Epigenetics in IBD: a conceptual framework for disease pathogenesis

Natasha G, Matthias Zilbauer

ABSTRACT

The global incidence and prevalence of paediatric inflammatory bowel disease (IBD) is increasing, with a notable emergence in developing countries with historically low rates. This suggests that environmental and epigenetic factors may play an important role in the pathogenesis and progression of IBD. Epigenetics refers to the study of biological mechanisms that result in a change in phenotype, without an change in the underlying DNA sequence. Epigenetic mechanisms drive many biological processes that occur in health, such as development and ageing, and are also implicated in disease, including cancer and other inflammatory diseases. Importantly, identification of cell-type-specific epigenetic mechanisms could lead to the identification of molecular disease subtypes allowing a personalised treatment approach. In this short review, we provide a summary of epigenetic mechanisms operative in mammals, and their potential involvement in IBD pathogenesis. Furthermore, we discuss key challenges associated with investigating epigenetics in IBD and provide potential strategies to overcome these, such as through the use of ‘omics’ and organoid technologies.

INTRODUCTION

Paediatric inflammatory bowel disease (IBD) is a chronic debilitating condition with a rising global incidence and prevalence, and a notable emergence in countries with historically low rates such as the Middle East and Asia.1–3 IBD is an umbrella term that includes Crohn’s disease (CD), ulcerative colitis (UC) and IBD-unclassified. The pathogenesis of this systemic, relapse-remitting disorder is complex and is hypothesised to result from the disruption in homoeostasis between the intestinal epithelium, microbiome and mucosal immune system.4 Family history of IBD is a strong independent risk factor to develop IBD. Indeed, twin and family studies have shown that there is a genetic predisposition for the development of IBD. Furthermore, genome-wide association studies (GWAS) have identified more than 200 risk loci for IBD, many of which involve genes that correspond to loss-of-function variants in immune components.5,6 While IBD is more likely polygenic, very
early onset IBD (VEO-IBD), defined as IBD in children diagnosed under 6 years of age, has been linked with monogenetic variations and are associated with primary immunodeficiencies. Although genetic variation undoubtedly plays a role in determining the risk of developing IBD in some individuals, the rising incidence of IBD in recent decades, particularly in low/middle-income countries that are becoming increasingly ‘Westernised’, suggest that environmental and epigenetic factors may be as or even more important in the pathogenesis of IBD. Furthermore, epigenetic mechanisms could be the driver for determining disease onset and progression, especially in the absence of genetic changes.

THE PRINCIPLES OF EPIGENETICS

The ‘epigenetic landscape’ was first described by Conrad Hall Waddington in 1942. As a developmental biologist, Waddington used the term ‘epigenetics’ to explain interactions between the environment and genes ultimately leading to the development of phenotype. Today, epigenetics can be defined as the study of heritable changes in phenotype, such as gene expression, that are not attributed to alterations in the fundamental DNA sequence. This is in contrast to genetics, which is the study of heritable changes in gene expression or function due to the direct changes to the genetic code, including point mutations, insertions, deletions and translocations. Epigenetic marks can be heritable, enabling changes in the epigenome to be passed onto daughter cells during mitosis, or from one generation to the next—a phenomenon described as transgenerational epigenetic inheritance. A major role for epigenetic mechanisms has been demonstrated for several fundamental biological processes including cellular differentiation, cell-type-specific gene transcription and cellular function, as well as immunological memory. With technological advances enabling researchers to study epigenetics on a genome wide scale in high resolution, there is increasing evidence for the role of epigenetics is health and in a multitude of diseases, including those with complex processes such as cancers, autoimmune and inflammatory diseases.

There are three main mechanisms of epigenetic control known to be operative in mammals: DNA methylation, post-translational histone modifications and expression of non-coding RNAs (ncRNAs) (figure 1).

DNA methylation is the most extensively studied epigenetic mechanism, and refers to the addition of a methyl group onto the 5’ position of cytosine, forming a 5-methylcytosine. This usually occurs in the context of a CpG site, in which a cytosine immediately precedes a guanine nucleotide and is a process catalysed by enzymes called DNA methyltransferases. In the human genome, most CpGs are methylated, with an exception being CpG islands, a region usually within promoter regions with a high proportion of CpG motifs. In principle, hypermethylation of a promoter region results in reduced gene expression and vice versa. Interestingly, DNA methylation is a process that...
is dependent on cofactors, dietary substrates and nutrients. For instance, folate and vitamin B₁₂ act as methyl donors and cofactors, contributing to DNA methylation.¹⁵ ¹⁶

Chromatin refers to the complex of DNA and histone proteins. Post-transcriptional histone modifications enable chromatin to be either tightly packed (ie, heterochromatin) or more accessible (ie, euchromatin). Consequently, the degree of chromatin accessibility determines transcriptional activity and hence gene expression and phenotype.¹⁷

ncRNAs include housekeeping ncRNAs (eg, tRNAs, rRNAs and snRNAs) and regulatory ncRNAs. The latter group can be further subdivided based on nucleotide length—transcripts shorter than 200 nucleotides are termed short ncRNAs (sncRNAs) and those exceeding 200 nucleotides in length are termed long ncRNAs. Among the sncRNAs, microRNAs are of increasing interest in their role in post-transcriptional regulation of gene expression in response to cellular or environmental changes through complex gene regulatory networks.¹⁸

EPIGENETICS AS A CONCEPTUAL FRAMEWORK FOR IBD PATHOGENESIS

Development of the human intestine relies on exposure to a wide range of environmental factors. These include nutrients, microbes, as well as other antigens capable of activating the mucosal immune system. In addition to allowing immune tolerance to develop, such environmental factors are also critical for the successful colonisation of the intestine by a healthy, diverse gut microbiome. The list of factors that have the potential of altering the physiological process of gut and intestinal microbiome development is much longer. Examples include exposure to pathogens (including enteric bacterial and/or viral infections), toxins (eg, food emulsifiers or preservatives) or medical drugs such as antibiotics. Any of these factors, if encountered during a critical time of development, has the potential to alter physiological progression and may even predispose to disease (figure 2).

The complex interaction between genetic, immune and environmental factors has been implicated in in the disease pathogenesis of IBD. Large-scale genome GWAS has been successful at identifying disease susceptibility gene loci, such is IL23R, IL12B, JAK2 and STAT3, as well as, pathways involving T-helper (Th)17 and interleukin (IL)-12/IL-23.⁷ ¹⁹ While genetic changes may account for modest proportion of the disease variance, epigenetic factors could potentially account for ‘hidden heritability’ in IBD.⁷ Assuming the human genome to be highly stable even over centuries, the major increase in the incidences of IBDs in recent decades could be seen as further evidence in support for a key role of environmental factors in IBD pathogenesis. Lastly, the rapid emergence of IBDs in countries adapting to a western lifestyle, also referred to undergoing ‘Westernisation’, further illustrates the striking correlation between environmental changes and the rising global incidence of IBD.¹⁻³

**Figure 2**  Schematic illustrating factors (genotype, epigenotype and environment) contributing to IBD pathogenesis. Created with BioRender.com. IBD, inflammatory bowel disease.
Although these concepts have long been established based on clinical observations and epidemiological studies, our understanding of underlying molecular mechanisms capable of explaining these observations remains limited. Epigenetics provides a plausible framework by being capable of mediating exposure to environmental factors into stable, heritable changes of cellular phenotypes without changing the underlying DNA sequence. The potential impact of environmental factors on cellular epigenome as well as their stability depends on several factors. Perhaps the most important among them is the degree of epigenetic plasticity, or in other words the ability for the cellular epigenome to be modifiable. Epigenetic plasticity has been shown to be directly linked to the developmental stage of a cell type or an entire organism. As such, the cellular identity is retained. Other factors determining the degree of cellular epigenetic changes include the type of environmental factors and their duration. Applying these concepts to the development of the human intestine, exposure at early developmental stages, for example, in utero or in the early postnatal period, might represent particularly vulnerable period during which specific environmental factors have the potential to alter the epigenetic programming of the intestine. Either prolonged and or repeat exposure to certain environmental factors could lead to the development of stable epigenetic changes, which predispose to an individual to the development IBD.11 24

The concept of repeat and or prolonged exposure to environmental factors causing stable epigenetic changes, ultimately leading to altered cellular function may also explain the variation in the timing of disease onset. Approximately one-third of patients with IBD manifest symptoms during childhood or early adulthood, although the majority are diagnosed in adolescence. Excluding rare forms of VEO, monogenic IBD, genetic risk alone does not sufficiently explain this distinct difference in the age of disease onset. As such, we hypothesise that variations in the timing, duration and specific nature of environmental factors might determine the occurrence of epigenetic changes and ultimately the onset of disease.

Howell et al demonstrated that in primary human epithelial cells obtained from mucosal biopsies from paediatric IBD compared with non-IBD control patients, genome wide analysis showed gut segment and disease specific differences DNA methylation and transcriptional changes. Furthermore, these DNA methylation marks remained stable over time in an ex-vivo organoid model. Studies have also supported the developmental origin of IBD—methylation analysis on the human epithelium of paediatric IBD patients compared with fetal and paediatric controls, showed an overlap significantly with those undergoing methylation changes during the development of the fetal intestine, including key genes in intestinal epithelial immune defence.26

IBD is also known to increase the risk of colorectal cancer (CRC), and this process could also be in part epigenetically driven. Rajamäki et al showed that in IBD associated CRC (IBD-CRCs) compared with sporadic CRC clustered separately, and there was global hyper-methylation in IBD-CRCs. This was not found to be associated with younger age at diagnosis or differential expression of the enzymes regulating methylation, hence suggesting these methylation changes are driven by inflammation.27 28

Overall, these examples lend support to the role of epigenetics in the pathogenesis of IBD, and these signatures potentially serve as new diagnostic and prognostic biomarkers.

Challenges in demonstrating the role of epigenetics in IBD

Despite the highly promising existing evidence in support of epigenetic mechanisms in IBD pathogenesis, reports on the identification of disease associated, cell-type-specific epigenetic alterations and their impact on causing stable changes of cellular function remain scarce. This is likely to be at least in part due to the many challenges that researchers and clinicians face when investigating epigenetics in IBD. Examples include the identification of relevant cell types, choosing specific epigenetic marks while considering that epigenetic mechanisms are closely connected, the timing of sampling and the limited access to human tissue. Outlining these aspects in detail is far beyond the scope of this article, and we will therefore focus on highlighting selected aspects. Perhaps the most important challenge, and often the most difficult to overcome, is the confounding factor that tissue samples have a heterogenous cellular composition, and hence, making it difficult to dissect cell-type-specific epigenetic changes. As a result, performing epigenetic profiling on mixed cell tissues, such as mucosal biopsies or whole blood samples, will not provide information of actual cell-type-specific epigenetic changes. Instead, epigenetic signatures are a ‘merged’ summary of all cell types present and therefore vary vastly according to cellular composition.28 29 This is important as a comparison between tissue samples with differences in cellular composition will yield differences in epigenetic profiles that are driven by difference in cellular composition. Even the application of in-silico cellular decomposition tools is unable to reliably detect cell-type-specific, disease-associated epigenetic changes.

One approach to overcome this challenge is to purify specific cell types of interest. However, such methods are extremely labour intensive, and could still be potentially confounded by cellular contamination. Single cell profiling could also circumvent this
problem and recent cutting-edge methodologies have demonstrated the possibly of performing genome wide epigenetic profiling on a single cell level such single cell Assay for Transposase Accessible Chromatin sequencing or single cell DNA methylation profiling. Indeed, the possibility of performing simultaneous genome wide transcriptional and epigenetic profiling on a single cell level is hugely exciting and has great potential of delivering novel insights.

Another elegant way of investigating epigenetic mechanisms in human cells is the use of patient-derived human organoids. Since the development of the human intestinal epithelial organoid (IEO) culture model by Hans Clevers just over a decade ago, these versatile translational research tools have become widely used in the field of gastrointestinal research including IBD. IEOs can be generated from stem cells obtained from mucosal samples, and expanded in culture into three dimensional structures which closely mimic the intestinal epithelium in vivo (figure 3).

Our group and others have demonstrated that organoids retain epigenetic (specifically DNA methylation) profiles and are key to determining patient and gut segment specific cellular function. Furthermore, comparing DNA methylation profiles of IEOs derived from human fetal with paediatric gut revealed striking differences in their epigenetic plasticity with fetal cell displaying major epigenetic changes in culture. Taken together, these early studies demonstrate the utility of patient-derived organoids as a reductionist model to investigate epigenetic mechanisms in human gut health and IBD.

Future directions in epigenetics of IBD research

Despite all the challenges associated with investigating the role of epigenetic mechanisms in IBD pathogenesis, the future potential for major findings remains high. The emergence of novel high throughput and high-resolution technologies, in particular single cell molecular profiling, spatial transcriptomics and the use of patient-derived organoids are capable of addressing some of the key questions in this area. For example, are there stable, cell type specific epigenetic marks that can either cause or contribute to the chronic, relapse-remitting gut inflammation in IBD? If so, what are the specific cell types are affected, and which environmental factors are directly involved in causing these epigenetic changes? Another important and promising area of research refers to the use of epigenetic signatures as clinical biomarkers—diagnostic, prognostic, predictive or therapeutic. For example, Ventham et al have demonstrated hypomethylation of ribosomal protein S6 kinase A2 (RPS6KA2) could predict complicated structuring and/or penetrating CD and extensive UC. In particular, stable epigenetic marks such as DNA methylation, which can reliably be analysed on extracted DNA has some advantages over other, less stable molecular profiles (eg, gene transcription). Furthermore, in the right context, performing epigenetic profiling of mixed cell tissue with the aim of generating clinical biomarkers may be justifiable. However, the identification of underlying mechanisms will still require the demonstrating of cell-type-specific stable epigenetic marks. Importantly, demonstrating epigenetic, cell-type-specific variation among patients diagnosed with the same condition (ie, UC or CD) could provide an opportunity to develop novel, molecular disease subtypes that could explain the major phenotypic variation, thereby allowing a more personalised treatment approach.

CONCLUSION

Epigenomics is an emerging field, that could mediate interactions between the genome and environment. Epigenetic mechanisms provide a plausible and attractive framework that is capable of filling in the gaps in our understanding of IBD pathogenesis, and in particular, aspects specific to patients diagnosed in childhood. Despite the challenges in performing translational epigenetic research, the availability of novel, cutting-edge research tools, including single cell epigenetic profiling and patient-derived gut organoids, is indeed exciting and holds huge potential to deliver compelling evidence in support of this largely theoretical framework. Overall, identifying epigenetic signatures in IBD could pave way for the development of new clinical biomarkers of IBD, identify new drug targets and hence could ultimately lead to major advances in IBD patient care.
Contributors NG and MZ designed and wrote the article together.

Funding MZ was funded by a Medical Research Council (MRC) New Investigator Research Grant (MR/T001917/1). The funder had no role in the design, creation or decision to submit this work for publication.

Competing interests None declared.

Patient consent for publication Not applicable.

Provenance and peer review Commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

ORCID iD Matthias Zilbauer http://orcid.org/0000-0002-7272-0547

REFERENCES