] and which is functionally linked to bacterial recognition. Variants of *IL23R* [25] and *IL12B* [26] are associated with both Crohn's disease and ulcerative colitis and are involved in immune system activation.

As for many complex disorders, the identified genetic variants cannot explain the entire disease risk: In Crohn's disease, currently 140 variants are known to be disease associated, and similarly, in ulcerative colitis the number of currently identified variants is 133 [27]. In this context, one has to keep in mind that the probability of accumulating all the variants at once in one single genome is extremely low, especially since many of those variants have very low frequencies. Consequently, the disease risk explained by genetics for a given genome is of purely theoretical nature. Twin concordance rates, which are higher than the currently explained disease risk [28], indicate that several variants are not identified yet. The resulting gap is generally referred as missing heritability [29]. However, the space beyond this gap is even less explored.

By definition, complex disorders are influenced not only by the genetic background. Environmental factors, such as nutrition, toxin exposure, or the intestinal microbiota—to name but few—are being discussed as potential contributors to disease risk and manifestation. Similarly, a high family concordance rate does not necessarily have to be attributed to the genetic background exclusively. Shared environment, nutrients, or toxins could also explain part of the family concordance. Finally, all these factors may interact leading to additional events of pathophysiological relevance [30].

One integral part of this disease risk which cannot be explained by the genetic background exclusively is epigenetic modifications. Traditionally, epigenetic events are defined as heritable modifications in DNA expression without changing the DNA sequence in itself [31]. Besides DNA methylation, histone modification and nucleosome positioning are integrated in this definition. More recent definitions include micro-RNAs as regulators of gene activity in the absence of DNA sequence variation [8]. In complex disorders, a combination of heritable as well as de novo events is being considered potentially disease relevant.

Epigenetic Events in Complex Diseases: Heading the Way to Inflammatory Disorders

Several scenarios, most of them with an oncological background, are known where epigenetic modifications lead to disease manifestation. A popular example is the global hypomethylation often observed in cancer cells [32]. In the same line, it has

been shown that hypomethylation of several genes (e.g., 16^{INK4a}–$p14^{ARF}$(*CDKN2A*) and *MGMT*) can be a causal event in early tumorigenesis [33]. Following the expanded definition of epigenetics, miRNAs which are widely downregulated in human tumors [34] as a result of hypomethylated miRNA promoters may play an important epigenetic role in cancers [35]. Beside the large number of studies in cancer, various other diseases have been the target of epigenetic research, showing the pathophysiological relevance of epigenetic modifications and their interactions to environmental factors [9, 36–39]. Interestingly, only very few studies address epigenetic events in inflammatory diseases, where a regulatory network of signal-specific and gene-specific functions is required controlling appropriate responses [40]. Initial studies have shown a link between the hypomethylation of Toll-like receptor 2 (TLR2) and increased proinflammatory response to bacterial peptidoglycan in cystic fibrosis [41]. Bacterial infection as an environmental factor was shown to have impact on the epigenetic status of the genome [42], while a recent study presented a functional map of the psoriasis epigenome [43], illustrating how this potentially could be linked to the transcriptome. Similar transcriptional control is provided by micro-RNAs, who are believed to target up to 30 % of all genes [44]. In concordance with DNA methylation, micro-RNAs have been shown to have significant impact on diseases, including inflammatory disorders [45, 46].

Disease-Associated DNA Methylation in Inflammatory Bowel Disease

Taken together, this illustrates the potential impact epigenetic modifications may have on disease risk, manifestation, and progression in Crohn's disease and ulcerative colitis. In fact, several studies have addressed this issue. First approaches in 1996 showed that DNA hypomethylation is a common pattern observed in the rectal mucosa of ulcerative colitis patients [47]. Interestingly, this effect was observed in patients with long-standing ulcerative colitis, supporting the hypothesis that epigenetic modifications in a given tissue are increasing over time. Epigenetic maturation and its potential impact on the onset of disease which is in early adulthood have been studied in mouse models indicating that mucosal epigenetic maturation continues after early adulthood in mouse, which could play a role in age-associated increase in colitis susceptibility [48].

Most studies in this field focused on the methylation of individual inflammation or immune-process-associated target genes. IFNγ methylation was investigated in various cell types present in the human gut, concluding that its methylation status is relevant for the modulation of cytokine secretion in the mucosa [49]. This subject was followed up in 2011, where IFNγ methylation levels correlated with immune response to microbial components and expression of IFNγ in ulcerative colitis patients, suggesting a categorization of patients based on this response [50]. Quantification of DNA methylation of the promoter region of interferon regulatory factor 5 (*IRF5*) aimed to create a link between epigenetics and genetics, since A 5-bp insertion-deletion (indel) polymorphism in the promoter of *IRF5* has been

associated with inflammatory bowel diseases [51]: However, the results implicate that epigenetic dysregulation of the *IRF5* promoter is unlikely to be associated with IBD [52].

Recently, evolving technology enabled assessment of disease-associated methylation in tissues derived from patients inflammatory bowel disease on a broader scale: Quantification of CpG methylation in a set of 1,505 CpG sites corresponding to 807 genes identified seven sites being differentially methylated between healthy and disease individuals [53]. This was expanded to a genome-wide level in Crohn's disease, where 50 methylation sites were identified to be epigenetically modified, including several genes involved in immune activation such as *MAPK13*, *FASLG*, *PRF1*, *S100A13*, *RIPK3*, and *IL21R* [54]. We have recently published a first epigenome-wide DNA methylation analysis (EWAS) combining 27k Illumina, MedIP-Chip and expression arrays from intestinal biopsies of twins discordant for UC. The integrated analysis identified 61 epigenetic disease loci, which were validated in a larger case-control cohort of unrelated individuals [62].

One of the major drawbacks in current approaches investigating the pathophysiological impact of epigenetic modifications is the lack of tools to specifically validate single CpG modifications in a model system. Currently, only demethylation agents, such as azacitidine and decitabine, which have been used in the treatment of myelodysplastic syndrome, are available [55, 56]. By inhibiting methyltransferases, these agents work genome wide. Consequently, it is unclear to which extent the observed cellular effects can be attributed to primary modifications of the methylation of target genes, or to secondary effects, or to interactions of all these.

Regulatory miRNA Networks in the Pathophysiology of Inflammatory Bowel Diseases

In contrast to DNA methylation, epigenetic research in the field of micro-RNAs (miRNAs) has access to such target-specific tools: Sequences, complementary to the micro-RNAs, so-called anti-miRs (or antagomirs), can be used to modulate endogenous miRNA levels. In addition, reporter gene assays represent a powerful tool to validate miRNA findings in model systems. After their discovery in 1992 [57], miRNAs have been found in all eukaryotes, and recent genome-wide computational screens for miRNA targets in humans predict that at least 10 % [58] to 30 % [44] of all genes are regulated by iRNAs. Several studies indicate that miRNAs play an important role in inflammatory scenarios [59–61], including the hypothesis that miRNAs are required to control and balance a specific inflammatory response [62]. Several miRNAs were identified to play a potential pathophysiological role in inflammatory bowel diseases, especially when addressing the disease subtypes specifically: In Crohn's disease miRNAs were associated with ileal and colonic manifestations [63], suggesting that the specificity of miRNA patterns may help to identify disease subtypes. In ulcerative colitis, variants in a noncoding region were shown to alter miRNA functionality [64], providing an explanation on how these variants could exhibit their functional effect. Similarly, a variant in the *IL23R* gene,

which is associated with IBD, has been reported to result in inhibition of miRNA binding to this allele, altering the control of this gene which finally may lead to sustained IL23R signaling, promoting the chronicity of IBD [65]. This was followed by recent approaches creating genome-wide maps of circulating miRNAs in ulcerative colitis, supporting the hypothesis that many previously identified variants located in noncoding regions might contribute to disease susceptibility by altering miRNA sequences [66]. Interestingly, some abnormally expressed miRNA could be linked to inactive colonic mucosa of patients with IBD [67], suggesting that not only an active inflammation results in dysregulation of miRNAs.

In summary, the results of studies targeting DNA methylation as well as miRNAs in inflammatory bowel disease represent not only a set of independent disease-associated mechanisms but also create a link between variants in noncoding regions and effects on pathophysiologically relevant target genes. Finally, epigenetics might help to answer the question whether we not only inherit the genetic background of our ancestors but also the footprints of their lifestyle.

Dialogue Between Epigenetics, Environmental Influences, and the Intestinal Microbiome in Inflammatory Bowel Disease

Due to the increasing prevalence of IBD in industrialized societies, the question arises which environmental factors lead to changing manifestation of disease, as this observation cannot be attributed to changes in genetic background of the respective populations [20]. While many factors have been discussed, the most drastic lifestyle changes within the last century are likely related to childhood infection rates (due to vaccination and antibiotics), increased hygiene in general and nutritional habits. It has been shown in epidemiological studies that improvement of hygienic conditions (such as warm water or water toilets) is positively correlated with incidence rates for Crohn's disease [68, 69]. Likewise in Europe there is a striking north–south and west–east gradient of IBD prevalence, and immune-mediated diseases in general are much more common in larger cities than in rural areas and are related to the presence of bacterial antigens [70–72]. Of course, it could be speculated that all these observations are influenced by mere confounding and the true factors are yet to be identified. Still, several striking hypotheses have been raised by genetic studies as well as functional underpinnings that point to a crucial role of the balance of intestinal host–microbiome interactions, and it is tempting to speculate that this long-term influencing factor actually is a major determinant of epigenetic profiles along the entire gastrointestinal tract. For this hypothesis several facts about this type of stable host–microbe interaction are important. Large international efforts have been made to systematically profile the properties and functional repertoires of human microbial communities [73, 74]. These studies have clearly shown a huge diversity of microbial species that is specific to the body region as well as to the individual (microbial "fingerprint"). Even after drastic life history events, e.g., intestinal infections or courses of combination antibiotic

therapies, intestinal microbial consortia display evidence of resilience, i.e., after a certain time the specific consortia return to their previous diversity that is similar to the one before the event. It has been proposed that only few stable states of the human intestinal core microbiome exist, the so-called enterotypes [75]. These metagenomic states, representing differences in core metabolic activities and pathways, could be caused by the genetic (and epigenetic) makeup of the host, but on the other hand the enterotypes together may also imprint on the long-term epigenetic (and thus functional) profiles in the different cellular compartments of the intestinal mucosa [76]. Exciting data point to long-term influences of dietary modifications on microbial communities that in turn cause functional changes in the human host. This principle was first described in animal models of obesity, where microbial communities that were transplanted from obese individuals led to increased energy harvest and weight gain in lean individuals [77]. This principle of microbiota-transmissible susceptibility has now been expanded to a number of immune-mediated diseases including IBD. In a genetic model of amino acid malnutrition resulting in dysbiotic microbial communities and increased susceptibility to colitis, it has been shown that long-term dietary supplement with chemically modified tryptophan resulted in changes of antimicrobial peptide profiles and decreased inflammatory responses [78]. Interestingly, the inflammatory phenotype could be transmitted to germfree wild-type animals by stool transplantation pointing to a crucial role of the microbiome in exerting this long-term effect. Further, the state of the intestinal microbiome has been shown to imprint on long-term functional properties of natural killer cells that result in different outcomes after experimental induction of colitis [79]. This effect was only restricted to a defined "vulnerable" period in the immunological life history and linked to changes in DNA methylation patterns making it likely that changes in the cell-type-specific epigenomes may modulate inflammatory responses in the long term. Along this line, in a larger cohort of monozygotic twins, stable correlations between the presence of distinct bacterial species and certain host transcripts or transcript profiles have been shown [80]. In IBD twins, this stable correlation is lost, which points to a gradual loss of epigenetic control of this two-way interaction.

It will thus be interesting to link the more classical view of nutrigenomics that is regularly defined as the investigation of how food components impact on phenotype–genotype interactions [81] with the "other" dimension of our intestinal genome, the microbiome, and related epigenetic marks. The advent of large-scale sequencing now allows for time and cost-efficient investigation of different sequence spaces, including the many epigenomes of the intestinal tract and their potential functional consequences (see Fig. 9.1). For the first time, the hypothesis that epigenetic modifications are the missing connection between genetic predisposition, environmental influences, and disease manifestation can be tested and put into a functional and clinical perspective. Several consortia have been launched within the framework of the International Human Epigenome Consortium (IHEC) including the BLUEPRINT [82] and DEEP networks that exactly address these questions in the different cellular compartments of the intestinal mucosa.

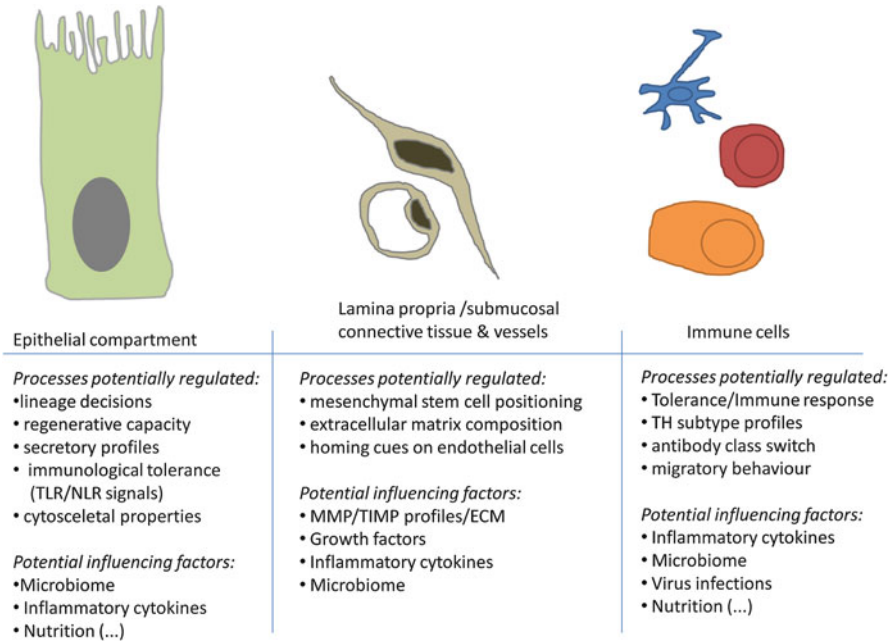

Fig. 9.1 Scheme of cellular compartments of the intestinal mucosa that are potentially influenced by epigenetic alterations in IBD

Is Epigenetics the Missing Link Between Inflammation and Cancer?

Tissue damage, wound healing, and continuously increased cell proliferation are only a few mechanisms of inflammation, which are believed to contribute to the initiation and development of cancer [83]. However, many elements of this link are still not understood. Since tumor tissue is often found to be globally hypomethylated and locally hypermethylated [32] which is believed to inactivate tumor suppressor genes [84], a key question is how inflammation can promote such changes in methylation. A general principle discussed in this context is inflammation-mediated cytosine damage: DNA damage caused by inflammatory agents such as reactive oxygen species can lead to inappropriate methylation, finally resulting in the development of cancer [85]. One pioneering study in this field demonstrated that epigenetic modification of the promoter of E-cadherin is associated with ulcerative colitis in patients undergoing colectomy [86], hypothesizing about its role in the progression from chronic inflammation to cancer. Interestingly, assessment of the methylation of 11 genes comparing 48 ulcerative colitis-associated cancers, 21 ulcerative colitis-associated dysplasias and 69 sporadic colorectal cancers could not show that epigenetic modifications lead to more aggressive clinical courses [87]. In contrast to that, other studies linked altered methylation in several target genes to

predisposition, manifestation, or progression of colorectal cancer in patients with ulcerative colitis as well as in model systems: Studies on the alternate reading frame p14 (ARF) [88], WNT signaling pathway genes [89], DNA mismatch repair genes [90], genes coding for the tumor suppressors ESR1 and N33 [91], and death-associated protein kinase DAPK [92] supported this concept.

Similarly, several miRNAs have been shown to potentially play a role in inflammatory bowel-associated neoplastic transformation: miRNA-31 dysregulation was presented as a candidate in the context of chronic inflammation progressing into tumor. This micro-RNA has been also shown to increase with disease progression in IBD patients [93]. Neurotensin, which promotes inflammation and colon cancer by activating neurotensin-1 receptor, has been shown to stimulate the expression of miR-21 and miR-155, suggesting a functional link. Furthermore, tissue levels of both micro-RNAs correlated with tumor stage in human colon tumor samples [94]. Recent studies have identified several miRNAs being regulated during the progression from dysplasia to cancer in patients with IBD (miR-122, miR-181a, miR-146b-5p, let-7e, miR-17, miR-143) [95].

As the potential development of colorectal cancer is one of the most serious complications for patients with IBD, the need of a more detailed understanding how inflammation can progress is evident. Epigenetic modifications, especially DNA methylation, may represent an inflammatory memory in the intestinal mucosa. However, as many of the studies provide mostly an exemplary view on a selected group of patients, drawing conclusions on the validity of the results for larger cohorts should be undertaken carefully. Further functional studies, documenting both the functional background and the clinical validity, will be required to further progress in this field.

Outlook: Epigenetic Strategies in Diagnostics and Treatment of Inflammatory Bowel Disease

Biomarkers to classify diseases or disease subtypes have been always a major goal in epigenetic research. However, providing validated biomarkers of adequate diagnostic value is a challenging and considerably expensive endeavor. Most publications are descriptive and use the term biomarker in the context of an observed molecular pattern, without the final validation which could demonstrate the validity of this observation in a clinical setting. In fact, most current approaches cannot afford taking their findings into very large cohorts. Such cohorts could be only created in joint efforts between academia and industry [96]. As epigenetic modifications in IBD have been linked to cancer, several therapeutical avenues from the field of cancer drugs could be potentially of interest in the therapy of IBD. However, it has been recently questioned whether premature claims on the effectiveness of some drugs are the result of peer pressure rather than the result of validated clinical research [97].

Independent of these shortcomings, several studies aimed to utilize the specificity of observed epigenetic patterns. Easily accessible biomaterials, such as peripheral blood, are of particular interest in this context: In children, where noninvasive methods are often favored, a study has presented 11 CD-associated serum miRNAs potentially suitable for diagnostic purposes [98]. Similarly, differentiating active ulcerative colitis from Crohn's disease was possible using a defined set of miRNAs from peripheral blood in adults [99], further supporting the hypothesis of specific patterns. A recent study confirmed this concept with a different and reduced set of miRNAs in circulating blood [100], which suggests that the number of specific signals is substantially larger than the number of biomarkers currently published.

A major issue of the upcoming large-scale epigenomic studies in IBD will be the elucidation of cellular specificity of such epigenetic events. As epigenetic profiles are highly cell-type specific [16, 17, 101], most of the previous studies aiming to develop clinical biomarkers or novel therapeutic principles suffer from the fact that sum signals (i.e., the entire mucosa or whole peripheral blood) were investigated. Even reproducible changes could thus reflect secondary differences in cellular composition rather than pathophysiologically relevant differences in epigenetic profiles. If epigenetic marks are to be translated to clinical therapies, the molecular chain of events has to be detected and linked to defined cell populations. It is evident that epigenetic variation may have broad consequences on cellular phenotypes in all functional compartments of the intestinal mucosa (see Fig. 9.1). It is thus important to experimentally understand the impact of certain marks in a functional context, e.g., how are lineage decisions influenced by epigenetic alterations in intestinal epithelial stem cells? What is the impact of epigenetic modifications on tolerance to microbial stimuli in professional migratory immune cells? Is there a trans-generational effect of inflammatory effects that can be attributed to epigenetic principles? If these aims can be reached, it is likely that we can start looking for epigenetic marks (that could possibly be linked to microbiome changes) even prior to clinical manifestation of disease. It will be a challenge to identify therapeutic principles that specifically target single epigenetic modifications; so far compounds like HDAC inhibitors completely lack target specificity, but still have been found efficacious in defined inflammatory indications like systemic sclerosis. Patterns of epigenetic marks represent a dynamic picture into etiology. The ultimate goal of EWAS is to merge high-resolution information on epigenetic variation such as differential DNA methylation or miRNA levels with functional consequences on mRNA regulation and clinical phenotypes into a molecular risk map that will contribute to a clearer understanding of the etiology of IBD. This map will help bridging the gap between unexplained disease susceptibility and disease manifestation and may lead to the identification of novel diagnostic and therapeutic targets. Broadening the scope of such studies to longitudinal studies that follow high-risk populations (e.g., IBD kindred cohorts) into manifestation may even result in biomarkers for identifying susceptible individuals prior to disease manifestation. Applying targeted preventive measures (e.g. modification of the intestinal microbiome) in such high-risk individuals would—for the first time—aim for a causative intervention, which in the end may only be possible before the onset of clinically overt disease.

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