Personal viewpoint

Epigenetics of inflammatory bowel disease

Summary
Understanding the causes and molecular mechanisms of Crohn's disease and ulcerative colitis, the two forms of idiopathic chronic inflammatory bowel disease (IBD), is a major challenge in gastroenterology research. Significant effort has been invested in uncovering genetic and environmental factors which may increase the risk of IBD, but progress has been slow, and no IBD specific factors have been detected so far. In this article we suggest that in addition to mutant genes and a hazardous environment, epigenetic factors may be relevant in understanding the aetiopathogenesis of Crohn's disease and ulcerative colitis. This hypothesis is based on a number of clinical and molecular findings in IBD studies such as: (i) the maternal effect in transmission of IBD from affected parent to offspring; (ii) parental differences in the degree of genetic anticipation; (iii) discordance of monozygotic twins affected by IBD; (iv) genetic association studies demonstrating evidence for aberrant regulation of expression of cytokine genes; (v) sex effects in association with HLA alleles and haplotypes; and (vi) epigenetic aspects of IBD treatment. It is suggested that inherited and/or acquired epigenetic defects, or epimutations, may be of aetiological and pathogenic importance in IBD. Epigenetic studies of Crohn's disease and ulcerative colitis may have a significant impact on the field of IBD research.

Introduction

Crohn's disease and ulcerative colitis are two forms of chronic idiopathic inflammatory bowel disease (IBD). Despite numerous clinical and experimental studies, the aetiopathogenesis of IBD remains contentious, although it has been generally accepted that IBD is a multifactorial disease caused by the interplay of genetic, environmental, and immunological factors.[1] Genetic epidemiology studies provided evidence that inherited factors may contribute to individual susceptibility to IBD. Familial risk of IBD[2–4] in combination with a higher concordance rate for IBD in monozygotic compared with dizygotic twins[5 6] suggest that genetic factors play an important role in the origin of IBD. Evidence for a genetic component in IBD evoked an avalanche of molecular genetic linkage and association studies in the past decade. A number of loci have shown strong or suggestive evidence for linkage to IBD.[7–10] Polymorphisms in genes encoding proinflammatory and regulatory cytokines as well as the HLA system have been investigated in genetic association studies of ulcerative colitis and Crohn's disease. Although with some degree of controversy, the association studies provided preliminary evidence that several genes may have an impact in determining predisposition, severity, and prognosis of these diseases.[8 9] In addition to predisposing genes, epidemiological studies have suggested that various exogenous factors such as bacteria, smoking, and diet may contribute to the risk of being affected with IBD.[11 12] Many more studies, however, are required to elucidate the exact mechanisms of how these factors predispose to IBD.

In addition to genetic and non-genetic studies, there is accumulating evidence that epigenetic, or gene activity regulating factors may play a role in the aetiopathogenesis of IBD. To our knowledge, epigenetic aspects of IBD have never been systematised. An overview of various clinical and molecular IBD findings with their epigenetic interpretation as well as a brief introduction into epigenetics are provided below.

Brief description of epigenetics

By definition, epigenetics pertains to modifications in gene expressions that are controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure.[13] DNA methylation and chromatin conformation are two "anatomically" different but functionally related molecular substrates of epigenetic regulation.[14] DNA methylation is achieved by methylation of cytosine residues. A large proportion of genes exhibit correlation between gene expression and the degree of methylation—that is, the lower the degree of DNA methylation in the gene regulatory region, the larger the number of mRNA molecules this gene would synthesise.[15 16] Although the cause-effect relationship between epigenetic status of a gene and gene expression has been questioned,[17] there are experimental data supporting the idea that epigenetic factors play a gene regulatory role (see Siegfried and colleagues[18]).

In a similar way to DNA methylation, a high degree of gene expression correlates with histone hyperacetylation, while a low degree of such expression is linked to hypoacetylation of histones.[19 20] Recently the relationship between DNA methylation and histone acetylation has been established.[21] It was found that a methylcytosine binding protein (MeCP2) that binds to methylated DNA also attracts histone deacetylases (HDAC1 and HDAC2) which hypoacetylate histones and thereby inhibit access of transcription factors.[21]

Over recent decades, epigenetics has differentiated into a separate field of molecular biology with numerous experimental techniques. A wide variety of epigenetic mechanisms that control various parameters of gene expression, genetic recombination, DNA repair, and DNA mutagenesis have been identified in bacteria, plants, animals, and humans.[22] It is also important to note that epigenetic DNA modification may be directly involved in the change in DNA sequence as methylated cytosines are known to exhibit the highest degree of mutability in comparison with other nucleotides, and such mutations are frequently detected in disease genes.[23]

A major difference between epigenetic and DNA sequence based factors lies in the ability of the former to change in comparison with the latter. Epigenetic factors may exhibit only partial stability ("metastability") while DNA sequence demonstrates nearly complete interclonal fidelity. Epigenetic patterns undergo significant reorganisation during gametogenesis, development, and aging.[24 25] This is applicable to various tissues including the colon.[26 27] Due to this metastability, as a rule, the epigenetic status of a gene may exhibit interindividual, tissue specific, and intercellular (within the same tissue) variation. In contrast, epigenetic signals are relatively stable, especially in somatic cells, and the epigenetic status of mitotically active special-

Abbreviations used in this paper: IBD, inflammatory bowel disease; SCFAs, short chain fatty acids.

ised cells is quite robustly transmitted to the daughter chromatids of dividing cells.[28] The term "dual inheritance" which emphasises the presence of epigenetic information in addition to the four nucleotide based DNA strand has been suggested.[29] Interestingly, in some cases epigenetic factors can be transmitted from one generation to another,[30] and the idea of "dual" inheritance is therefore applicable to the germline as well.

Despite the fact that epigenetic mechanisms play a significant role in controlling gene activity, and failure of such epigenetic regulation may negatively affect functioning of cells and tissues, disease related epigenetic changes, or epimutations,[31] have been sought in only a very small proportion of human diseases. Thus far, the dominating paradigm in genetics of complex diseases is concentrated on the damaging effects of DNA mutations on protein structure while epigenetic factors have been ignored. Below are summarised the clinical and experimental data that suggest that epigenetic factors may be of aetiopathogenic relevance to IBD.

Parent-of-origin effect in IBD transmission

Clinical evidence for a parent-of-origin effect was recently shown in the analysis of 135 families in which both a parent and an offspring had IBD.[32] In the majority of families the transmission of susceptibility to disease occurred from mother to child, and the difference between maternal versus paternal transmissions was highly significant ($p=0.00001$).[32] Interestingly, the maternal effect was observed only among non-Jewish pairs with Crohn's disease in which affected mother-offspring pairs comprised 85% of the sample ($p=0.00007$). The maternal effect suggests several possibilities, such as mitochondrial transmission, X linked effects, and genomic imprinting. None can be excluded at present. Given the relatively late onset of IBD as well as remissions and relapses of this disease, a defect in mitochondrial genome is not likely to be the main cause of the maternal effect. X linked factors are of interest as an association of IBD with Turner's syndrome (X monosomy) has been identified,[33 34] and evidence of IBD linkage to markers on the X chromosome was recently detected.[35] The third possibility is genomic imprinting, or differential expression of homologous genes depending on their parental origin. Molecular mechanisms of genomic imprinting pertain to epigenetic DNA methylation and chromatin remodelling.[36 37] It has been shown that aberrant imprinting can lead to a wide variety of clinical disorders ranging from tumours to pronounced growth abnormalities and from mental retardation to developmental disorders.[38] The effect of genomic imprinting can be detected in linkage studies when cosegregation of maternal alleles is investigated separately from paternal alleles.[39] Alternatively, genomic imprinting can be diagnosed when sex specific recombination fractions for maximal lod score (Z_{max}) at the putative disease locus are incompatible with meiotic recombination rates for that specific region in the reference map.[40]

Parent-of-origin effects in genetic anticipation studies of IBD

Parent-of-origin effects were also detected in genetic anticipation studies of IBD. Genetic anticipation is a biological phenomenon that exhibits an earlier age of disease onset and increased severity in affected offspring in comparison with their affected parents.[41] A new wave of anticipation studies in human diseases has arisen after trinucleotide repeat expansion-type mutations were detected in several neurological diseases, and it was shown that the degree of expansion correlates with the degree of intergenerational age difference. Anticipation studies, however, are not straightforward because a number of ascertainment biases may occur, and it has been shown that the rate of false positives can be very high if inappropriate sample collection criteria are used.[42] Cautionary notes regarding genetic anticipation studies in IBD have been expressed in several recent review articles.[43 44] On the other hand, any improvements in the design that allow for bypassing methodological complexities are of interest as evidence for genetic anticipation may have both clinical and molecular implications.

One heuristic approach for differentiating between artefactual anticipation and a genuine one uses the comparison of parental effects on the degree of anticipation.[45] The rationale of this idea is that if anticipation occurs as a consequence of ascertainment bias, it would be equally apparent in father-offspring and mother-offspring pairs, while anticipation confined to parents of one sex indicates some specific genetic or epigenetic event.[45] This approach was applied in a sample of 61 parent-offspring pairs, both with Crohn's disease, and it was found that the degree of anticipation was significantly greater for father-child pairs (20.6 (3.2) years; n=17) than for mother-child pairs (11.7 (2.1) years; n=44).[46] Parental effects were also detected for severity where disease was more severe in the younger generation when the affected parent was the father (13 of 16 pairs) and not the mother (two of 11 pairs).[47]

Several other studies of genetic anticipation were less conclusive, with the trend towards higher degrees of anticipation in transmitting fathers[48] and transmitting mothers.[49] Discrepancies between the studies are not surprising given the differences in ascertainment criteria, ethnic background of IBD patients, and the high degree of genetic heterogeneity of IBD. Some IBD genes may exhibit maternal effects while others may lead to a higher degree of anticipation in paternal transmissions, and clinical observations will depend directly on the ratio of the two types of genes in a specific sample. In this situation, parent-of-origin specific genetic anticipation studies in a subgroup of families that exhibit a relatively strong evidence for linkage to a specific IBD locus (for example chromosome 16 or 12) may be of interest. Another possibility is to group IBD parent-offspring pairs according to their clinical characteristics which may also reduce the degree of genetic heterogeneity.

Parent-of-origin effects in genetic anticipation in families affected with IBD, if confirmed, would be of interest for several reasons. Firstly, the presence of genetic anticipation suggests instability of trinucleotide repeats.[50] A different degree of genetic anticipation in maternal versus paternal transmissions implies a differential mutagenic effect on the unstable DNA mutation in oogenesis versus spermatogenesis. This feature can be used for cloning of disease specific trinucleotide repeat expansions, as the search for repeat expansion in the pairs where the transmitting parent exhibits a higher degree of genetic anticipation would be more likely to reveal larger and therefore easier detectable intergenerational differences in the length of trinucleotide tracts.

Secondly, a parent-of-origin effect in genetic anticipation is important from the point of view of basic science as such an effect implies a mechanistic role of epigenetic factors in trinucleotide repeat expansion during gametogenesis and/or embryonic development. It is somewhat surprising that this aspect of DNA instability has been neglected in human genetics. A strong parent-of-origin effect in the unstable DNA diseases[51] has been known for more than a decade, but the role of epigenetic factors in trinucleotide repeat instability has not yet been explained.

Finally, in addition to expansion of repetitive sequences, purely epigenetic mechanisms of genetic anticipation are

possible. Experimental studies in plants and animals suggested that epigenetic factors were directly involved in intergenerational changes in gene activity,[52] which is consistent with genetic anticipation. In transgenic mice studies, a genetically stable transgene locus *TKZ751* exhibited gradual intergenerational changes in DNA methylation in the offspring of such mice.[53] The degree of DNA methylation increased or decreased in the subsequent generations depending on the genetic background of the non-transgenic parent (BALB/c or DBA/2, respectively). DNA methylation correlated with decreasing expression of the transgene across generations, and was spreading by 6–10 kb with each subsequent generation.[53] In plants, a mutant strain of *Arabidopsis thaliana*, *ddm1*, demonstrated a progressively more severe phenotype in subsequent generations.[54] Phenotypic changes correlated with gradual loss of DNA methylation across generations which in the absence of DNA sequence differences suggests that epigenetic changes may be responsible for the delayed onset and progressive severity of the morphological defects.[54]

Discordance of monozygotic twins for IBD

Although IBD twin studies detected a significantly higher concordance rate among monozygotic twins for both ulcerative colitis and Crohn's disease compared with dizygotic twins,[5 53] concordance of monozygotic twins for IBD was relatively low. In a combined Swedish and British sample, the two largest IBD twin datasets, concordance of monozygotic twins reached only 13% for ulcerative colitis and 30% for Crohn's disease.[5 55] Environmental factors are thought to cause phenotypic discordance of monozygotic twins as these twins are considered to be genetically identical. A large variety of hazardous "external" and "internal" environmental factors such as occupational, dietary, infectious, psychological, and others[56 57] may play a role in the aetiology of IBD but it is almost impossible to trace such factors for each specific twin affected with IBD. In contrast, the genetic identity of monozygotic twins has been challenged on several occasions,[58 59] but identification of disease related genetic differences has not been very productive thus far.

In this situation, epigenetic developments may be of interest. To some extent the epigenetic status of a gene represents an "interface" between the DNA sequence and the intra- and extracellular environment. DNA modification and chromatin conformation is subject to change due to the influence of cellular-environment factors, and therefore epigenetic status represents a combination of both inherited and acquired epigenetic factors. Monozygotic twins, although carrying identical (or very similar) DNA sequences, may be very different from an epigenetic point of view.[60] Under the influence of genetic, environmental, and/or stochastic factors, epigenetic signals may exhibit only partial stability when transmitted from parent to daughter cells. As mentioned above, epigenetic patterns undergo changes during development and aging. Inborn and acquired epigenetic defects, or a combination of the two, may progress after birth and reach the level of epimutation (that is, clinical IBD) in only one twin, while the other twin may remain below such a "threshold" and therefore remain unaffected or clinically asymptomatic. This epigenetic feature provides a new perspective on a number of non-Mendelian features of complex diseases including the discordance of monozygotic twins.

Epigenetics and genetic association studies in IBD

Numerous studies have been performed investigating putative genetic associations of IBD with various candidate genes, such as genes for cytokines and HLA haplotypes. Some of the tested polymorphisms are located within the regulatory regions of candidate genes, for example the gene for tumour necrosis factor α, *TNF-α*, contains a polymorphic G(−308)A site in the promoter region. The frequency of the uncommon *TNF-α* allele 2 (−308)A was decreased in patients affected with ulcerative colitis compared with controls (0.15 *v* 0.25 in ulcerative colitis and controls, respectively; p=0.044).[61] Carriage of the same allele 2, however, was increased in a subgroup of patients with ulcerative colitis who were also positive for antineutrophil cytoplasmic antibodies.[62] In another study, *TNF-α* haplotypes that included the G(−308)A polymorphism, detected that haplotype *TNF-α*-C was associated with progression of the extent of ulcerative colitis (p=0.003).[63] In Crohn's disease, the frequency of *TNF-α* allele 2 was modestly reduced compared with healthy subjects (13.2% and 21.3% in patients and controls, respectively; p=0.04)[64] although no association was found for fistulising Crohn's disease.[65] Although the above positive results have to be replicated before firm conclusions can be drawn, evidence for association with a polymorphism at the *TNF-α* promoter region suggests that regulation of *TNF-α* expression but not the protein coding sequence may be impaired in IBD. This assumption immediately raises a question about epigenetic regulation of *TNF-α*. Epigenetic factors may act synergistically with the DNA sequence in the regulation of gene expression as the polymorphic G(−308)A site is located within a transcription factor AP2 binding site, and AP2 is sensitive to DNA methylation.[66 67] In addition, epigenetic regulation may be of primary importance, as a functional role of the polymorphic nucleotide on the transcription of *TNF-α* is not clear yet (see Wilson and colleagues[68] and Bouma and colleagues[69] *v* Brinkman and colleagues[70]).

The same rationale for epigenetic studies is applicable to other candidate genes. The C(−511)T polymorphic site in the promoter region of the gene for interleukin 1B (*IL*1B) was investigated in IBD. In some[71 72] although not all[73 74] studies, an association between *IL*1B C(−511)T polymorphism and IBD was detected. In another association study, several polymorphisms at the kinin B1 receptor gene were investigated, and the only polymorphism that demonstrated association with IBD was a G/C polymorphism located in the promoter region.[75]

Several other experimental findings support the hypothesis of dysregulation of cytokine genes. Gene expression studies detected significantly increased mRNA levels of several cytokines, including *IL*1B and *TNF-α* in both Crohn's disease[76] and ulcerative colitis[77] compared with controls. Another study demonstrated that in addition to local dysregulation of cytokine genes, the increase in cytokine production may also be due to increased concentration of transcription factors. In both IBDs, but particularly Crohn's disease, increased activation of NFκB, a factor that controls transcription of various genes including the cytokine genes, was detected, and it has been suggested that this may be involved in the regulation of the inflammatory response.[78] Epigenetic factors may be important in this case also as NFκB is sensitive to DNA methylation—that is, attachment of this factor to DNA is inhibited if the NFκB binding site is methylated.[79]

HLA association studies in IBD detected that HLA alleles and haplotypes are differentially distributed depending on the sex of the proband. For example, DRB1*15 was increased only in females affected with ulcerative colitis (53% *v* 24%; p<0.0001; odds ratio (OR) 3.5) but not in affected males.[80 81] In another study, the frequency of DRB1*0301 DQB*0201 (DR3DQ2) was reduced in females affected with ulcerative colitis (9.8% *v* 26.3% in controls; p=0.037), particularly in those with distal disease (2.3%; p=0.001 *v* controls).[82] The molecular mechanisms

of sex effects on the differential risk of HLA to IBD are likely to be mediated by androgens and oestrogens. The importance of epigenetics derives from the fact that differential effects of hormones, including sex hormones, have a significant impact on gene expression, and this is achieved by changing chromatin conformation[83 84] and/or the local pattern of gene methylation.[85 86]

Epigenetics and IBD treatment

Additional evidence for the importance of epigenetic factors in IBD derives from the mechanisms of action of some medications used for the treatment of IBD. There is increasing evidence that glucocorticoids change the chromatin structure of genes that code mediators of inflammation. Glucocorticoids can increase transcription of anti-inflammatory genes which results from the uncoiling of DNA wound around histone and this is achieved by acetylation of the histone residues.[87] Glucocorticoids may also lead to deacetylation of histones of genes of proinflammatory cytokines, resulting in tighter coiling of DNA and reduced access of transcription factors to their binding sites, thereby suppressing gene expression.[87]

In a similar way, the therapeutic effect of butyrate and other short chain fatty acids (SCFAs), bacterial products in the gut lumen which play a major role in maintenance of colonic integrity,[88] may be mediated by epigenetic modification of some IBD related genes. Butyrate inhibits strong histone deacetylase[89] and selectively affects gene expression.[90] A series of clinical trials showed that butyrate and other SCFAs provide an effective primary and/or adjunctive treatment in patients with mild to moderate distal ulcerative colitis (reviewed by Kim[91]).

Methotrexate, a common drug used to treat IBD, inhibits dihydrofolate reductase and therefore reduces folic acid which is an important mediator of biological methylation, including DNA methylation.[92] According to this mechanism, methotrexate should generally reduce DNA methylation although epigenetic changes in various cells and genes may not be straightforward. DNA from peripheral blood mononuclear cells of arthritis patients treated with methotrexate exhibited a higher degree of methylation in comparison with untreated patients.[93] The epigenetic status of cytokine genes before and after methotrexate treatment has not yet been investigated.

Evidence for any epigenetic changes at the candidate genes for IBD may lead to new DNA modification based therapies. Recently, a compound protein consisting of DNA methyltransferase and zinc finger protein was constructed.[94] The mechanism of action of this protein consists of recognition of a specific DNA sequence by a zinc finger protein specific for that DNA sequence, and subsequent epigenetic modification (methylation or demethylation) of the surrounding cytosines by a DNA methylation or DNA demethylation enzyme. This design may exhibit a number of advantages in comparison with the more traditional therapeutic approaches. Such a compound protein should exhibit high specificity and no other genes would be affected, and therefore this approach would have fewer side effects in comparison with non-specific treatment with glucocorticoids and methotrexate. In addition, such an approach would be less toxic as the compound protein is generated by proteins that are present in each mammalian cell. Epigenetic therapies can also be applied to prevention of IBD associated neoplastic formations. Hypermethylation of the promoter region of *p16INK4a*, a tumour suppressor gene, is a frequent and early event in the malignant transformation of cells in ulcerative colitis,[95] and therapeutic demethylation of this gene may potentially reverse the pathological process.

Final notes

Epigenetic studies carry significant heuristic potential and may provide new insights into our understanding of the aetiopathogenesis of IBD. Numerous clinical and molecular findings summarised in this article strongly argue that epimutations are operating in Crohn's disease and ulcerative colitis. Various epigenetic experiments that are new to the field of IBD research can be applied to detection of IBD epimutations. Discovery of epigenetic changes in Crohn's disease and ulcerative colitis may lead to the development of new therapies in IBD.

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