Inflammation, DNA methylation and colitis-associated cancer

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Inflammation can result from a range of sources including microbial infections, exposure to allergens and toxic chemicals, autoimmune disease and obesity. A well-balanced immune response can be anti-tumorigenic; however, a sustained or chronic inflammatory response is generally harmful as the immune response becomes distorted. A causal link between chronic inflammation and cancer is now well accepted and many chronically inflamed organs of the gastrointestinal tract show this association. For example, patients with inflammatory bowel disease (IBD), including both ulcerative colitis and Crohn’s disease, have a 2- to 3-fold greater lifetime risk of developing colorectal cancer compared with the general population. The development of colitis-associated cancer (CAC) is thought to be multifaceted and is probably due to a combination of genetic factors, epigenetic factors and the duration, extent and severity of disease. Recently, epigenetic alterations, in particular alterations in DNA methylation, have been observed during inflammation and inflammation-associated carcinogenesis. The mediators of this, the significance of these changes in DNA methylation and the effect this has on gene expression and the malignant transformation of the epithelial cells during IBD and CAC are discussed in this review. The recent advances in technologies to study genome-wide DNA methylation and the therapeutic potential of understanding these molecular mechanisms are also highlighted.

Introduction

Inflammation is the body’s response to acute tissue damage and is characterized by leukocyte infiltration to the affected area as a result of microbial infections, exposure to allergens and toxic chemicals, autoimmune disease and obesity. An acute inflammatory response is normally beneficial and a well-balanced immune response can be anti-tumorigenic. However, a chronic inflammatory response, which is characterized by persistently activated immune cells, DNA damage and tissue destruction, is harmful as the immune response becomes distorted. In fact, chronic inflammation is strongly associated with approximately one-fifth of all human cancers (1–3). Consistent with this, treatment with non-steroidal anti-inflammatory agents decreases the incidence of and the mortality that results from several tumor types. Therefore, a causal link between chronic inflammation and cancer is now well accepted (4,5). This link is evident in a number of organs and tumors of the gastrointestinal tract provide an excellent model to study this (6) as many chronically inflamed organs of the gastrointestinal tract show this association. For example, inflammatory bowel disease (IBD), Helicobacter pylori infection, Barretts esophagus, chronic gastritis and chronic pancreatitis confer a predisposition to malignant transformation of benign epithelia (7–10). This review will examine the link between inflammation and cancer, and the molecular mechanisms promoting this, with a particular focus on colitis-associated cancer (CAC). Recent advances on the potential role(s) of epigenetics, in particular DNA methylation, in CAC will also be discussed.

Colitis-associated cancer

IBD, including both ulcerative colitis (UC) and Crohn’s disease, is characterized by chronic uncontrolled inflammation of the intestinal mucosa due to an overactivated innate and adaptive immune response, which results in disruption of the intestinal epithelium (11,12). The peak age of onset of IBD is in the second and third decades of life and the incidence and prevalence of IBD are increasing with time. Furthermore, patients with IBD have a 2- to 3-fold greater lifetime risk of developing colorectal cancer (CRC) and the most important risk factors for CAC are duration, severity and extent of IBD (7,13,14). Combined these factors are problematic in the long-term management of patients with chronic IBD and so an understanding of the mechanisms involved in the promotion of CAC during IBD is critical. In addition, as there is a growing body of evidence that inflammation is the root cause of many cancers, CAC serves as an excellent model to study the general principles underlying inflammation-associated cancer (15).

The availability of biopsies from IBD patients attending endoscopy clinics for monitoring of disease and the large number of animal models available also make this disease amenable to study (16,17).

The development of CAC is thought to be multifaceted and due to a combination of genetic and epigenetic factors, host and microbial influences (18–25). Research indicates the underlying molecular mechanisms responsible for these changes include increased cytokines, chemokines, growth factors, matrix-degrading enzymes and reactive oxygen and nitrogen species, which can induce genomic instability. In addition, inflammation is thought to play a role at all stages of tumor development (20,26–31) (Figure 1). To understand the mechanisms involved in the development of CAC, investigators examined tumors from IBD patients for evidence of the molecular characteristics that are important in the pathophysiology of sporadic CRC. Sporadic CRC develops from an adenomatous polyp and is caused by the progressive accumulation of genetic and epigenetic events that lead to the malignant transformation of normal colonic epithelium. Three main molecular pathways are involved in this process: chromosomal instability, microsatellite instability and the CpG island methylator phenotype (CIMP). The combination of genetic and epigenetic changes as a result of these processes results in alterations in the expression of tumor suppressor genes (TSGs) and oncogenes leading to the progression from a benign adenoma to malignant adenocarcinoma and finally distant metastasis (20,32). Malignancy in patients with IBD develops through a dysplasia–carcinoma sequence and the cancer originates in field precursor cells localized in or close to the dysplastic mucosa. The three main molecular pathways involved in sporadic CRC (chromosomal instability, microsatellite instability and CIMP) are also thought to play a role in CAC but they differ in their timing and frequency. Epigenetics, in particular DNA methylation, is also assuming increasing attention as a potential molecular mechanism contributing to CAC (19,20,33–35). Therefore, the molecular mechanisms involved in sporadic CRC and CAC appear similar but the underlying inflammation in IBD alters the timing and frequency of malignancy.

The role of inflammation in tumorigenesis

During inflammation, the fate of the cell is dependent on the balance between pro- and anti-tumorigenic immune responses and it is now believed that inflammation affects the three stages of cancer—tumor initiation, tumor promotion and tumor progression. Tumor initiation is the process by which a normal cell becomes pre-malignant. The
inflammatory environment, which consists of an increase in cytokines, chemokines and reactive oxygen and nitrogen species, results in DNA mutations, epigenetic changes and genomic instability that can contribute to tumor initiation (1,36,37). Tumor promotion involves the proliferation of genetically altered cells and chronic inflammation promotes this by inhibiting apoptosis and acceleration of proliferation and angiogenesis (1,31,36,38–41). Finally, tumor progression and metastasis, which involves an increase in tumor size, additional genetic changes and the spreading of the tumor from its primary site to multiple sites are also influenced by inflammation. In this case, the close interaction of cancer cells, immune cells and stromal elements and the factors produced by each of these cell types can act to promote metastasis (36,42–44). Therefore, it is evident that there is a close link between inflammation and cancer at all stages of tumorigenesis.

Mediators of CAC

Much of the current information known on the mediators of CAC has been discovered through the use of animal models. However, there is no ‘perfect’ animal model because colitis-associated disease is complex in humans and relapses are spontaneous. Therefore, it is important to choose the most appropriate animal model for the question being asked. For example, some models better represent UC such as the azoxymethane/dextran sulfate sodium model, whereas other models better represent Crohn’s disease such as that induced by peptidoglycan–polysaccharides or interleukin (IL)-10 deficiency. Therefore, using the most appropriate animal model can help in understanding the disease process at the molecular level and ideally, these data should be confirmed in human IBD samples to allow translation to man (17,45,46). The use of animal models combined with information from humans has so far demonstrated that the main theme underlying CAC promotion is a complicated immune network and recent studies in genetically modified mice have helped to dissect and characterize the main players involved in these processes. Cytokines, gastrointestinal tract microbiota, chemokines and cell surface receptors are all critical in this process.

Cytokines can generally be considered to exert pro-inflammatory or anti-inflammatory effects. The balance between the levels of these cytokines plays an important role in inflammation-associated carcinogenesis (17,47). The pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) is a well-established player in chronic inflammation and during IBD, the levels of TNF-α are elevated in the blood and colonic mucosa (27). TNF-α is also important in cancer where it acts as a tumor initiator by stimulating the production of molecules that lead to DNA damage and mutations, such as reactive oxygen and nitrogen species and as a tumor promoter by altering cell proliferation and cell death (26,48). Consistent with its critical role in chronic inflammation, humanized monoclonal antibodies against TNF-α, such as infliximab, have proven to be effective in the treatment of IBD (49). Furthermore, in a mouse model of colitis, infliximab reduced the development of tumors (39). Therefore, a critical role for TNF-α in chronic inflammation and CAC has been established. IL-6 is a pro-inflammatory cytokine that plays an important role in a wide variety of inflammation-associated diseases and IL-6 levels are increased during IBD, CRC and CAC. IL-6 is important in CAC development by promoting the survival of the neoplastic colon epithelial cells (10,30,31,50,51). Using a mouse model of colitis, it was suggested that inhibition of IL-6 signaling could be a useful therapeutic system for the treatment of CAC (52). IL-6 also plays a role in promoting growth and tumorigenesis of cancer cells through altering the epigenome. For example, in colon cancer cells, IL-6 promotes colon tumorigenesis through DNA methyltransferase 1 (DNMT1)-mediated TSG silencing (53). Therefore, IL-6 appears to play a critical role in inflammation-associated carcinogenesis and the molecular mechanisms responsible for this are still being elucidated. The anti-inflammatory cytokine IL-10 is also an important regulator of the immune response and IL-10−/− mice develop colitis and CRC (54). The anti-inflammatory action of IL-10 is thought to be due to the ability of IL-10 to block nuclear factor-kappaB (NF-κB) activity and regulate the Jak-Stat signaling pathway (55,56). In addition, IL-10 can downregulate TNF-α, vascular endothelial growth factor and IL-6 production, and this may also account for its inhibitory effect on the tumor stroma (28). Therefore, both pro- and anti-inflammatory cytokines are critical to both inflammation and inflammation-associated carcinogenesis and it is the incorrect balance of both these groups of cytokines that results in a dysregulated immune response. This dysregulation is highly associated with cancer.

Microbiota are also critical to intestinal health and disease, and it is thought that commensal bacteria play an important role in the progression of IBD. This has been highlighted by the fact that humans with IBD respond to antibiotic treatment and in animal models, pre-treatment with antibiotics alleviated subsequent intestinal
inflammation. Furthermore, commensal bacteria are required to induce colitis in mice. Therefore, it is evident that a complex interaction of microorganisms, both symbiotic and pathogenic, with the host immune system is required in the intestine (57–59). Toll-like receptors (TLRs), which act to detect microbial infection, play a critical role in this process. This has been highlighted in MyD88−/− (an adapter molecule essential for TLR-mediated induction of inflammatory cytokines) mice administered dextran sulfate sodium where severe mortality, ulceration and epithelial injury were observed when compared with control mice. However, it is not yet completely understood how TLRs can distinguish between symbiotic and pathogenic bacteria (60,61). The NF-κB pathway can also be triggered by microbial infections and it is a major signaling pathway downstream of TLRs, which may act to provide survival signals for the epithelial cells and to control the interaction between the mucosal immune system and the microbiota (62–64).

Inflammatory mediators activate oncogenic transcription factors such as NF-κB and STAT3, both of which play critical roles in linking inflammation and carcinogenesis. The transcription factor NF-κB is triggered in response to infectious agents and pro-inflammatory cytokines, resulting in the altered expression of many genes, which ultimately provides an environment that can promote tumorigenesis if an immune response is sustained (65). Consistent with its role in inflammation, activated NF-κB is detected in macrophages and epithelial cells of patients with IBD (66), and activated NF-κB is also found in CRC (67). A study using a mouse model of CAC with a conditional disruption of the IKKβ gene found that NF-κB activation in intestinal epithelial cells was essential for the development of colonic tumors, confirming the critical role that NF-κB plays in linking inflammation and cancer (63). The transcription factor STAT3 is induced by many cytokines, including IL-6, and by growth factors, such as epidermal growth factor (68,69). Active STAT3 is increased during inflammation and is constitutively activated in many cancers. Consistent with this, in a mouse model of colitis, IL-6 activated STAT3 to promote tumorigenesis. The critical role of STAT3 in this process was demonstrated by inhibiting STAT3 in the intestinal epithelial cells, which resulted in inhibition of CAC induction and growth (31,65). SOCS3, an inhibitor of STAT3 that is itself regulated by DNA methylation, provides an additional layer of regulation to this transcription factor. If the DNA methylation pattern of SOCS3 is altered, persistent activation of STAT3 that is itself regulated by DNA methylation, provides an additional layer of regulation to this transcription factor. If the DNA methylation pattern of SOCS3 is altered, persistent activation of STAT3 that is itself regulated by DNA methylation, provides an additional layer of regulation to this transcription factor. If the DNA methylation pattern of SOCS3 is altered, persistent activation of STAT3 that is itself regulated by DNA methylation, provides an additional layer of regulation to this transcription factor. Active STAT3 is increased during inflammation, cytokines, including IL-6, and by growth factors, such as epidermal growth factor (68,69). Active STAT3 is increased during inflammation and is constitutively activated in many cancers.

Chemokines and growth factors are also critical in inflammation and inflammation-associated carcinogenesis. The specific role(s) that these factors play have been discussed elsewhere and will not be expanded upon here as it is not the focus of this review. However, it is important to note that the combined activity of all these mediators is responsible for disrupting the previously balanced immune response and ultimately causing the immune response to become pro-tumorigenic. It is also important to mention that although chronic local inflammation can lead to cell transformation, tumor cells also increase the inflammatory response and so a positive loop between inflammation and cancer is created (29,36,71).

Molecular mechanisms involved in the malignant transformation of epithelial cells

It is now evident that chronic inflammation during IBD and the factors produced during inflammation promote CAC. However, it is still unclear if certain individuals are predisposed to developing IBD, which subsequently places these individuals at a higher risk of developing CRC. Genetic factors do play a role in susceptibility to IBD and nucleotide-binding oligomerization domain 2 (NOD2) was the first gene identified to be strongly associated with IBD (72,73). Further research has identified ~100 other genetic loci that are significantly associated with IBD (24,25). However, the significance of these loci is still questionable because the risk of IBD conferred by them individually is small (11). Therefore, it appears that IBD is heterogeneous and genetically complex and so cannot be explained by a single genetic model. This has prompted research into potential environmental factors, epigenetic factors and transcriptome analysis in order to gain a better understanding of the molecular mechanisms involved in IBD and CAC (25,37,74,75).

Epigenetics, which includes histone modifications and DNA methylation, is defined as a process that alters gene expression without changing the DNA sequence, effects that can be transmitted to daughter cells. Much cross talk also exists between histone modifications and DNA methylation and those epigenetic factors have been found to be highly dysregulated in diseases, particularly in cancer (76,77). Epigenetic alterations are also observed during inflammation and inflammation-associated carcinogenesis (37,75,78).

The molecular events that underlie epigenetic regulation take place at nucleosomes, which are the basic structural subunits of chromatin, consisting of DNA wrapped around histone proteins in a compacted form. The amino terminal tails of the histone proteins protrude from the nucleosome body and are subject to post-translational modifications including acetylation, methylation, phosphorylation, ubiquitination and sumoylation. These modifications act to regulate gene expression in addition to the role chromatin plays in compacting DNA (79). The importance of histone modifications in regulating gene expression during disease can be observed in mouse models of colitis in which the use of histone deacetylase inhibitors provides protective effects (80). Furthermore, histone deacetylase inhibitors suppress cancer cell proliferation and clinical trials on their use are currently underway in patients with leukemia (81,82).

DNA methylation involves the addition of a methyl group to the fifth carbon position in the pyrimidine ring of cytosine located in the context of CpG dinucleotides. DNA methylation is one of the most studied epigenetic processes and is maintained through replication by the enzyme DNMT1, whereas de novo DNA methylation is believed to be prominently mediated by DNMT3A and DNMT3B. However, the roles of DNMT1, DNMT3A and DNMT3B do overlap and are not totally exclusive (83–85). DNA methylation plays a vital role in embryonic development, maintenance of pluripotency, X chromosome inactivation and genomic imprinting through the regulation of transcription, chromatin structure and chromosome stability (86,87). DNA methylation regulates gene expression by displacing transcription factors that normally bind DNA and also by recruiting histone modifications associated with gene silencing and chromatin compaction. This type of regulation is particularly evident at CpG islands, which are CpG-rich areas of the genome that are usually found in regulatory regions of the genome, mainly promoters and 5’ coding regions. CpG islands are usually unmethylated in normal cells and this allows for gene transcription. However, in diseases such as cancer, these CpG islands become hypermethylated, which has important consequences on tumorigenesis. For example, when TSGs become hypermethylated in their CpG islands, this results in gene silencing of the TSG and tumor promotion. Other changes in DNA methylation also occur during cancer including hypomethylation of CGs outside of the CpG islands, which is associated with genomic instability (86–89) (Figure 2). In addition, the levels of DNMT enzymes are also increased in some cancers, including CAC (53,90,91).

Chronic inflammation and DNA methylation

In addition to alterations in cancer, aberrant DNA methylation patterns have also been observed in many chronic inflammatory conditions including chronic biliary tract inflammation, Barrett’s esophagus, H. pylori infection and IBD. In some of these cases, the methylation status of particular genes correlated with inflammatory status, dysplasia and malignant transformation, which suggests that epigenetic changes are involved in inflammation-induced carcinogenesis (37,92–96). However, results on DNA methylation status and its importance in IBD and CAC are conflicting. On the one hand, a number of genes are hypermethylated in colitis-associated disease (Table I) and multiple studies have found aberrant promoter methylation for p14ARF, p16INKA and estrogen receptor genes in human patients with UC. These genes are
also commonly hypermethylated in sporadic CRC (18,22,23,97). In various animal models of colitis, inflammation also resulted in aberrant DNA methylation patterns. One study found that this aberrant DNA methylation pattern was directed by the polycomb complex and was also present later in the malignant tissue. In another animal model, the aberrant DNA methylation pattern had a protective effect during inflammation of the mucosal epithelium, whereas in yet another animal model, the DNA methylation pattern was found to increase from the time of inflammation to tumor development, and T and B cells were dispensable in this (37,102,103). In a more recent study in which oxidative stress was induced in a human cell line, DNMT1 was recruited to the damaged chromatin and formed a repressive complex with other proteins and relocalized to GC-rich areas. Similar results were found in an animal model of colitis in the same study (104). In a human colon cancer cell line, the pro-inflammatory cytokine IL-6 stabilized DNMT1 and silenced a subset of TSGs by promoter methylation, which led to a more neoplastic cell phenotype. Consistent with this, an increase in DNMT1 expression was higher in human CAC samples when compared with sporadic CRC samples. Interestingly in this case, nuclear DNMT1 was detected both in the tumor and the peritumoral stroma. This suggests that altered DNA methylation in peritumoral stromal cells might affect the malignant transformation of the epithelial cells (53) (Figure 3). Consistent with this, a role for macrophages in inflammation-associated cancer has been reported and DNA methylation has been identified as a regulator of cell fate and identity in the immune response (38,105,106). Therefore, these data indicate a role for DNA methylation in the development of CAC. A proposed mechanism as to how the immune cells and epithelial cells may interact during inflammation and result in the malignant transformation of epithelial cells is provided (Figure 4).

In contrast to these results, it has also been reported that epigenetic instability is relatively uncommon in CAC. The CIMP, in which a set of genes identified to have altered methylation in their CpG islands, which leads to epigenetic inactivation of these genes, was found less frequently in CAC than sporadic CRC (107–109). It was instead suggested that in UC, accelerated age-related CpG island methylation occurred. However, these studies focused on a subset of loci in CpG islands that are commonly methylated in sporadic CRC samples and much larger studies are required to precisely define the CIMP (93,107). Moreover, the set of genes contributing to the CIMP will likely vary across different types of cancer and diseases and as the progression, timing and molecular mechanisms underlying CAC and sporadic CRC are not identical, it is likely that epigenetic factors are also different. In summary, although results on aberrant DNA

![Fig. 2. Modifications of the cytosine base and the effect(s) of these modifications on gene expression. (A) Structure of cytosine and the modifications that occur by the addition of a methyl group or a hydroxymethyl group. (B) In normal cells, repetitive DNA is methylated (represented in black) and the promoter region of a gene is normally hypomethylated (represented in grey). This methylation pattern allows for the recruitment of RNA polymerase II and associated cofactors to the promoter region to allow for transcription of target genes. (C) In cancer cells, the repetitive DNA becomes hypomethylated resulting in chromosomal instability and the promoter region becomes hypermethylated, which blocks transcription of target genes. The precise differential and overlapping roles of 5mc and 5hmc have yet to be fully defined in these regions. The role of DNA methylation outside of these regions also requires further study.](https://academic.oup.com/carcin/article-abstract/33/4/723/2463444/fig2)

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methylation and its functional consequences during colitis-associated disease are conflicting, at this time significant evidence of a pathogenic role in CAC for DNA methylation has accumulated.

Based on the more recent studies in inflammation-associated carcinogenesis, the key mediators of these inflammation-induced DNA methylation changes appear to be oxidative stress and increased pro-inflammatory cytokines, including IL-6, IL-1β, TNF-α and interferon-γ (37,53,102,104,110,111). The mechanism(s) for how these factors alter the DNA methylation pattern during inflammation is still not completely clear but recent research has begun to elucidate this. For example, DNMT1 protein levels are increased in human CAC, and IL-6, which is increased in expression during CAC, can stabilize DNMT1 protein levels in human colon cancer epithelial cells (53). Interferon-γ was found to increase nuclear 5’ methylcytidine in human intestinal epithelial cell culture and this was explained by an increase in DNMT3B messenger RNA levels (102). IL-1β expression was concordant with methylation induction in an animal model of colitis; this was also observed in H.pylori infection in which IL-1β and TNF-α paralleled the temporal changes of methylation levels (103,111). Finally, in a colon cancer cell line exposed to oxidative stress, DNMT1 and repressive factors were recruited to GC-rich regions of the genome (104). Therefore, factors produced during inflammation can alter the mediators of DNA methylation and so the aberrant DNA methylation patterns are likely a cause of the inflammatory environment itself rather than a result of a response to insult. This is consistent with the fact that in a mouse model of colitis, aberrant DNA methylation was found when no macroscopic tumors were present and this methylation gradually increased until macroscopic tumors developed (103). This phenomenon also appears to be true in other inflammatory conditions, for example in gerbils infected with H.pylori, altered DNA methylation was observed as a result of chronic inflammation and not acute inflammation (111). Therefore, the duration of inflammation is an important factor in aberrant DNA methylation, which is consistent with the duration of IBD being a risk factor for the development of CAC. Further investigations on these factors individually are required to fully comprehend this. In addition, it is not known if an inflammation-induced increase in DNA methylation is targeted to specific genomic

Fig. 3. Greater expression of CD68 and DNMT1 in IBD-associated than in sporadic colon cancers. (A) Hematoxylin and eosin-stained, anti-CD68-stained and anti-DNMT1-stained sections of sporadic and IBD-associated CRCs. (B) Matrix scatterplot showing the abundance scores of the immunohistochemical staining for DNMT1 in tumors and peritumoral areas and for CD68 and age. The lines represent best-fit linear regressions of the three variables for IBD-CRC (black) and sporadic CRC (grey). Taken from Foran et al. (53) with permission from American Association for Cancer Research.

Fig. 4. Proposed molecular mechanism on how the inflammatory environment during IBD results in the malignant transformation of epithelial cells. During inflammation, NF-κB is increased in the immune cells and this results in increased TNF-α and IL-6, which can increase NF-κB and STAT3 in the epithelial cells, resulting in inhibition of apoptosis and increased proliferation of epithelial cells. In addition, the increased IL-6 can also increase DNMT levels in the epithelial cells, which can alter gene expression such as that of TSGs. Overall, this provides an environment conducive to malignant transformation.
regions or if this has functional consequences on gene expression throughout the genome.

For these reasons, a genome-wide approach to study DNA methylation is required to determine the extent of changes in DNA methylation may play in the development of CAC. This requirement has been further highlighted by the fact that global DNA methylation levels measured by a long interspersed element-1 assay were significantly higher in CAC than sporadic CRC (108). Until recently, very little was known about the effect of DNA methylation on gene expression, outside of CpG islands. Recent studies have reported that gene body methylation is positively correlated with gene expression and that CpG island shores, which are regions within 2000 bp of the promoter but outside a CpG island, are involved in tissue-specific differential methylation (112,113). In addition, a more recent report found that altered DNA methylation in the immune system occurred predominantly at CpG islands within gene bodies and an increase in DNA methylation there correlated with silencing of the associated gene (106). It has also been demonstrated that methylation of specific CpG s outside of CpG island promoters may also impact on the transcription of nearby genes (77,96). Therefore, recent advances have altered our perceptions on how DNA methylation regulates gene expression and these data further highlight the requirement to study beyond the promoter region to identify the biological influence of CpG methylation in gene regulation.

Genome-scale methylation analyses are now available by combining microarrays or high-throughput DNA sequencing with immunoprecipitation-based methods, bisulfite-based methods or digestion with methylation sensitive restriction enzyme methods (114–116). All methods have their own advantages and disadvantages in genome coverage, resolution, reproducibility, sensitivity and cost. Therefore, the choice of technology should be based on the biological question, cost, amount and quality of sample (117,118). Currently, methods to study genome-wide DNA methylation are rapidly improving and these advances combined with bioinformatics should allow us to examine the role of DNA methylation throughout the genome at a much faster pace in the future. However, it is important to consider that because DNA methylation varies by cell type, the use of highly purified cell populations will be needed to reveal the most useful information. In the case of CAC, the contribution of altered DNA methylation in the epithelial cells and the stromal cells (53) and the effect this has on the malignant transformation of the epithelial cells with DNA methylation of this interest is clear. In addition, the integration of methylation data with transcriptome and genomic analysis and other epigenetic modifications will also provide additional insights (77,96,119).

Adding to the complexity of DNA methylation and the effect this has on gene expression is the recent discovery of 5-hydroxymethylcytosine (5hmC; Figure 2). Enzymes of the ten-eleven translocation (TET) family can convert 5mc to 5hmC and the unique distribution of 5hmC in mouse embryonic stem cells suggests that these enzymes provide an additional layer of epigenetic control. Pastor et al. (120) identified an enrichment of 5hmC at the transcription start site when compared with 5mc and Stroud et al. (121) found 5hmC enriched in enhancers and gene bodies, thus suggesting a role for 5hmC in gene regulation. High levels of 5hmC are observed in embryonic stem cells, which decrease upon differentiation, indicating that more research is required to fully comprehend the role that 5hmC plays in development, normal tissue homeostasis and disease (120–124). The abundance of 5hmC is reduced in cancerous colorectal tissue and in myeloproliferative neoplasms compared with normal tissue (125,126) but the implications of this change in the disease state has yet to be determined. More recently, Ito et al. (127) demonstrated that Tet proteins can also generate 5-formylcytosine (5fc) and 5-carboxylcytosine (5cac) from 5mc in an enzymatic-dependent manner. Both 5fc and 5cac were detected in gDNA from mouse embryonic stem cells and organs but the functional significance of these additional modified cytosines is as yet unknown (127). Therefore, the complex nature of DNA methylation, its modifications, biological consequence(s) and functional significance are only beginning to be uncovered.

DNA methylation— a friend or foe during inflammation?

Taking all these factors into consideration, it is now important to consider the net biological effects of DNA methylation during chronic inflammation. In animal models of colitis, aberrant DNA methylation was common, but one study found that this provided protective effects during inflammation of the mucosal epithelium. In contrast, other studies found an increase in DNA methylation during inflammation and this continued to increase as tumors developed. In addition, the altered methylation pattern was also present in the malignant tissue, suggesting that aberrant DNA methylation is causally associated with CAC (37,102,103). It is possible that aberrant DNA methylation may initially prove to be protective to the epithelium, but prolonged exposure to the mediators of inflammation could promote further aberrant DNA methylation patterns that are conducive to tumor development. If this is the case, the methylation status of particular genes could potentially be used as biomarkers. To fully comprehend this, epigenome-wide association studies need to be conducted at various time points and in particular tissue types to examine aberrant DNA methylation patterns over time and the resulting effect on gene expression.

Targeting of DNA methylation for anticancer therapies

The use of DNA methylation inhibitors in anticancer therapy is based on the principle that the inhibitor will cause demethylation and re-expression of TSGs. There are currently two main types of DNA methylation inhibitors: nucleoside and non-nucleoside analogs. Nucleoside analogs have a modified cytosine ring and decrease methylation when the inhibitor is integrated into the DNA. 5-azacytidine and 5-aza-2'-deoxycytidine are the two main analogs in use and both are Food and Drug Administration approved for the treatment of myelodysplastic syndrome. Non-nucleoside analogs are small molecular inhibitors, which inhibit DNMT catalytic activity without being incorporated into the DNA and these include procainamide and hydralazine, among others. However, compared with nucleoside analogs, these are relatively weak hypomethylators. Although the overall effect of decreasing methylation in cancer appears to be positive, it is important to consider that DNA methylation inhibitors act non-specifically and so cause DNA hypomethylation genome wide, effects that could increase genomic instability. In addition, the use of DNA methylation inhibitors for anticancer therapies cannot reach their full potential until the precise role(s) that DNA methylation plays in regulating gene expression in each part of the genome, for example in gene bodies, promoters, CpG island shores etc., is fully elucidated (87,128–130). As our understanding of how DNA methylation regulates gene expression and also how it interacts with other epigenetic factors is increased, so too will the potential use of DNA methylation inhibitors as an anticancer therapy.

Future directions

The importance of understanding how chronic inflammation can lead to cancer is highlighted by the fact that approximately one-fifth of all human cancers are associated with chronic inflammation. Research so far has indicated that the progression to cancer in an inflammatory setting involves a complicated immune network; however, much remains to be understood on this and more recent evidence indicates that epigenetics, in particular DNA methylation, may also be involved. The recent advances made in genome-wide DNA methylation analyses will allow investigations into the potential role(s) that DNA methylation plays in inflammation-associated cancers at a much faster pace in the future; in addition, this will also allow for the specific methylation patterns of multiple cell types to be elucidated. Furthermore, the availability and reduced cost of massively parallel sequencing will allow for transcriptome and genomic analyses to be done in parallel. Combined these will ultimately increase the significance of the data obtained in these assays and so provide a greater potential to exploit this knowledge for use as biomarkers or in anticancer therapies. The
recent discovery of the cytokine derivatives 5hmC, 5fc and 5cac and the role that they may play in regulating gene expression also needs to be characterized. Epigenetic alterations are reversible and so by increasing our understanding of these processes in development, normal tissue homeostasis and disease, we can exploit this knowledge to stop or potentially reverse the disease process through targeted therapeutic approaches in the future.

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References
16. Feagins,L.A. et al (2002) Review article: how relevant to human inflammatory bowel disease is the recent discovery of the cytosine derivatives 5hmC, 5fc and 5cac and the role that they may play in regulating gene expression also needs to be characterized. Epigenetic alterations are reversible and so by increasing our understanding of these processes in development, normal tissue homeostasis and disease, we can exploit this knowledge to stop or potentially reverse the disease process through targeted therapeutic approaches in the future.

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