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COLORECTAL CANCER – A MODEL FOR EPIGENETIC TUMORIGENESIS

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5-AZA, 5- azacytidine; CIMP, CpG island methylator phenotype; DMR, differential methylated region; DNMT, d*e novo* methyltransferase; H3-K9, lysine 9 residue of histone H3; HAT, histone acetyl transferase; HDAC, histone deacetylase; HNPCC, hereditary non-polyposis colorectal cancer; LOI, loss of imprinting; lys, lysine; MBD, methyl-CpG binding protein; MSI, microsatellite instability.

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ABSTRACT

Recent advances in basic and clinical science have driven epigenetics to the forefront of cancer research. Together with genetic changes, the disruption of epigenetic mechanisms is now established as a hallmark of human cancer. Colorectal cancer, long a classical model for the genetic basis of cancer, is now providing researchers with the opportunity to view epigenetic events in the context of human neoplasia. Knowledge of the heritable changes in gene expression that result from epigenetic events is of increasing relevance to clinical practice, particularly in terms of diagnostic and prognostic molecular markers, as well as novel therapeutic targets.

INTRODUCTION

Colorectal cancer, for many years a prototypic model for the genetic basis of cancer, is now increasingly cited as an exemplar of the role of epigenetic alterations in tumorigenesis. In part, this is because colorectal neoplasia provides a wide range of accessible lesions, from aberrant crypt foci to carcinoma. But colorectal neoplasia serves also as a poster child for epigenetic change because of the likely role that DNA methylation plays in the initiation and progression of this disease. For both of these reasons, it also provides an excellent opportunity to understand how epigenetics and genetics collude to produce malignancy.

This review will provide a broad overview of common epigenetic processes as they occur in the normal cell as well as in the cancer cell, and will highlight recent findings in the epigenetics of colorectal neoplasia. It will briefly discuss the clinical implications of epigenetic changes, in terms of both the identification of disease predisposition, and the therapeutic opportunities that a better understanding of these changes may provide. The term epigenetics, while variously defined (1), will be used in this review to describe those heritable changes in gene function that do not entail a change in DNA sequence (2). By way of context, the key historical milestones in the field of cancer epigenetics are shown in Table 1.

Table 1. Milestones in cancer genetics and epigenetics, in relation to the clinical management of colorectal cancer.

Decade	Genetics (3)	Epigenetics (4)	Clinical
1940	Proposed existence of cancer stem cells	CH Waddington coins the terms epigenetics and epigenome	Dukes' staging 1932
1950	Two hit hypothesis		No touch technique for colon surgery
1960	Chromosomal translocations	Description of X chromosome inactivation	Flexible sigmoidoscopy and colonoscopy
1970	First human oncogene Tumour suppressor genes	5-methylcytosine as mechanism of gene control in mammals	Therapeutic polypectomy
1980	Oncogene cooperation	Global hypomethylation of cancer cells Hypermethylation of *RB* Chromatin modification linked to DNA methylation	Total mesorectal excision for rectal cancer
1990	Genetic basis for cancer predisposition	Invention of bisulphite technique First imprinted genes identified DNA methylation involved in genomic imprinting Loss of imprinting in cancer	Adjuvant chemotherapy introduced
2000	Cancer gene expression profiling	Human drug trials target the epigenetic modifications in DNA	Biological and targeted therapies

EPIGENETIC EVENTS IN NORMAL HUMAN CELLS

While the nucleotide sequence of the human genome has long been recognised as the blueprint from which all macromolecular structures are derived, it has also been apparent that there are other factors within the material of the cell nucleus that can also determine gene expression, and hence the structure and function of the cells and tissues that they form. Because these factors are heritable, in that they can be passed from cell to cell, they have been referred to as the "epigenetic code". Ingeniously, this code marks the DNA sequence in ways which do not involve modification of the DNA sequence itself. The repertoire of epigenetic marks includes modifications to histone proteins, methylation of DNA and the phenomenon of RNA interference as described in plants (5) and fungi (6), and possibly in mammalian cells (7) (Figure 1). While the genetic code provides the blueprint for all cellular elements, the epigenetic code controls elaboration of that blueprint, including the particular suites of "luxury" proteins that set apart one differentiated cell from the next (8). In effect, this means that individuals have a single genome but many "epigenomes".

Histone modifications and the histone code

Much of the epigenetic code is carried through chemical modifications of individual amino acids on the tails of proteins called histones. The basic unit of human chromatin, the nucleosome, consists of a 146 base pair loop of DNA wrapped over an octamer of core histones (H2A/H2B dimers and H3/H4 tetramer). Covalent modifications of histone proteins can change densely compacted, inactive heterochromatin to the open and active configuration of euchromatin, and *vice versa* (Figure 2). These modifications, which include acetylation, methylation, phosphorylation, and ubiquitinylation, are reversible events that occur at the N- and C-terminal domains of all core histones. Each of these modifications can be subjected to further variations that can alter function. For instance, methylation of arginine can involve the addition of 1, 2 or 3 methyl groups each conferring subtly different functional consequences. The histone modifications are made possible by a number of families of enzymes, including histone acetyltransferases (HATs), histone deacetylases (HDACs) and histone methyltransferases. The balanced activity of these enzymes and related proteins is pivotal to normal cellular function, and alteration in their function is known to cause diverse and often profound disorders (9).

Generally, the active chromatin structure corresponding to increased transcriptional activity is associated with increased histone acetylation (Figure 2). HATs such as P300 and CBP are known to catalyze acetylation of lysine (lys) residues on H3 and H4 (10). Acting

antagonistically to HATs, HDACs produce transcriptional repression by a complicated mechanism that involves interaction with DNMTs (11, 12) and MBDs (13, 14). Likewise, methylation and phosphorylation of histones are also involved in regulation of the activation state of chromatin (9). Methylation at lys 4 and lys 14 as well as phosphorylation of serine 10 on H3 have all been linked to gene activation, whereas methylation of lys 9 on H3 has been associated with gene silencing (15). Taken together, it is this pattern of histone modifications that is said to constitute the 'histone code', and that complements the primary DNA sequence in defining transcription states (16, 17).

DNA Methylation

Among all mechanisms of epigenetic modification, enzymatic modification of cytosine bases in DNA to form 5-methylcytosine is perhaps the most widely studied and the best understood (Figure 3). In the mammalian genome, methylation of cytosine residues occurs most commonly at the 5'-CG-3' dinucleotides (also termed CpG dinucleotides) and occasionally at 5'-CA-3' or 5'-CT-3' residues(18). The resultant base, 5-methylcytosine, is relatively unstable, and prone to spontaneous deamination to form thymine (Figure 3), and in this way DNA methylation can be seen as an endogenous mutagen. Over 70% of all CpG dinucleotides in the human genome are heavily methylated (19), and the remainder are typically seen in CpG rich regions of 200 bp or more that span the promoters and sometimes the first exons of genes. These regions, known as CpG islands (20), are found in association with about 60% of all human genes. It is thus apparent that the configuration of CpG methylation in the genome produces a recognisable pattern of non-methylated CpG islands scattered on a background of DNA that is methylated at low density (Figure 4). These genomic patterns of CpG methylation are reprogrammed in the early embryo, but maintained with considerable fidelity thereafter, and are of great functional relevance in normal cells. Patterns of methylation cooperate in the regulation of the differential expression of genes, such as the silencing of genes on the inactive X chromosome, and the production of age-related and tissue-specific gene expression (21).

Genomic imprinting is a variant of the process of DNA methylation that allows monoallelic gene expression in a parent-of-origin specific manner. Over 80 imprinted loci have now been described, and they are typically characterised by tissue and stage-specific patterns of expression (22). This is clearly a key epigenetic process, and one which has been extensively reviewed elsewhere (21, 23).

Patterns of genomic methylation are of vital important in both health and disease, and an understanding of the mechanism by which methylation leads to transcriptional silencing is developing rapidly (24). However, there is relatively less known about the factors that determine the positioning and *de novo* development of these epigenetic marks within the genome. There has been considerable interest in the role of transposable elements in inducing methylation events, but there is limited data to support this contention in higher organisms (25). Others have suggested that *de novo* development of DNA methylation is the result of loss of transcription from the gene itself (26).

EPIGENETIC EVENTS AND MECHANISMS IN COLORECTAL CARCINOGENESIS

Given the powerful role of epigenetic changes in altering gene expression, as well as their close relationship to development, it is not surprising that cancer cells show a significant alteration in the configuration of epigenetic marks on their genome (8, 27, 28). Historically, much work has focused on the changes in DNA methylation patterns seen in cancer, both in terms of global hypomethylation and focal hypermethylation at CpG islands. More recently, work has begun to elucidate the changes to chromatin structure seen in this disease. Both of these will be reviewed briefly.

DNA hypomethylation

In the late 1970s, a number of workers showed that the genome of tumour cells showed a progressive and global decrease in the number of cytosine bases that had been methylated to form 5-methylcytosine (28-31). This phenomenon, usually referred to as DNA hypomethylation, is a typical finding in all neoplasms, both benign and malignant (4). In the particular case of colorectal neoplasia, global hypomethylation has been found in lesions across the neoplastic spectrum, from adenomatous polyps to carcinomas (32), as well as in hyperplastic polyps (33).

Hypomethylation has been linked to a number of mechanisms that could drive neoplastic progression. In contrast to normal cells, hypomethylation in tumour cells typically occurs at the repetitive sequences residing in satellite or pericentromeric regions. This pattern of hypomethylation may make chromosomes more susceptible to breakage, and therefore lead directly to genomic instability (34, 35). Hypomethylation can also result in reactivation of previously silenced retrotransposons, leading to the disruption of normal gene structure and function (36, 37). Furthermore, it is possible that the activity of transposable elements governs the methylation state of their neighboring genes through the phenomenon of transcriptional

interference, which has been observed in maize and wheat (38, 39) but not to date in animals. DNA hypomethylation can also lead to the activation of oncogenes; an event that has been documented with the S100A4 metastasis-associated gene in colorectal carcinoma, as well as *cyclin D2* (40) and *Maspin* (41) genes in gastric carcinoma. Finally, decreased methylation of DNA can lead to loss of imprinting, and this can drive cellular proliferation in cancer. The clearest example of this phenomenon is the loss of imprinting at *IGF2*/*H19* region as a result of hypomethylation at the differentially methylated region (DMR) of *IGF2* (42), an event seen in over 40% of colorectal cancer (43).

Hypermethylation

In concert with global hypomethylation, focal hypermethylation at CpG islands is also regarded as a critical event in cancer development (44-46) (Figure 4). Not surprisingly, research in this area has focused on tumor suppressor genes, as promoter silencing by hypermethylation provides a mechanism other than sequence mutation for the inactivation of these key genes. Since the demonstration of methylation-induced silencing of the *RB* gene in cancer (47), many more tumour suppressor genes have been identified as targets for this process, including *p16INK4A, VHL, APC, CDH1 (*E-cadherin*)* and *MLH1* (4). Yet silencing of tumour suppressor genes is not the only mechanism by which hypermethylation can favour the development of cancer. Hypermethylation can also lead to loss of imprinting in cancer. In Wilms' tumour, for example, hypermethylation at the *IGF2* DMR causes loss of imprinting of the normally silenced maternal allele of *IGF2* (48, 49). Similar events are seen with the *p73* gene in haematological malignancies (50), and *ARH1* in follicular carcinoma of the thyroid (51)

CpG island methylation is of course a common epigenetic event in colorectal neoplasia, with *MLH1* promoter methylation representing a classical example of this phenomenon. A long list of hypermethylated genes has been associated with colorectal neoplasia, including tumor suppressor, mismatch repair and cell cycle regulatory genes (Table 2). This list is likely to grow as methods for the discovery of methylation targets are improved. Importantly, and as recently summarised by Baylin and Ohm, these genes have been drawn from many the key functional groupings that define the cancer phenotype, including Wnt signaling (*SRFP* genes), mismatch repair (*MLH1*), cell cycle regulation (*CDKN2A*), epithelial differentiation (*GATA4,5*), *p53* mediated damage responses (*HIC1*) and cell-matrix interactions (*TIMP3*) (52).

Table 2. Some of the genes silenced by promoter methylation in colorectal neoplasia

Gene	Function	Frequency (%)	Reference
APC	Signal transduction, beta-catenin regulation	10-50	(53-58)
CDH13	Cell signalling (cell recognition and adhesion)	30-40	(59)
CDKN2A	Cell cycle regulation	15-30	(56, 60, 61)
CHFR	Mitotic stress checkpoint	30-40	(62, 63)
HIC1	Regulation of DNA damage responses	~80	(64, 65)
HPP1	Transmembrane TGF-beta antagonist	~80	(66)
LKB1	Cell signalling, cell polarity	5-10	(67)
MGMT	Repair of DNA guanosine methyl adduct	30-40	(56, 57, 68-70)
MLH1	Mismatch repair	10-20	(57, 71-73)
$P14^{ARF}$	Cell cycle regulation	20-30	(56, 74, 75)
RASSF1A	DNA repair, cell cycle regulation	>50	(56, 76, 77)
SOCS-1	Cell signaling	5-10	(59)
THBS1	Angiogenesis	10-20	(56, 57)
TIMP3	Matrix remodeling, tissue invasion	10-30	(56, 78)

Interesting recent observations have challenged the dogma that hypermethylation is confined to discrete CpG islands. Frigola *et al*. showed many colorectal cancers exhibited epigenetic silencing of an entire 4 Mb band of chromosome 2. This finding demonstrates that epigenetic silencing can be a regional phenomenon with an impact on the expression of multiple rather than single genes (79).

Dysregulation of histone modification

In comparison to DNA methylation, current knowledge regarding dysregulation of the histone code in cancer is less advanced. At a simplistic level this involves replacement of histones with variants, or changes in the decorations on the histone tails through chemical modifications of individual amino acids. Certainly, aberrant methylation of tumour suppressor genes is accompanied by two key modifications in the histone code, namely deacetylation and methylation of the lysine (K) 9 residue of histone H3 (H3-K9). These two moieties are mutually exclusive, since they affect the same position. Acetylation of H3-K9 correlates with gene expression, whereas methylation of this residue is associated with gene silencing and acts by recruiting heterochromatin-associated proteins (80). Changes of these types are well documented in colorectal cancer(81-83). More recently, a pattern of changes to the core histone H4, characterised by the loss of both monoacetylation from lys 16 and trimethylation

from lys 20, has been proposed as universal markers for malignant transformation (84). Other workers have demonstrated overexpression of a putative histone methyltransferase *SMYD3* that methylates H3 lys 4 in colorectal cancer (85). Since methylation of H3 lys 4 has been associated with gene activation (4), this suggests that the increased activity of *SMYD3* can potentially promote transcription of oncogenes, homeobox genes and cell-cycle regulatory genes. These types of changes in histone modification are characteristic of many human tumours (86). Individual histones may be replaced by histone variants such as H3.3 for the canonical H3 histone (87) or the H2A.Z variant for H2A. The latter histone variant plays a crucial role in embryogenesis (88), and also by depositing at the 5' end of genes can retain the boundaries that prevent the spread of heterochromatin into euchromatic regions (89). It is possible that inappropriate inclusion of histone variants disturb the boundaries between euchromatin and heterochromatin. The disturbances in the epigenetic machinery that induce these changes are the focus of much current research (84, 86), as are the consequences of such changes.

RELATIONSHIP BETWEEN EPIGENETIC EVENTS, GENETIC CHANGE AND PATHOLOGY IN COLORECTAL NEOPLASIA

The epigenetics of microsatellite and chromosomal instability

It is apparent that there is a close interplay between genetic mutations and epigenetic modifications within the neoplastic cell. For example, by silencing one allele of a tumour suppressor gene, methylation can work in concert with sequence mutation of the other allele to fulfill Knudson's two hit hypothesis. Yet while it is possible to consider the epigenetic events seen in colorectal cancer in isolation, it is perhaps more informative to see these changes within the existing framework of established pathways for the development and progression of colorectal neoplasms.

Current paradigms of colorectal cancer progression suggest at least two distinct pathways for progression, the traditional chromosomal instability pathway, and the more recently elucidated microsatellite instability (MSI) pathway (90). These pathways represent divergent patterns, in terms of underlying genetics as well as tumour biology, including precursor lesions and morphology (91) (Figure 5). Epigenetic events are clearly at work in the chromosomal instability pathway, with hypomethylation establishing opportunities for chromosomal instability, and for activation of oncogenes such as c-*myc* (92). However, it is the MSI pathway, characterised by early loss of mismatch repair activity within the tumour

clone, and thus by the accumulation of errors at microsatellite loci, that serves as an exemplar of epigenetic carcinogenesis.

The microsatellite instability pathway was recognised largely because of its occurrence in the cancer predisposition syndrome of hereditary non-polyposis colorectal cancer (HNPCC), and it was only several years later that CpG island methylation was recognised as being critical to the development of the 15% of sporadic cancers that also followed this pathway (93). It is now well established that biallelic methylation of *MHL1* followed by transcriptional inactivation of the gene is seen in nearly all sporadic MSI cancers. Like HNPCC tumours, sporadic MSI colorectal carcinomas have distinctive clinicopathological features, including poor differentiation, intra-epithelial lymphocytic infiltrates and location in the proximal colon (94). Curiously however, they occur predominantly in elderly women, and a recent systematic review has confirmed they have a significantly better outcome than those with microsatellite stable cancers of similar stage and grade (95).

While MLH1 methylation is the hallmark of the MSI pathway for colorectal cancer and epigenetic silencing of other genes is common (Table 2), it is noteworthy that these tumours also show particular types of genetic change. For instance, activating mutations of the *BRAF* gene are very common in sporadic MSI cancers (96-99), even though this gene is rarely if ever mutated in cancers arising in individuals with HNPCC. Likewise an interdependence has been reported between MGMT hypermethylation and TP53 mutations (100).The precise interrelationship between genetics and epigenetics in the MSI pathway, including the chronology of key events, remains to be elucidated.

The CpG island methylator phenotype (CIMP)

In 1999, Toyota, Issa and colleagues identified a set of CpG islands that could be methylated in tumours (MINTs), but that were not methylated in normal epithelial cells (101). They were able to show that many of these loci were heavily methylated in a subset of colorectal cancers, and they coined the acronym CIMP to describe those tumours characterised by multiple, concordant methylation events (101). Subsequent population-based studies of colorectal cancer patients have suggested that CIMP tumours are clinically, pathologically and genetically distinct. They are characterised by many of the features typical of MSI tumours, such as right-sidedness, high grade, mucinous type, and increased frequency in the elderly and in females (102-104). However, over half of the tumours which display widespread CpG island methylation are microsatellite stable (Figure 5). There is also evidence to suggest that they may be unique in terms of behaviour. Our group has reported difference in outcome

between subgroups of CIMP tumours depending on microsatellite status (105), and highlighted the poor prognosis of individuals with CIMP positive, microsatellite stable tumours.

The CIMP concept has not been accepted by all researchers in this field, and over the past few years there has been much debate as to whether CIMP tumours represent a biologically distinct group of colorectal cancers, or are an artificially selected group from a continuum of tumours showing different degrees of methylation at particular loci (106). Underpinning this debate is the important issue of whether the cell(s) that give rise to CIMP tumours have a definable alteration in their machinery of methylation that produces what Issa has referred to as “epigenetic instability” (107), and whether this is integral to tumour initiation and progression. This is an important question, since if it were true, then a better understanding of CIMP tumours would shed more light on the mechanisms that control of CpG island methylation, and potentially on the appropriate management of this type of cancer. An affirmative answer would also support the concept that predisposition to CIMP may in part be hereditary, an observation initially suggested by some (108) but not confirmed in larger studies (104, 109). At present, it is clear that issues regarding the operational definition of CIMP are limiting the attainment of consensus on these important matters (107), and the biological basis of CIMP remains uncertain.

Chronology of genetic and epigenetic events in colorectal cancer

Research over the past decade has shown consistently that epigenetic changes such as promoter hypermethylation (68, 110, 111) and loss of imprinting (43) can occur in histologically normal colonic epithelium, and that these changes are more likely in individuals with CIMP or MSI cancers (112). The early occurrence of these epigenetic events, and their relevance to the emerging field of stem cell biology, serve to highlight their theoretical significance in neoplastic development. Baylin and Ohm have recently advocated the primacy of epigenetic events in colorectal neoplasia(52), arguing that such epigenetic alterations in stem cells may predetermine the nature of subsequent genetic events. Such a concept, if true, would help to explain the distinctive pattern of genetic changes in colorectal carcinogenesis made famous by Vogelstein and Fearon (113). Feinberg has also recently highlighted the early role of epigenetic change in neoplastic progression, suggesting that epigenetic modifications within stem cells and their progeny are responsible for forming a polyclonal cellular milieu from which neoplastic clones can develop (114).

CAUSES OF EPIGENETIC CHANGES IN COLORECTAL NEOPLASIA

Clearly, if epigenetic events are present at the earliest stages of colorectal tumorigenesis, then this holds important implications for both the recognition of cancer predisposition, and possibly for the chemoprevention of this disease. At a minimum, it appears important to understand the factors that may induce epigenetic alterations.

Environmental factors influencing epigenetic changes in colorectal neoplasia

The influence of environmental factors on the epigenetic state of cells (epimutagens) is a rapidly expanding field, and will only be discussed briefly in this review. With regards to dietary factors, folate is perhaps the best-studied link to colorectal neoplasia. As an essential donor of one-carbon units, folate is important in methylation reactions as well as DNA synthesis and repair. Epidemiological and experimental studies have both shown that dietary folate correlates inversely with risk of colorectal neoplasia (115-117), but the effect of folate intake on tumorigenesis remains complex, and may depend in part on the stage of tumor development (118, 119). From an epigenetic viewpoint, increased methylation secondary to dietary folate supplementation may have contradictory effects, from the beneficial restoration of gene hypomethylation to the disadvantageous silencing of genes. The complexity of this situation is compounded by related dietary factors such as alcohol consumption, which may abrogate the protective role of folate (120, 121). Finally, in considering dietary factors, it must be recognised that early maternal nutrition impacts significantly on epigenetic patterning in the fetus, and it has been hypothesized that this in turn can influence adult phenotypes, through the persistence of epigenetic changes at susceptible loci (122).

Advancing age also correlates closely with epigenetic changes in normal colorectal mucosa. In these tissues, methylation of many genes including the *ESR1* (112, 123), *MLH1* (71), *HIC1* and *IGF2* (124) have been shown to increase progressively with age. For at least some of these genes, this process appears to be accelerated in individuals with colorectal cancer (101, 125). These epigenetic changes may reflect the clinical truism that colorectal carcinoma is a disease of the elderly.

Inherited factors in the epigenetics of colorectal cancer

Given that epigenetic changes are stable and potentially heritable through meiosis, it is worth considering some of the ways in which inheritance may influence epigenetic changes associated with colorectal neoplasia. As discussed above, there has been considerable interest, albeit scant supporting evidence, for the proposition that the changes that underpin CpG

island methylator phenotype, be they genetic or epigenetic, may be hereditable. Perhaps a clearer example of inherited epigenetic risk is seen in the case of loss of imprinting at *IGF2*. Certainly, individuals with widespread LOI for this gene have an increased risk of developing colorectal cancer (43), and are also more likely to have a family history of colorectal neoplasia (126). However, it is unclear whether LOI is a germline or somatic event.

Our group has recently found germline epimutations of *MLH1* which predispose to young onset MSI tumours in the large bowel and at extra-colonic sites (127, 128). These germline epimutations manifest as soma-wide uniparental methylation of the *MLH1* promoter in the absence of an intragenic sequence mutation (127), and cause transcriptional silencing of the affected allele (128). These observations indicate that germline epigenetic change can mimic hereditary cancer syndromes, and may be hereditable (127-131). To date, such soma-wide epimutations have not been found in other genes such as APC (131), and further research on the family members of individuals with this abnormality is required to better understand this phenomenon.

CLINICAL IMPORTANCE OF EPIGENETIC CHANGE IN COLORECTAL NEOPLASIA

Given the increasing recognition of epigenetic changes in histologically normal colorectal mucosa, as well as in precursor lesions such as aberrant crypt foci, adenomas and serrated polyps, it is clear that these changes may serve as a marker for individuals at risk of colorectal cancer (112). Epigenetic markers are also increasingly being used in screening tests for colorectal neoplasia (110), yet much work remains before such observations can be meaningfully translated into routine clinical practice.

It is also possible that the epigenetic events in colorectal cancer may soon come to influence treatment decisions. For instance, while still controversial (132), there is growing evidence from retrospective analyses that MSI tumours respond differently to traditional chemotherapeutic agents (133, 134), and indeed that outcomes for some individuals with these cancers may be worse with standard treatments (135). Such observations may reflect fundamental differences in drug responsiveness that are driven not by MSI *per se*, but rather by underlying and as yet unrecognised epigenetic mechanisms (136, 137).

Not surprisingly, a better understanding of the epigenetic events in carcinogenesis, and the recognition that these events are potentially reversible, has brought with it a plethora of potential “epigenetic” therapies. Currently there are two broad classes of epigenetic drugs, designed to inhibit either DNA methylation or histone deacetylation. At least some of these

drugs are in current clinical practice, and many more are in the clinical trials pipeline. Although only transient(138), inhibitors of DNA methylation such as 5- azacytidine (139) inactivate DNA methyltransferases, and can thus revert methylation-induced silencing (140), Inhibitors of histone deacetylase, such as SAHA and newer derivatives (9) have been slower to emerge, and it is likely that combination therapy approaches may also be beneficial, given the interdependence of these epigenetic processes (9). Whether these treatments will have sufficient specificity in practice to provide a useful therapeutic window awaits the outcome of current and future trials. Nevertheless, the experience gained in this process is likely to inform the mechanism of action of these drugs, and indeed the significance of epigenetic events in colorectal carcinogenesis.

CONCLUSIONS

While our knowledge of the molecular genetics of colorectal neoplasia has developed rapidly over the past several decades, it is only in recent years that we have begun to understand the epigenetic events that underpin neoplastic initiation and progression in the large bowel. Currently, colorectal cancer epigenetics is a bourgeoning field, and as was the case with the genetics of cancer, the lessons learned from colorectal neoplasia are serving to throw light on the epigenetics of other common cancers. It is difficult to predict the extent to which knowledge of epigenetics gained over the next decade will transform our understanding of the disease and its precursors, but it is clear that it does have the potential to entirely rework our current paradigms of cancer development, if not management.

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Competing interest statement:

The authors have no competing interests.

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LEGENDS FOR FIGURES

Figure 1: Schematic of the interrelated cellular processes that constitute the epigenetic code. RNA modification includes the roles of RNA interference and microRNA in altering gene expression.

Figure 2: A model of epigenetic modifications and their effect on transcription.

The nucleosome is assembled from DNA and histones, and chemical modification of histone tails induces conformational changes that can cause either activation or repression of transcription. Repressive modifications include H3K9, H3K27 and H4K20 methylation, in association with DNA methylation. Changes such as acetylation at H3K9 (shown) are associated with open chromatin formation (euchromatin).

Figure 3: Methylation of cytosine residues and its consequences.

De novo methyltransferase (DNMT) catalyses the methylation at position 5 of cytosine, using S-adenosylmethionine (SAM) as the methyl donor. Spontaneous deamination of 5-methylcytosine results in its conversion to thymine, an event which is in itself mutagenic, and which has caused progressive depletion of cytosine bases from the eukaryotic genome throughout evolution.

Figure 4: Organisation and consequences of CpG methylation in normal and cancer cells.

The upper panel shows a normal cell, in which a cluster of C-G dinucleotides (CpG island) remains unmethylated (pale pins), while scattered cytosines elsewhere are methylated (red pins). In the absence of methylation of this CpG island, DNA in the promoter region remains accessible to transcription factors, and the gene is expressed. In the lower panel, a cancer cell shows characteristic CpG island methylation, with concomitant compact chromatin structure in the promoter region, causing silencing of gene expression.

Figure 5: Proposed pathways for colorectal tumorigenesis and their relationship to the CpG island methylator phenotype (CIMP).

A working model of the dichotomy between chromosomal instability and microsatellite instability pathways in colorectal carcinogenesis, and the common morphological and genetic changes that accompany each subtype. A subgroup of tumours is shown in the centre of the figure that are characterised by CpG island methylation (CIMP +ve) and microsatellite stability (MSS). It is not clear whether these tumours arise from either or both of the main

pathways, or whether they develop separately. MSI - microsatellite instability; Serrated polyp - hyperplastic polyp or serrated adenoma.

Figure 1

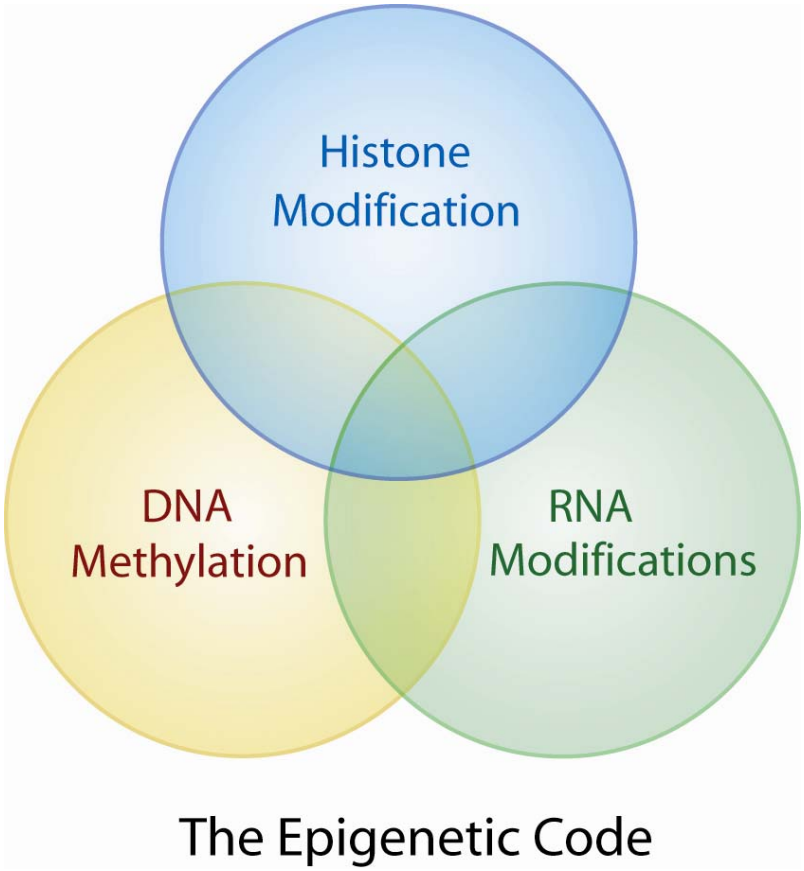
Histone
Modification
DNA
Methylation
RNA
Modifications
The Epigenetic Code

Figure 2

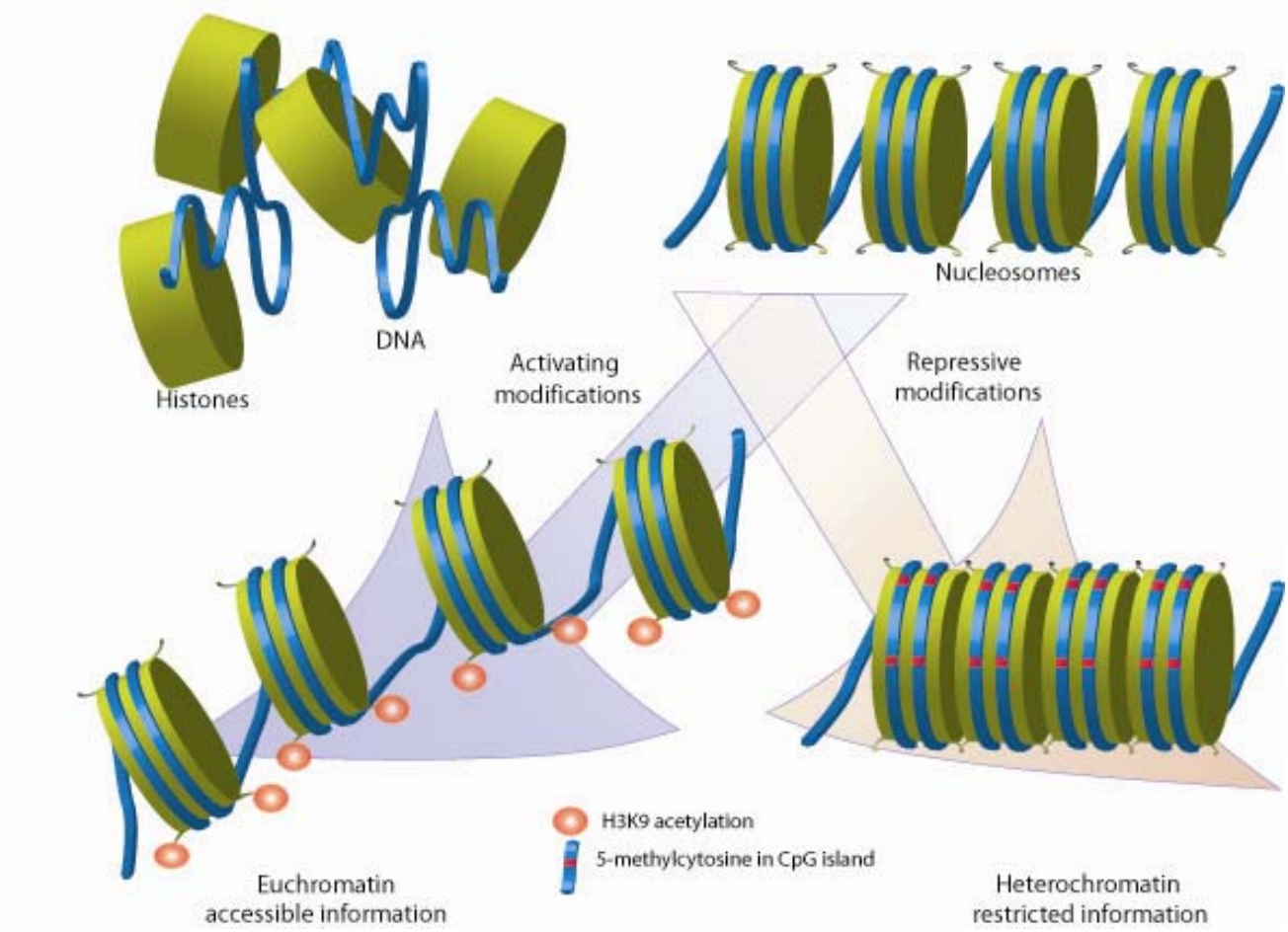

Figure 3

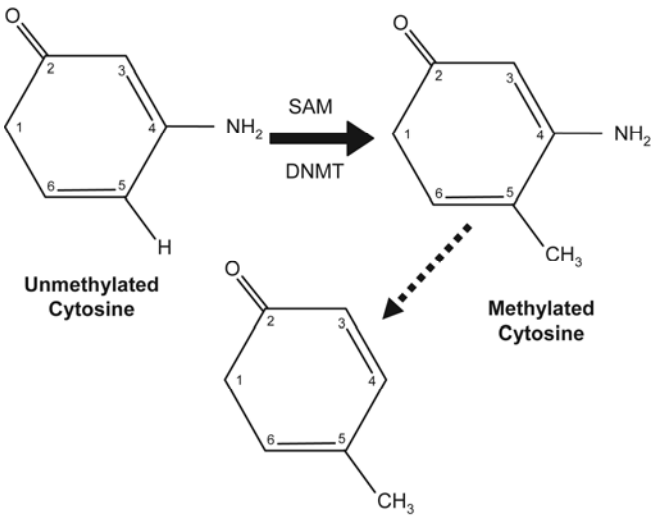

Figure 4

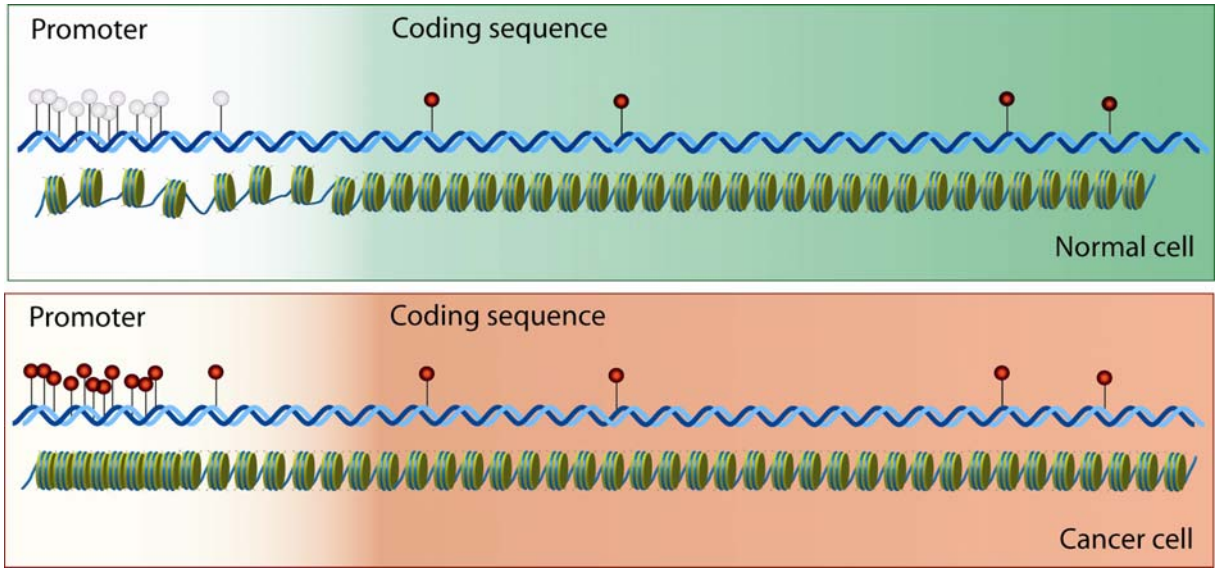

Figure 5