

Epigenetic Markers in Rectal Cancer

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DNA methylation changes in rectal cancer may serve as a new screening marker and a tool for monitoring recurrence. Importantly, these changes may also function as a predictive marker to allow appropriate exclusion of (neo)adjuvant therapies in patients at low risk for disease recurrence, sparing them from potential treatment-related morbidities. *Clin Cancer Res*; 16(10); 2699–701. ©2010 AACR.

In this issue of *Clinical Cancer Research*, de Maat and colleagues reported the quantitative MINT locus methylation profile and its association with local recurrence in 325 of 924 rectal cancer patients undergoing total mesorectal excision (TME) only in the Dutch study (1).

Rectal cancer has both an insidious propensity for local invasion with risk of pelvic recurrence and loss of anorectal function as well as systemic spread resulting in profound patient suffering, morbidity, and mortality. Over the past 25 years, significant progress has been achieved in the treatment of patients with localized rectal carcinoma. The adoption of preoperative radiation therapy or chemoradiation followed by TME into the routine management of these patients has resulted in reduced local recurrence rates and improved disease-related outcomes.

The relative contributions of these two treatment modalities to outcomes were evaluated in the Dutch Colorectal Cancer Group trial, which randomized 1,861 patients with clinical stage I to III rectal cancer to short course preoperative radiation therapy followed by TME or TME alone (2). The 5-year local recurrence risk of 873 patients undergoing radiation therapy and macroscopically complete TME was 5.6% versus 10.9% in 875 patients undergoing TME alone ($P < 0.001$), although this number may underestimate the benefit of radiation therapy through the inclusion of early stage patients at very low risk of developing pelvic recurrence following TME alone. Although there was clinically meaningful and statistically significant reduction in local recurrence, no difference in 5-year overall survival rates were seen between the two treatment arms.

Continued analysis of patients given radiation therapy + TME versus TME alone has identified which patients have higher rates of acute and late side effects, complications,

and perturbations in health-related quality of life including sexual function (3). Considering the satisfactory outcomes in a relatively high percentage of patients undergoing TME only and the potential risk of radiation therapy-related morbidity, there are continuing efforts to better identify patients at low risk for pelvic failure and, therefore, optimal candidates for TME alone. Improved imaging techniques, refinements in pathological analysis and staging, assessment of response to preoperative therapy, and more recently, molecular biomarkers have been examined to enhance selection of patients.

Using patient tumor and normal rectum-containing archival material from the Dutch study, investigators have undertaken a variety of hypothesis generating analyses. These include examining the expression of molecular biomarkers (cyclooxygenase 2, epithelial HLA-DR, PIK3CA mutations, p53 mutation, intrinsic apoptosis, gene expression profiles), comparing their expression to the development of local recurrence and distant metastases following treatment (4). Some of these biomarkers have shown positive associations with clinical outcome whereas others have not. To date, no prospective clinical trial using a molecular biomarker as a critical element in its design has evolved from these analyses.

Molecular studies of many types of cancer have revealed that tumors carry multiple genetic and epigenetic abnormalities, including gene mutation, amplification, and aberrant promoter hypermethylation. Together, these aberrant genetic and epigenetic changes result in the activation of oncogenes and inactivation of tumor-suppressor genes. The aberrant methylation of the CpG islands located at the promoter region of specific genes is established as a common and early event involved in cancer development. Genes involved in essentially all facets of tumor development and progression have been shown to be targeted by aberrant methylation in human malignancy (Fig. 1; refs. 5, 6). DNA methylation has shown great promise as a marker that can be used to predict outcome or response to therapy in cancer patients and also as a mechanism for early cancer detection.

The prognostic value of DNA methylation biomarkers has been shown for a number of different cancers. In colon cancer, aberrant DNA methylation of specific

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doi: 10.1158/1078-0432.CCR-10-0559

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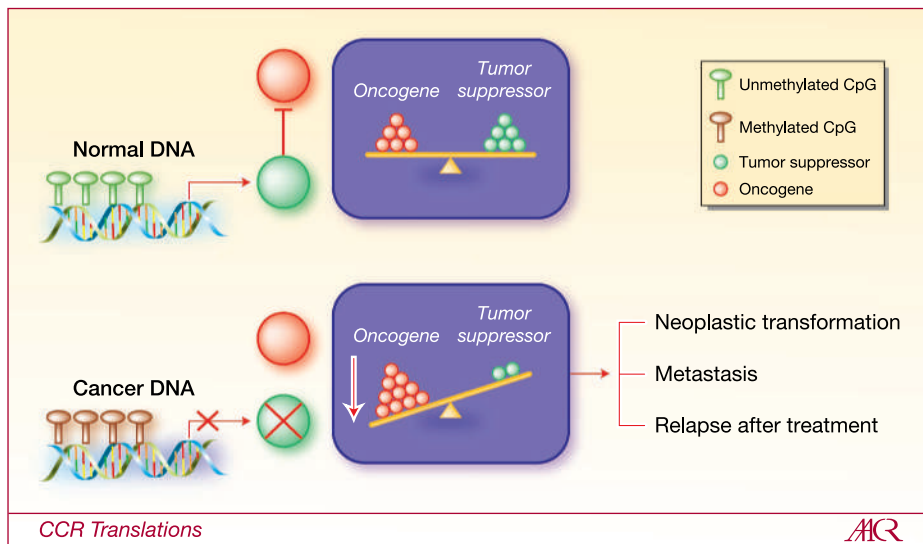


Fig. 1. CpG islands in normal epithelial cells are generally unmethylated. Cancer cells often show *de novo* methylation of CpG islands and it can result in the transcriptional silencing of tumor suppressor genes and tip the balance between oncogenes and tumor suppressors. This result can have a causal role in initiate tumorigenesis, tumor metastasis, and tumor recurrence after therapy in numerous forms of human cancers.

loci has been identified in the earliest precursor lesions for colon adenocarcinomas, aberrant crypt foci (ACF). *MINT1*, *MINT31*, *SLC5A8*, and *MGMT* methylation has been found in ACFs and in adenomas (7) and has been shown to drive the initiation and progression of colon cancer (8). Recent studies have shown that epigenetic silencing of genes involved in Wnt signaling is an alternative mechanism in colorectal carcinogenesis (9). In addition, many studies reported epigenetic silencing of genes involved in tumor growth, angiogenesis, and metastasis (6, 8). However, very few studies have evaluated DNA methylation as a prognostic marker and biomarker for response to treatment in rectal cancer.

In a previous study from Hoon's group, they measured methylation levels at multiple loci and identified two specific MINT loci, possibly related to rectal tumor formation, and which may serve as surrogate markers of distant metastases development in stage I and II TME patients. They found that the methylation status of *MINT2*, *MINT3*, and *MINT31* is involved in the adenomatous transformation phase, suggesting that these events occur during initiation of carcinogenesis (10). Building on these initial findings, de Maat and colleagues, in this issue of *Clinical Cancer Research*, propose that on the basis of absolute quantitative methylation levels of the *MINT3* and *MINT17* loci, rectal cancers with a high risk of local recurrence can be identified. This finding is intriguing given the high standards of surgery and pathology review of these TME patients.

One of the major obstacles to highly efficacious cancer therapy is the propensity for malignant tumors to become resistant to treatment and develop recurrence. Biomarker studies to correlate disease recurrence and metastasis development are critically needed. An important issue in biomarker study is the accessibility and stability of samples. There are several advantages of

methyated genes as biomarkers for response to therapy (11). First, methylated DNA can be detected in tumor-derived DNA found in the serum of cancer patients, as well as in samples obtained from cancer patient stool, vaginal secretions, or peritoneal fluids (12). Therefore, obtaining the sample is a noninvasive procedure and does not require bowel preparation, and would have a much higher patient acceptability. Second, sample transportation and handling protocols are not as strict as those required for RNA or protein expression analysis. The easy accessibility and handling of samples makes it possible to detect early and closely monitor disease recurrence.

On the basis of the two studies from this group, DNA methylation changes in rectal patients may serve, on the one hand, as a possible new screening marker for rectal cancer and, on the other hand, as a tool for monitoring recurrent disease in rectal cancer patients after treatment. Importantly, it may also serve as a predictive marker to allow appropriate exclusion of (neo)adjuvant therapies in patients at low risk for disease recurrence, sparing them from potential treatment-related morbidities. However, although intriguing, as pointed out by the authors, this biomarker-oriented approach to individualize therapies requires confirmation in prospective trials, given that disease recurrence is often unsalvageable and leads to disease-related mortality in the majority of patients. Nonetheless, these and other data continue to provide valuable insight in the quest to better individualize therapies on the basis of a given tumor's molecular profile.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received 03/23/2010; accepted 03/24/2010; published OnlineFirst 05/11/2010.

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