Epigenetic events in the colorectum and in colon cancer

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Abstract

Colon cancers arise from benign neoplasms and evolve into adenocarcinomas through a stepwise histological progression sequence, proceeding from either adenomas or hyperplastic polyps/serrated adenomas. Genetic alterations have been associated with specific steps in this polyp–adenocarcinoma sequence and are believed to drive the histological progression of colon cancer. Recently, epigenetic alterations, which include CGI (CpG island) DNA methylation, have been shown to occur in colon polyps and colon cancer. The aberrant methylation of genes appears to co-operate with the genetic alterations to drive the initiation and progression of colon polyps to colon cancer. CGI DNA methylation is an epigenetic mechanism that represses gene transcription in normal cellular processes, but it becomes excessive and aberrant in many neoplasms. The aberrant DNA methylation affects CpG-rich regions, called CGIs, in the 5’ region of genes and results in transcriptional silencing through effects on transcription factor binding and associated changes in chromatin structure. These hypermethylated genes are not only probable pathogenic events affecting colon-cancer formation, but also neoplasm-specific molecular events that may be useful as molecular markers for colon tumours. Furthermore, aberrant DNA methylation of tumour-suppressor genes may occur secondary to a genetic predisposition or to a field-cancerization effect in the colon and may be useful as molecular markers for the risk of developing colon cancer.

Introduction

Colorectal cancer develops as the result of the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colon adenocarcinoma. The fact that colon cancer develops over 10–15 years and progresses through parallel histological and molecular changes has permitted a detailed analysis of the events that are involved in its initiation and progression. Consequently, the last 20 years have been witness to an explosion of information that has revealed the specific nature of many of these alterations, and to the emergence of the appreciation that epigenetic alterations participate in the pathogenesis of colon cancer. The subsequent effect of these genetic and epigenetic alterations on the cell, and the molecular biology of the cancer cells in which they occur, has also begun to be revealed in the last decade. From the analysis of the molecular genesis of colon cancer, key themes concerning the molecular pathogenesis of cancer have been established. The first is that cancer emerges via a multistep progression at both the molecular and the morphological levels [1]. The second is that genetic and epigenetic alterations are key pathogenic events in cancer formation that drive the initiation and progression of the polyp–cancer sequence [2]. The third is that hereditary cancer syndromes frequently correspond to germline forms of key genetic and epigenetic defects, whose somatic occurrences drive the emergence of sporadic colon cancers [3].

Consistent with these themes, colon cancer is most commonly initiated by alterations in the signalling elements in the Wingless/Wnt signalling pathway and then progresses as the result of the accumulation of sequential events that either activate oncogenes or inactivate tumour-suppressor genes. Some of the alterations that have been convincingly shown to promote colon carcinogenesis affect KRAS2, TP53, and elements of the TGF (transforming growth factor)-β signalling pathway, such as TGFBR2 and MADH4/SMAD4. Epigenetic alterations, particularly aberrant CGI (CpG island) methylation, appear to affect genes whose inactivation can promote tumour formation by creating genomic instability [e.g. MLH1 (mutL homologue 1)] or through the primary inactivation of the methylated gene itself (e.g. CDKN2A). The identification of the specific genes that are altered by these genetic and epigenetic events is currently an area of intense investigation. These studies have provided targets for the development of new therapies for the prevention and/or treatment of colon tumours throughout their progression from normal epithelium to adenocarcinoma. Indeed, pharmaceutical and biological agents that target such alterations as oncogenic KRAS2 and TP53 are currently in clinical trials, as are agents that target aberrant DNA methylation and histone deacetylation [4].

Polyp–carcinoma sequence

The evolution of normal epithelial cells to adenocarcinoma usually follows a predictable progression of histological...
changes and concurrent genetic and epigenetic changes. These alterations provide a growth advantage and lead to clonal expansion of the altered cells. Subsequent alterations with waves of clonal expansion then occur as a consequence of progressive events that provide other growth advantages to the cells, such as loss of cell contact inhibition.

The earliest identifiable lesion in colon-cancer formation appears to be the ACF (aberrant crypt focus). The true neoplastic potential of this lesion is still undetermined, but it does appear that some of these lesions can progress to frank adenocarcinoma and harbour mutations in Kras2 or APC (adenomatous polyposis coli). In particular, dysplastic ACFs frequently carry mutations in APC and appear to have the highest potential for progressing to colon cancer. Thus alterations in APC, which result in over-activation of the Wingless/Wnt signalling pathway, appear to initiate tumour formation in the colon. Subsequent alterations in other genes then play a role in tumour growth and the eventual acquisition of other malignant characteristics, such as tissue invasiveness and the ability to metastasize.

More recently, evidence has accumulated that demonstrates that some colon cancers arise from hyperplastic polyps that can evolve into adenocarcinomas through a serrated adenoma intermediate [5]. Interestingly, this hyperplastic polyp-serrated adenoma–adenocarcinoma is more common in the proximal colon, and these tumours more often show increased CGI methylation and mutations in BRAF [6].

Epigenetic alterations and cancer

DNA methylation is present throughout the majority of the genome and is maintained in relatively stable patterns that are established during development [4]. In humans, approx. 70% of CpG dinucleotides are methylated. Interestingly, the CpG dinucleotides are under-represented throughout the human genome, presumably because of suppression secondary to increased susceptibility to mutations. However, there are regions that contain higher proportions of CpG dinucleotides called CGIs, which are 0.2–3 kb-long sequences and by definition are composed of greater than 50% cytosines/guanines. They are present in the 5′ region of approx. 50–60% of genes and are normally maintained in an unmethylated state. In cancers, many of these CGIs become aberrantly methylated, and this aberrant methylation can be accompanied by transcriptional repression. The significance of these epigenetic alterations has been a point of significant controversy, as to whether these DNA methylation changes are simply passenger events accompanying genetic events that are driving this process, or whether they are pathogenic themselves. This issue of whether aberrant methylation is a cause or an effect of cancer formation remains unresolved because the mechanism responsible for aberrant DNA methylation has yet to be identified, and because it is a well-known fact that transcriptional inactivity can lead to DNA methylation [7,8].

However, the finding of aberrant MLH1-promoter methylation in sporadic colon cancers with MSI (microsatellite instability) dramatically illustrated the role of epigenetic changes as potential pathogenetic alterations in cancer [9–11]. Aberrant methylation of the cancer genome, and associated silencing of the genes whose promoters demonstrated such methylation, has been well described at multiple genetic loci [12,13]. Reversion of the methylation using demethylating agents, such as 5-deoxy-azacytidine, frequently restores expression of these, demonstrating that methylation may in fact drive the gene silencing. As inactivation of MLH1 presumptively plays an initiating role in the pathogenesis of MSI colon cancers, the finding of aberrant methylation of MLH1 in sporadic MSI colon cancers, and the restoration of MLH1 expression by demethylating the MLH1 promoter in cell lines derived from such cancers, strongly suggests that such aberrant methylation could be a cause rather than a consequence of colon carcinogenesis [9–11]. Fine-structure analysis of the methylation status of specific CpGs in the MLH1 promoter has shown that the methylation status of small clusters of CpGs in the 5′ region of the MLH1 promoter appears to dictate the transcriptional status of the gene [14]. Additional genetic support for a primary role for aberrant methylation in gastrointestinal carcinogenesis was provided by Grady et al. [15] when they demonstrated loss of expression of E-cadherin concurrent with CpG methylation of the wild-type CDH1 allele in tumours that occur in the setting of hereditary diffuse gastric cancer, a cancer family syndrome caused by germline mutations in CDH1. It also appears that the epigenetic and genetic changes cooperate to promote cancer formation [12]. Moreover, it is likely that the aberrant hypermethylation of 5′ CpG dinucleotides, which has been demonstrated to silence a variety of tumour suppressor genes including CDH1, CDKN2A/p16, thrombospondin-1 (TSP1), MLH1 and GSTP1, may be similarly pathogenetic in the tumours in which these changes have been identified [10–12,16–18]. In particular, although mutation of CDKN2A/p16 has not been described in colon cancer, methylation of CDKN2A/p16 is detected in 40% of colon cancers [17] and has been found not only in colon cancer, but also in colon adenomas, as has methylation of other genes [19,20]. This observation demonstrates that aberrant promoter methylation is occurring early in the adenoma sequence, although it does not confirm that the aberrant CDKN2A/p16 methylation is a primary, rather than a secondary, event in the tumorigenesis process. More broadly, early work has suggested that colon cancers that hypermethylate MLH1 and/or CDKN2A/p16 may belong to a distinct subclass of colon cancers, termed the CIMP (CGI methylator phenotype), that demonstrate genome-wide aberrant methylation of gene promoters, and that may arise by a distinct and unique mechanism [17,18].

Also worthy of note, is recent progress in our understanding of mechanisms through which DNA methylation may affect transcription. DNA methylation may impair transcription by direct inhibition between methylated promoters and transcription factors, such as AP-2 (activator protein-2), CREB (cAMP-response-element-binding protein), E2F and NF-κB (nuclear factor κB) [4]. CpG methylation can also mediate transcriptional silencing by recruiting methyl-binding
proteins, MeCP2, MBD2 and MBD3, that recognize methylated sequences and recruit HDACs (histone deacetylases). The HDACs then induce changes in chromatin structure that impede the access of transcription factors to the promoter [12]. The relationship between DNA methylation and post-translational modification of histones appears to be complex; other studies have shown that changes in the methylation state of H3-Lys9 and H3-Lys4 precede changes in DNA methylation, suggesting that the histone-modification state and chromatin structure may cause the DNA methylation changes [4].

Epigenetic alterations in the polyp–cancer sequence

In addition to the interest in the role of epigenetic alterations in established cancers, the evidence of increased methylation in CGIs in non-neoplastic tissues has led to considerable interest in the role aberrant DNA methylation may have as a pre-neoplastic event. Indeed, there is evidence that aberrant CGI methylation may occur as the result of a genetic predisposition or a field effect. Ahuja et al. [21] have shown that aberrant CGI methylation occurs in histologically normal colon epithelium in an age-dependent fashion, and that half of the genes involved in this age-related methylation are the same as those involved in colon carcinogenesis. The cause of this age-related DNA methylation is unknown, but current models suggest that the methylation occurs as the consequence of local predisposing factors in DNA [e.g. methylation control centres, such as Sp1 (specificity protein 1) sites or tandem B1 elements], environmental exposures, and/or a genetic predisposition to DNA methylation. Indeed, Kim et al. [22] have demonstrated that tobacco-smoke exposure is significantly associated with methylation of CDKN2A/p16 in non-small-cell lung cancer, reinforcing the role of environmental agents in mediating this class of epigenetic alterations. It is also likely that the genetic and epigenetic alterations may co-operate to promote tumour formation and that detection of colon adenomas with methylation may identify colon epithelium that is at significant risk of acquiring genetic alterations that will lead to colon tumour formation [23].

As mentioned above, the transformation of normal colon epithelial cells to adenomas, and then to cancer, follows a predictable progression of histological changes, resulting in an adenoma–carcinoma progression sequence. This neoplastic progression sequence is believed to be an evolutionary process in which neoplastic cells acquire heritable genetic and epigenetic alterations that drive the carcinogenesis process [24]. These genetic and epigenetic alterations are typically gene mutations and aberrant DNA-methylation events that provide clonal heterogeneity with evolution by natural selection. Each major step in this evolutionary process is usually accompanied by a recognizable histological change that proceeds from a benign tubular adenoma to an advanced adenoma (e.g. tubulovillous or villous adenoma) and finally to invasive adenocarcinoma. In colon cancer, ‘intuitive frequency’ analysis has led to the concept that specific gene mutations initiate the formation of tubular adenomas (e.g. APC mutations) and others drive the malignant transformation of the adenomas (e.g. TPS3 mutations).

With regard to the epigenetic alterations that have been identified in colon cancer, CGI DNA methylation is the best-characterized epigenetic alteration that has been shown to affect tumour suppressor genes during the adenoma–carcinoma sequence. Aberrant DNA methylation of specific loci has been identified in the earliest precursor lesions for colon adenocarcinomas, ACF. MINT1, MINT31, SLC5A8 and MGMT have been found in ACFs and in adenomas [19,25,26]. Investigation of colon cancer has demonstrated that these alterations, as well as a variety of others, are present, suggesting that cells that acquire aberrantly methylated genes are favoured in the clonal evolution of colon cancer [4,7]. This model has led Toyota et al. [18] to propose that a subset of colon cancers display a hypermethylator phenotype that has been termed CIMP. Interestingly, the specific genes that are commonly found to be methylated in colon cancer differ from those commonly found to be methylated in other tumour types, suggesting that there is a selective process driving the occurrence of methylated genes in these tumour types.

Although the observation that aberrant DNA methylation occurs in the earliest lesions implicated in colon cancer formation is consistent with the concept that epigenetic alterations play a role in the initiation of colorectal cancer, it does not exclude the possibility that aberrant DNA methylation is a secondary phenomenon. Indeed, Bai et al. [27] have found that the methylation status of genes is established in the adenoma phase of the adenoma–carcinoma sequence, suggesting that these events are acquired during the initiation of the colon neoplasms and do not have a functional role in the progression of colon cancer. In contrast, Lee et al. [28] observed that a subset of genes (MLH1, RASSF1A, CDKN2A, GSTP1, THBS1 and TIMP3) were more commonly methylated in colon cancers compared with adenomas, suggesting that at least some genes may affect the transformation step in colon cancer formation. Lee et al. [28] did not find a difference in the proportion of genes methylated in progressively more advanced stages of adenocarcinoma, but they did not assess the frequencies of specific methylated genes in different stages of colon cancer, which would be more informative with regard to understanding the role of epigenetic events in the clonal evolution of colon cancer. Consequently, we assessed the methylation status of five genes, previously demonstrated to be aberrantly methylated in colon cancers (in a collection of colon neoplasms that included representative cases of adenomas, histologically advanced adenomas, early-stage colon cancers, late-stage colon cancers and colon-cancer metastases), in order to determine if the frequencies and patterns of aberrantly methylated genes vary among these different histological steps in the adenoma–carcinoma sequence. We have observed different frequencies and patterns of methylation of specific genes among these groups of neoplasms, suggesting that aberrant DNA methylation inactivates tumour-suppressor genes, and that the aberrant methylation of these genes affects tumour initiation or progression events, depending on the specific genes. The results found in our
laboratory, as well as in other laboratories, in conjunction with data demonstrating that the clear causal role of specific aberrant methylation events (such as MLH1 methylation) in colon cancer, provide significant evidence that the aberrant methylation of genes contributes to the initiation of adenomas and their progression to colon cancer.

Methylated genes: potential molecular markers of colon cancer

Aberrant CGI methylation may be a particularly effective molecular marker for sporadic colon cancer because of its frequency in colon cancer. Recently, aberrant hypermethylation of tumour-suppressor genes has been shown to be a common mechanism for silencing tumour-suppressor genes in a variety of cancers, including colon cancer. As mentioned above, CGI methylation has been shown to affect a number of genes in colon cancer, including CDKN2A/p16, MGMT, THBS-1, TIMP-3, p14ARF and MLH1 [7,19]. The percentage of colon cancers demonstrating CGI methylation appears to be as high as 58%, which is approximately the same frequency as colon cancers demonstrating CGI methylation appears to be a common genetic alteration seen in colon cancer, such as APC mutations [29]. Furthermore, the CGI methylation of CDKN2A/p16, O6-Methylguanine-DNA methyltransferase (MGMT) and MINT31 has been shown to occur early in the adenoma–carcinoma sequence, while the neoplasms are still benign, supporting the use of these alterations as early detection markers that could lead to the primary prevention of colon cancer [18,30]. In addition, MLH1 methylation is both a candidate marker for MSI colon cancers, since approx. 80% of sporadic MSI colon cancers are initiated by aberrant methylation of the MLH1 promoter, and potentially a marker for risk for developing MSI colon cancer [9,31].

In specific regard to the use of CGI methylation of tumour-suppressor genes as molecular markers of colon cancer, a sensitive and specific assay termed MSP (methylation-specific PCR), which appears to be useful in clinical applications, has been developed by Herman et al. [32]. We have developed novel MLH1, CDKN2A, and MGMT MSP assays that can detect as little as 10–50 pg of methylated DNA. Furthermore, we have employed a first-generation version of the MLH1 MSP assay to detect methylated MLH1 DNA in the serum of patients with sporadic MSI colon cancer [33]. We were able to detect methylated MLH1 DNA in the serum of individuals with tumours with methylated MLH1 DNA in 30% of the cases with 100% specificity, in a study of 20 patients [33]. In terms of clinical utility, MSP assays to detect aberrantly methylated MLH1, CDKN2A and MGMT DNA in faecal DNA from patients with sporadic colon neoplasms (for the early detection of colon cancer) have also been performed [20]. We have demonstrated that it is feasible to detect methylated genes in faecal DNA from individuals with colon polyps that carry methylated genes with high sensitivity (approx. 80%), although we have also identified a subset of individuals who have endoscopically detectable polyps, but who have methylated genes in their faecal DNA [20]. Thus evaluation of the clinical utility of these assays as early detection markers or potentially risk stratification markers remains under active investigation.

Conclusion

Epigenetic and genetic alterations participate in the initiation and promotion of colon cancer. Epigenetic alterations, specifically aberrant DNA methylation, are becoming increasingly recognized as a causal mechanism in colon carcinogenesis. Although the mechanism underlying the cause of these epigenetic alterations remains to be elucidated, the characterization of the genes affected by aberrant methylation in colon cancers is proceeding at a rapid pace. These methylated genes have the potential to be early-detection and prognostic markers for colon cancer.

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