Commentary

Aberrant DNA Methylation: Have We Entered the Era of More Than One Type of Colorectal Cancer?

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It has been known for over two decades that DNA from colorectal cancer (CRC) is hypomethylated compared to that from normal colonic epithelia.^{1,2} More recently it has also been recognized that specific CpG dinucleotide islands are often hypermethylated in this tumor type,³ giving rise to the term CpG island methylator phenotype, or CIMP+.⁴ Hypomethylation may contribute to the activation of oncogenes, whereas CpG-island specific hypermethylation has been linked to the transcriptional silencing of tumor suppressor genes. The relationship between these two epigenetic phenomena has not been examined, however, in CRC. Intuitively one might expect that tumors with severe global DNA hypomethylation might also show a lower frequency of CpG island hypermethylation due to a lower overall content of methyl groups. The meticulous study by Bariol et al⁵ in this issue of *The American Journal of Pathology* reveals this not to be the case. No association was apparent between global 5-methyl cytosine content and the number of methylated CpG sites in a relatively large and representative series of 97 premalignant and malignant lesions. With only one exception, all of the 22 CRCs investigated showed a lower 5-methyl cytosine content compared to normal mucosa from the same patient. Moreover, approximately 30 to 40% of hyperplastic polyps, adenomas, and CRCs showed no methylation at any of the five CpG sites examined.

This most recent study by the Ward laboratory follows on from their previous work in characterizing CIMP+ CRCs.⁶ It is also relevant to the notion put forward by these and other workers that CIMP+ and the closely related tumor subgroup with microsatellite instability (MSI+) originate in large hyperplastic polyps and serrated adenomas.^{7,8} Increasingly, it is becoming recognized that tumors with widespread DNA hypermethylation have distinctive clinicopathological and molecular features.^{4,6,9} Although the study by Bariol et al⁵ emphasizes and probably serves to justify the current interest in hypermethylation as opposed to hypomethylation, there is clearly much more to be learned about the general role of aberrant DNA methylation in the development and progression of CRC.

Technical Issues in the Study of Aberrant DNA Methylation

Proper characterization of the molecular features of cancer is often compromised by technical factors. A good illustration of this is the widely divergent results obtained for MSI+ tumors following the adoption of questionable molecular criteria for the classification of this phenotype.¹⁰ The evaluation of hypomethylation in the current study by Bariol et al⁵ was relatively straightforward and based on the level of incorporation of radio labeled methyl groups into the test DNA. Peripheral blood DNA was used as a reference standard and the results obtained were reproducible to within 10%. Evaluation of DNA hypermethylation was based on initial treatment with sodium bisulfite followed by methylation-specific PCR (MSP) to distinguish between methylated and non-methylated sequences. The frequencies of methylation observed by Bariol et al⁵ for *p16* and for the various MINT CpG sites are similar to other reports on CRC.^{4,9} There is, as yet, no consensus panel of CpG sites that should be analyzed to classify tumors as CIMP+; however, the combination of five sites used by Bariol et al (*p16* and four different MINTs) is likely to be as good as any. Since the degree of hypermethylation shows a skewed distribution in CRC, the most informative comparisons will be between tumors showing light methylation (0 or 1 site methylated) and those with heavy methylation (4 or 5 sites methylated).

The DNA hypo- and hypermethylation observed in CRC may be manifestations of more widespread aberrations in methyl group metabolism. Consequently, study of the level of intracellular folate intermediates involved in methyl group transfer reactions may also prove interesting, particularly for the further characterization of CIMP+ tumors. Two obvious candidates for investigation are the concentrations of 5,10-methylenetetrahydrofolate and tetrahydrofolate.

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Another technical issue relating to the study of aberrant DNA methylation or indeed to that of any molecular alteration in cancer is sample selection and size. Bariol et al⁵ have been careful to investigate representative cases of hyperplastic polyps, adenomas and CRCs for their study. Their work highlights the critical importance of large, population-based frozen tumor collections with appropriate patient consent and with comprehensive histopathological and clinical information. Funding agencies with an interest in translating advances in molecular oncology into improved cancer patient management would be well advised to consider the value of such tissue banks.

What Are the Factors That Influence DNA Hypomethylation in Normal Tissues?

Global DNA hypomethylation within the colonic mucosa may be a predisposing factor for the development of CRC, and hence the identification of environmental and genetic risk factors associated with this condition is an important area of research. Bariol et al⁵ found more hypomethylation with advancing age, but no apparent associations with gender or site in the large bowel. Other possible risk factors that merit investigation in future studies are the level of dietary folate intake, hormone status in women, and polymorphisms in methyl group metabolizing genes such as methylene tetrahydrofolate reductase (MTHFR) and thymidylate synthase. Hypomethylation of DNA from peripheral blood cells has been reported in individuals homozygous for the C677T polymorphism of MTHFR,¹¹ and hence it is reasonable to expect the existence of similar genotype-related associations in colonic epithelia. Some evidence for this has recently been put forward.¹²

Global Hypomethylation Does Not Distinguish CRC Subgroups

The study by Bariol et al⁵ confirms two earlier reports^{2,13} showing DNA hypomethylation in preneoplastic lesions compared to normal mucosa. Interestingly, the current study found no evidence for increased hypomethylation between polyps and invasive or metastatic cancers, suggesting this process does not contribute to tumor progression. In contrast to hypermethylation (see below), no associations were observed between hypomethylation and either the site of tumor origin in the large bowel or the degree of differentiation. The only hint of association was a trend between the extent of hypomethylation and the proliferative rate of a subgroup of hyperplastic polyps and small adenomas. Whether or not the level of global demethylation in CRC is related to other molecular factors such as the frequencies of loss of heterozygosity and p53 mutation remains to be determined. Also to be established is whether the degree of hypomethylation has prognostic or predictive value.

Frequent CpG Island Hypermethylation Appears to Be Associated with Hyperplastic Polyps

The extent of hypermethylation in an unselected series of sporadic hyperplastic polyps and adenomas is reported for the first time in the study by Bariol et al.⁵ Of the 41 hyperplastic polyps and small adenomas examined, two-thirds (27/41) showed methylation at none or only one of the seven CpG sites examined (*p16*, MINT-1, -2, -12, -31, hMLH1-A, and hMLH1-C). Importantly, of the eight lesions among this group that showed methylation at four or more sites, seven were hyperplastic polyps. The authors conclude from their data that widespread CpG island methylation occurs infrequently in adenomatous polyps and one could therefore speculate that it does not play a role in the progression of these lesions. In contrast, the heavy methylation observed in 35% (7/20) of sporadic hyperplastic polyps suggests this epigenetic alteration is involved in the development of such lesions and possibly their progression to CIMP+ invasive carcinoma. CRCs with heavy methylation are known to occur more frequently in tumors from older female patients, are often poorly differentiated and frequently arise in the right-sided colon.^{6,9} It would be interesting to know whether the seven hyperplastic polyps with heavy methylation described by Bariol et al⁵ also share these features.

The majority of sporadic MSI+ tumors are associated with methylation of the hMLH1 gene¹⁴ and occur almost exclusively in the right-sided colon. Interestingly, the frequency of MSI+ tumors in the proximal colon (approximately 25%) is very similar to that of heavily methylated tumors from the same region.^{6,9} These observations suggest that methylation of the promoter region within the hMLH1 gene occurs mostly in tumors with extensive CpG island hypermethylation and consequently this site may be one of the last to undergo methylation.

Concluding Remarks

The two major findings of the study by Bariol et al⁵ are first, that global hypomethylation and CpG-island specific hypermethylation occur independently of each other in CRC and second, hypermethylation occurs more frequently in hyperplastic polyps compared to similar sized adenomas, particularly in the proximal colon. The latter finding supports the concept that hyperplastic polyps may be precursor lesions for the subset of CRCs having widespread CpG island methylation and referred to as CIMP+.^{7,8} Apart from the obvious implications for the management of patients with this type of polyp, several other issues require consideration if one accepts there are at least two pathways leading to the development of CRC.

Although DNA hypomethylation does not readily identify distinct subgroups of CRC, the presence of widespread CpG island-specific methylation certainly does. CRCs with heavy methylation (CIMP+) are often poorly differentiated, located in the proximal colon and appear likely to arise from hyperplastic polyps. They have lower frequencies of p53 and K-ras mutations, a much higher

frequency of MSI+ and may also have worse prognosis compared to CIMP- tumors.^{4,6,9} These biological differences beg the question of whether the environmental and genetic risk factors for CIMP+ and CIMP- tumors are also different.¹⁵ Another vitally important but largely ignored issue is whether these tumor subgroups respond differently to therapeutic agents such as the 5-fluorouracil-based treatments commonly used against CRC. The marked difference in frequency of heavily methylated tumors between right- and left-sided CRC may be directly related to observations of tumor site differences in the survival benefit from 5-fluorouracil.^{16,17} It would seem we have reached the era where epidemiologists and medical oncologists, in line with molecular oncologists and pathologists, will in turn also need to consider the existence of more than one type of CRC.

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