

COLORECTAL CANCER: A MODEL FOR EPIGENETIC TUMORIGENESIS

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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 cancer in humans. Colorectal cancer, long a classic model for the genetic basis of cancer, is now providing researchers with the opportunity to view epigenetic events in the context of neoplasia in humans. 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.

BACKGROUND

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 changes in tumorigenesis. In part, this is because colorectal neoplasia provides a wide range of accessible lesions, from aberrant crypt foci to carcinoma. It also serves as a poster child for epigenetic change because of the possible role that DNA methylation has in the initiation and progression of this disease. For both these reasons, it 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 and 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 Table 1 shows the key historical milestones in the research on cancer epigenetics.

EPIGENETIC EVENTS IN NORMAL HUMAN CELLS

Although 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 other factors within the material of the cell nucleus can also determine gene expression, and hence the structure and function of the cells and tissues that they form. As 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 that 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 plants⁵ and fungi,⁶ and possibly in mammalian cells⁷ (fig 1). Whereas 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-bp 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 (fig 2). These modifications, which include acetylation, methylation, phosphorylation and ubiquitinylation, are reversible events that occur at the N-terminal and C-terminal domains of all core histones. Each of these modifications can be subjected to further variations that can change its 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 several families of

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

Decade	Genetics ³	Epigenetics ⁴	Clinical management
1940	Proposed existence of cancer stem cells	C H Waddington coins the terms epigenetics and epigenome	Dukes' staging 1932
1950	Two-hit hypotheses		No-touch technique for colon surgery
1960	Chromosomal translocations	Description of X chromosome inactivation	Flexible sigmoidoscopy and colonoscopy
1970	First human oncogene	5-Methylcytosine as a mechanism of gene control in mammals	Therapeutic polypectomy
	Tumour-suppressor genes		
1980	Oncogene cooperation	Global hypomethylation of cancer cells	Total mesorectal excision for rectal cancer
		Hypermethylation of the <i>RB</i> gene	
		Chromatin modification linked to DNA methylation	
1990	Genetic basis for cancer predisposition	Invention of bisulphite technique	Adjuvant chemotherapy introduced
		First imprinted genes identified	
		DNA methylation involved in genomic imprinting	
		Loss of imprinting in cancer	
2000	Expression profiling of cancer genes	Drug trials on humans target the epigenetic modifications in DNA	Biological and targeted treatments

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 any change in their function causes diverse and often profound disorders.⁹

Generally, the active chromatin structure corresponding to increased transcriptional activity is associated with increased histone acetylation (fig 2). HATs such as P300 and cellobiose phosphorylase (CBP) catalyse acetylation of lysine (lys) residues on histones H3 and H4.¹⁰ Acting antagonistically to HATs, HDACs produce transcriptional repression by a complicated mechanism that involves interaction with *de novo* methyltransferases^{11 12} and methyl-CpG-binding proteins.^{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 histone H3 have all been linked to gene activation, whereas methylation of lys 9 on histone H3 has been associated with gene silencing.¹⁵ Taken together, this is the pattern of histone modifications that constitutes the "histone code" and that complements the primary DNA sequence in defining transcription states.^{16 17}

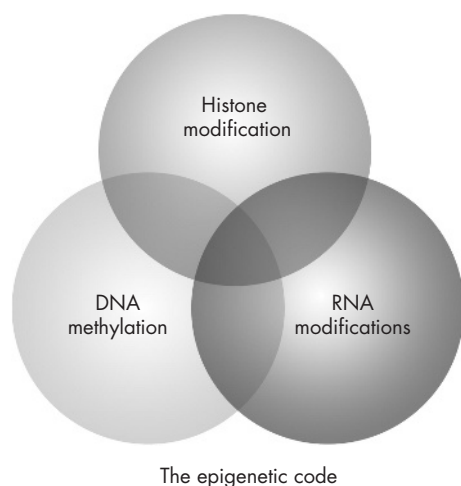


Figure 1 Schematic of the inter-related cellular processes that constitute the epigenetic code. RNA modification includes the roles of RNA interference and microRNA in changing gene expression.

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 (fig 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 (fig 3); in this way, DNA methylation can be seen as an endogenous mutagen. About 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 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. Apparently, 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 (fig 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. About 80 imprinted loci have now been described, and they are typically characterised by tissue-specific and stage-specific patterns of expression.²² This is clearly a key epigenetic process, and one which has been extensively reviewed previously.^{21 23}

Patterns of genomic methylation are of vital importance in both health and disease, and an understanding of the mechanism by which methylation leads to transcriptional silencing is developing rapidly.²⁴ However, less is known about the factors that determine the positioning and the *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 limited data support this contention in higher organisms.²⁵ *De novo* development of DNA methylation is suggested to be the result of loss of transcription from the gene itself.²⁶

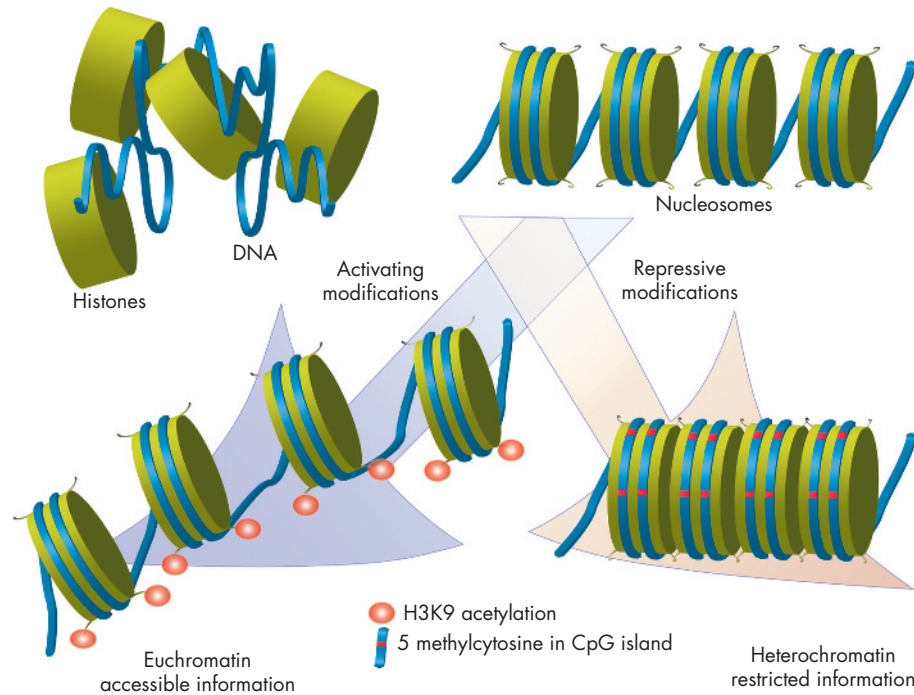


Figure 2 A model of epigenetic modifications and their effect on transcription. The nucleosome is assembled from DNA and histones, and the chemical modification of histone tails induces conformational changes that can cause either activation or repression of transcription. Repressive modifications include methylation of lysine 9 residue of histone 3 (H3K9), lysine 27 residue of histone 3 (H3K27) and lysine 20 residue of histone 4 (H4K20) in association with DNA methylation. Changes such as acetylation at H3K9 (shown) are associated with open chromatin formation (euchromatin).

EPIGENETIC EVENTS AND MECHANISMS IN COLORECTAL CARCINOGENESIS

Given the powerful role of epigenetic changes in changing gene expression, as well as their close relationship with development, it is not surprising that cancer cells show a marked

change 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 cells 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.

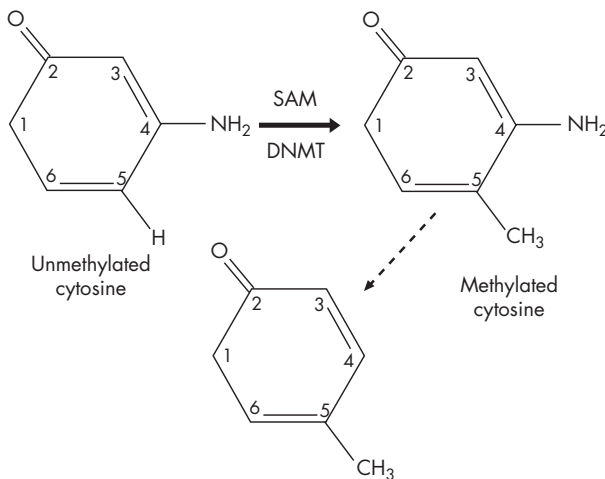


Figure 3 Methylation of cytosine residues and its consequences. De novo methyltransferase (DNMT) catalyses the methylation at position 5 of cytosine, using S-adenosylmethionine (SAM) as the methyl donor. Spontaneous deamination of 5-methylcytosine results in its conversion to thymine, an event which is in itself mutagenic, and which has caused progressive depletion of cytosine bases from the eukaryotic genome throughout evolution.

DNA hypomethylation

In the late 1970s, it was shown 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 several mechanisms that could drive neoplastic progression. In contrast with 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 the reactivation of previously silenced retrotransposons, leading to the disruption of normal gene structure and function.^{36 37} Further, the activity of transposable elements may govern the methylation state of their neighbouring genes through the phenomenon of transcriptional interference, which has been

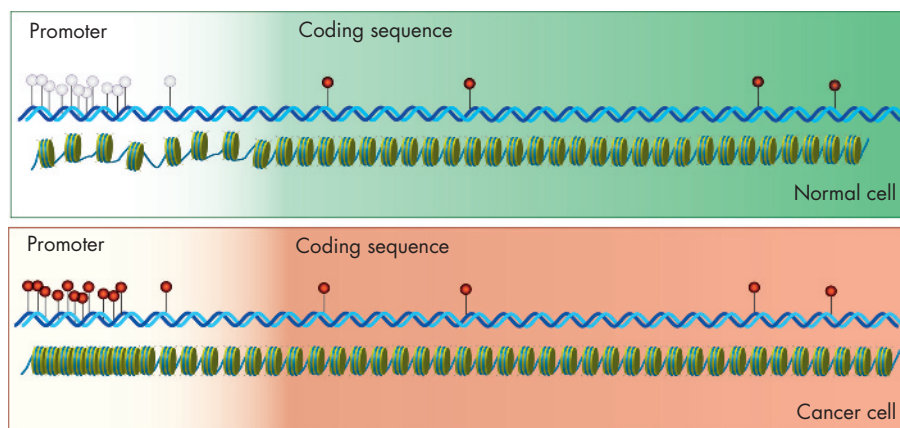


Figure 4 Organisation and consequences of CpG methylation in normal and cancer cells. The upper panel shows a normal cell, in which a cluster of CG dinucleotides (CpG island) remains unmethylated (pale pins), whereas scattered cytosines elsewhere are methylated (red pins). In the absence of methylation 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.

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, and in the cyclin *D2*⁴⁰ and *maspin*⁴¹ genes in gastric carcinoma. Finally, decreased methylation of DNA can lead to loss of imprinting (LOI), and this can drive cellular proliferation in cancer. The clearest example of this phenomenon is the LOI at the *IGF2/H19* region as a result of hypomethylation at the differentially methylated region of *IGF2*,⁴² an event seen in about 40% of colorectal cancer tissue.⁴³

Hypermethylation

In concert with global hypomethylation, focal hypermethylation at CpG islands is also regarded as a critical event in cancer development^{44–46} (fig 4). Not surprisingly, research in this discipline has focused on tumour-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 LOI in cancer. In Wilms' tumour, for example, hypermethylation at the *IGF2* differentially methylated region causes LOI of the normally silenced maternal allele of *IGF2*.^{48–49} Similar events are seen with the *p73* gene in haematological malignancies,⁵⁰ and the *ARH1* gene in follicular carcinoma of the thyroid.⁵¹

CpG island methylation is a common epigenetic event in colorectal neoplasia, with *MLH1* promoter methylation representing a classic example of this phenomenon. A long list of hypermethylated genes is associated with colorectal neoplasia, including tumour-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,⁷⁸ these genes have been drawn from many of the key functional groups that define the cancer phenotype, including Wnt signalling (*SRFP* genes), mismatch repair (*MLH1*),

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

Gene	Function	Frequency (%)	References
<i>APC</i>	Signal transduction, beta-catenin regulation	10–50	52–57
<i>CDH13</i>	Cell signalling (cell recognition and adhesion)	30–40	58
<i>CDKN2A</i>	Cell-cycle regulation	15–30	55, 59, 60
<i>CHFR</i>	Mitotic stress checkpoint	30–40	61, 62
<i>HIC1</i>	Regulation of DNA damage responses	~80	63, 64
<i>HPP1</i>	Transmembrane transforming growth factor (TGF)- β antagonist	~80	65
<i>LKB1</i>	Cell signalling, cell polarity	5–10	66
<i>MGMT</i>	Repair of DNA guanosine methyl adduct	30–40	55, 56, 67–69
<i>MLH1</i>	Mismatch repair	10–20	56, 70–72
<i>p14^{ARF}</i>	Cell-cycle regulation	20–30	55, 73, 74
<i>RASSF1A</i>	DNA repair, cell-cycle regulation	>50	55, 75, 76
<i>SOCS1</i>	Cell signalling	5–10	58
<i>THBS1</i>	Angiogenesis	10–20	55, 56
<i>TIMP3</i>	Matrix remodelling, tissue invasion	10–30	55, 77

cell-cycle regulation (*CDKN2A*), epithelial differentiation (*GATA4,5*), *p53*-mediated damage responses (*HIC1*) and cell-matrix interactions (*TIMP3*).

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

Dysregulation of histone modification

In comparison with 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. These two moieties are mutually exclusive as they affect the same position. Acetylation of lysine (K) 9 residue of histone H3 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 a universal marker for malignant transformation.⁸⁴ Overexpression of a putative histone methyltransferase *SMYD3* that methylates the lysine 4 residue of histone H3 in colorectal cancer has been reported.⁸⁵ As methylation of the lysine 9 residue of histone H3 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 histone⁸⁷ or the H2A.Z variant for H2A. The H2A.Z variant has a crucial role in embryogenesis⁸⁸; and also by depositing it at the 5' end of genes, it can retain the boundaries that prevent the spread of heterochromatin into euchromatic regions.⁸⁹ Inappropriate inclusion of histone variants possibly disturb the boundaries between euchromatin and heterochromatin. The disturbances in the epigenetic machinery that induce these changes are the focus of current research,^{84–86} as are the consequences of such changes.

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

Epigenetics of microsatellite and chromosomal instability

There is apparently 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 a sequence mutation of the other allele to fulfil Knudson's two-hit hypothesis. Yet, although 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 and tumour biology, including precursor lesions and morphology⁹¹ (fig 5). Epigenetic events are clearly involved in the chromosomal instability pathway, with hypomethylation establishing opportunities for chromosomal instability, and for activation of oncogenes such as *c-myc*.⁹² However, 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, serves as an exemplar of epigenetic carcinogenesis.

The MSI 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 distinct clinicopathological features, including poor differentiation, intraepithelial lymphocytic infiltrates and location in the proximal colon.⁹⁴ Curiously, however, they occur predominantly in elderly women, and a recent systematic review has confirmed that they have a considerably better outcome than those with microsatellite-stable cancers of similar stage and grade.⁹⁵

Although *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} although this gene is rarely if ever mutated in cancers arising in patients with HNPCC. Likewise, an interdependence has been reported between *MGMT* hypermethylation and *TP53* mutations.¹⁰⁰ The precise inter-relationship between genetics and epigenetics in the MSI pathway, including the chronology of key events, remains to be elucidated.

The CpG island methylator phenotype

In 1999, Toyota *et al*¹⁰¹ identified a set of CpG islands that could be methylated in tumours but were not methylated in normal epithelial cells. 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 tumours characterised by multiple, concordant methylation events.¹⁰¹ Subsequent population-based studies on patients with colorectal cancer 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 high incidences in elderly people and in females.^{102–104} However, about half of the

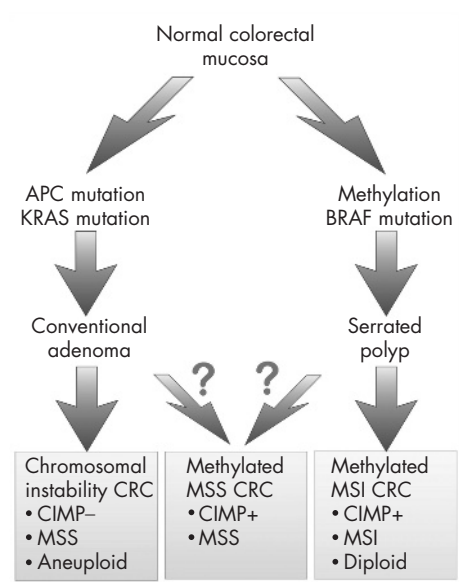


Figure 5 Proposed pathways for colorectal tumorigenesis and their relationship with the CpG island methylator phenotype (CIMP). A working model of the dichotomy between chromosomal instability and microsatellite instability (MSI) pathways in colorectal carcinogenesis (CRC), and the common morphological and genetic changes that accompany each subtype. A subgroup of tumours, which are characterised by CpG island methylation (CIMP positive) and microsatellite stability (MSS), is shown. 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.

tumours that display widespread CpG island methylation are microsatellite stable (fig 5). Evidence also suggests that they may be unique in terms of behaviour. Our group has reported difference in outcome between subgroups of the CIMP tumours depending on microsatellite status,¹⁰⁵ and highlighted the poor prognosis of patients 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 the 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 cells that give rise to the CIMP tumours have a definable change in their machinery of methylation that produces what Issa¹⁰⁷ has referred to as “epigenetic instability”, and whether this is integral to tumour initiation and progression. This is an important question; if this were true, then a better understanding of the CIMP tumours would shed more light on the mechanisms that control 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 the CIMP tumour may partly be hereditary, an observation initially suggested¹⁰⁸ but not confirmed in larger studies.^{104 109} At present, issues regarding the operational definition of the CIMP are clearly limiting the attainment of consensus on these important

matters,¹⁰⁷ and the biological basis of the CIMP remains uncertain.

Chronology of genetic and epigenetic events in colorectal cancer

Research over the past decade has consistently shown that epigenetic changes such as promoter hypermethylation^{67 110 111} and LOI⁴³ can occur in histologically normal colonic epithelium, and that these changes are more likely in patients with CIMP or MSI cancers.¹¹² The early occurrence of these epigenetic events, and their relevance to the emerging science of stem cell biology, serves to highlight their theoretical significance in neoplastic development. Baylin and Ohm⁷⁸ have recently advocated the primacy of epigenetic events in colorectal neoplasia, arguing that such epigenetic changes 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 *et al.*¹¹³ Feinberg *et al.*¹¹⁴ have also recently highlighted the early role of epigenetic changes 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.

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, the chemoprevention of this disease. At a minimum, it is important to understand the factors that may induce epigenetic changes.

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 be discussed only briefly in this review. With regard 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 in DNA synthesis and repair. Both epidemiological and experimental studies have 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 tumour 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 markedly affects the epigenetic patterning in the fetus, and it has been hypothesised that this in turn influences 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*,⁷⁰ *HIC1* and *IGF2*,¹²⁴ has been shown to increase progressively with age. For at least

some of these genes, this process seems to be accelerated in patients with colorectal cancer.^{101–125} These epigenetic changes may reflect the clinical truism that colorectal carcinoma is a disease in elderly people.

Inherited factors in the epigenetics of colorectal cancer

Given that epigenetic changes are stable and potentially heritable through meiosis, some of the ways in which inheritance may influence epigenetic changes associated with colorectal neoplasia should be considered. As discussed earlier, there has been considerable interest, albeit scant supporting evidence, for the proposition that the changes that underpin CIMP, whether genetic or epigenetic, may be inherited. Perhaps a clearer example of inherited epigenetic risk is seen in the case of LOI at *IGF2*. Certainly, patients with widespread LOI for this gene have an increased risk of developing colorectal cancer⁴³ 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 in *MLH1*, which predispose individuals to young-onset MSI tumours in the large bowel and at extracolonic sites.^{127–128} These germline epimutations manifest by somawide 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 changes can mimic hereditary cancer syndromes, and may be inherited.^{127–131} To date, such somawide 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 understand this phenomenon better.

CLINICAL IMPORTANCE OF EPIGENETIC CHANGES IN COLORECTAL NEOPLASIA

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

The epigenetic events in colorectal cancer may soon influence treatment decisions. For instance, although still controversial,¹³² growing evidence from retrospective analyses suggests that MSI tumours respond differently to traditional chemotherapeutic agents,^{133–134} and that outcomes for some patients 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 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-azacytidine¹³⁹ inactivate DNA methyltransferases, and can thus revert methylation-induced silencing.¹⁴⁰ Inhibitors of HDAC, such as suberoylanilide hydroxamic acid (SAHA) and newer derivatives,⁹ have been slower to emerge, and given the interdependence of these epigenetic processes, combination therapy approaches may also be beneficial.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

Although 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, epigenetics of colorectal cancer is a burgeoning field, and as was the case with the genetics of cancer, the lessons learnt 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 clearly has the potential to entirely rework our current paradigms of cancer development, if not management.

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