Moving Closer to a Prognostic DNA Methylation Signature in Colon Cancer

F. Javier Carmona and Manel Esteller

Although large panels of genes undergoing aberrant CpG island methylation in colorectal cancer have been identified, reliable predictors for clinical management in this tumor type remain elusive. A new DNA methylation signature affecting the extracellular matrix (ECM) pathway has been identified with potential prognostic value in colon cancer. Clin Cancer Res; 17(6); 1215–7. ©2011 AACR.

In this issue of Clinical Cancer Research, Yi and colleagues (1) report a widespread epigenetic inactivation affecting extracellular matrix (ECM) pathway genes in colon cancer. In addition, the authors have tested this signature as a prognostic indicator for individualizing stage II colon cancer patients at high risk of recurrence, who would benefit from adjuvant treatments (1).

An aberrant DNA methylation profile has been recognized as a common hallmark in all types of human neoplasia (2). DNA methylation is an essential process for the maintenance of homeostatic equilibrium in normal cells, and alterations in normal DNA methylation patterns in association with human disease affect almost all cellular pathways. Disruption of normal epigenetic patterns occurs from early stages of tumor development and accumulates throughout cancer progression. Research in cancer epigenetics has, so far, yielded a significant number of candidate genes whose methylation status is informative of cancer cell behavior. Specific DNA methylation profiles have been associated with particular types of cancer, and biomarkers exist with the potential to anticipate tumor formation and to predict metastatic progression or the risk of recurrence after surgical removal. Colon cancer, in particular, is a well-established model for DNA methylation studies (3). In colorectal tumorigenesis, the promoter CpG island methylation-associated silencing of 2 genes, the mismatch repair gene hMLH1 (4) and the 06-methylguanine-DNA methyltransferase gene MGMT (5), leads to 2 different mutator pathways characterized by insertion and/or deletion in many downstream targets and point transition mutations in K-ras and p53 (6, 7), respectively. The value of DNA methylation biomarkers lies in their stability in the face of subtle variations in the cell environment, which can nevertheless deeply affect transcription profiles, and in the availability of sensitive, simple, and cost-effective techniques to detect them. Importantly, gene hypermethylation is frequently associated with gene inactivation, and, given its plasticity, it is a modification that has the potential to be reverted by epigenetic drugs. Thus, aberrant DNA methylation has important implications for cancer research and management. However, despite all the efforts made, clinically relevant biomarkers valuable in the management of cancer patients remain scarce, with the exception of MGMT and GSTP1 hypermethylation in gliomas and prostate carcinomas, respectively (2).

In this issue of Clinical Cancer Research, Yi and colleagues (1) have adopted an integrative approach whereby they combine genomic data about gene mutations reported in colon and breast cancer (8) with epigenomic analyses to identify the pathways inactivated in colon cancer. As a result, the authors have found a series of genes undergoing epigenetic inactivation that result in the impairment of the ECM pathway (1). This pathway seems to be altered in the majority of colon cancers and probably facilitates cancer cell dissemination, thereby making it a poor prognosis factor. Alterations in ECM remodeling, as a major constituent of the microenvironment, have important implications for tumor biology and progression, providing a permissive milieu for cancer cell growth and dissemination (9). The results provided by the authors include a set of 29 genes featuring cancer-specific methylation, in other words, genes that are unmethylated in normal colon tissue and that are susceptible to genomic mutations that are enriched in ECM components. These genes seem to be more often epigenetically inactivated than genetically disrupted. Interestingly, 19 of these genes display frequent DNA hypermethylation in colorectal cancer, underlining the relevance of ECM impairment in cancer progression.

Promoter DNA methylation of selected genes (IGFBP3, EVL, CD109, NRCAM, NTNG, and FLNC) was assessed in relation to survival in 3 cohorts of colon cancer patients. The authors report an overall greater risk of mortality in
relation to the DNA hypermethylation of individual genes or of combinations of them (Fig. 1). Of special relevance is the signature composed of \textit{IGFBP3}, \textit{EVL}, \textit{FLNC}, and \textit{CD109}, whose CpG island methylation is associated with an 8-fold increase in mortality risk relative to that of patients with no DNA methylation of these genes. The results were similar in 1 of the 2 validation cohorts surveyed (John Hopkins University and the Netherlands Cohort Study on Diet and Cancer), most likely because of diverse clinico-pathologic and environmental factors influencing the populations examined. However, individual hypermethylation of \textit{CD109} kept its previous trend, and \textit{EVL} retained its prognostic significance in both cohorts. The signature proposed here has obvious potential to be of prognostic significance at a clinical level. This finding should be confirmed in prospective studies.

Furthermore, the authors describe an association between DNA methylation of 2 of the 6 selected ECM genes (\textit{IGFBP3} and \textit{CD109}) and a high risk of recurrence (Fig. 1). This finding was tested on a homogeneous cohort (John Hopkins University) and should be confirmed in independent series of patients. Another interesting report described LINE-1 hypomethylation levels to be of prognostic significance (10). Nevertheless, to date, few reports have provided conclusive results on the prognosis of colon cancer patients, and this is the first one to encompass both genomic and epigenomic events. Major translational implications arise from this study because it has the potential to enable stage II colon cancer patients to be categorized and those benefitting from adjuvant therapy to be selected (1).

Overall, this is a valuable report that proves the significance of a prognostic signature with potential clinical applicability. This and other studies of outcome prediction signatures should be reproduced by independent laboratories, and the findings confirmed by a wide range of techniques, and then borne in mind in the design of further studies. In addition to these analyses, it would also be interesting to investigate quantitative variation at the level of CpG island methylation in specific genes, because this might yield important clues about tumor progression. High-throughput, unbiased strategies aiming to complement currently available data are likely to provide

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Figure 1. CpG island hypermethylation in colon cancer and the proposed DNA methylation signature for prognosis.
meaningful biomarkers that increase the discriminatory power of the available predictive signatures. Molecular markers to predict recurrence in stage II colon cancer patients are available in the literature based on DNA methylation (10), gene expression (11), or microRNA profiling (12). Nevertheless, no prognostic signature so far has been transferred to the clinic, and standards for clinical management are still based on clinico-pathologic parameters (node involvement, tumor infiltration, etc.) and limited molecular clues, MSI microsatellite instability being the best example. Furthermore, many of the current screening guidelines entail invasive protocols that are traumatic for the patient. Predictive imprints need to be validated in extensive prospective studies in order to develop (epi) genetic-based prediction models to help guide the selection of adjuvant treatments for patients. The signature reported here should follow similar standardized pipelines to prove its clinical usefulness. Furthermore, ongoing trials evaluating available signatures or single indicators in stool and blood from colon cancer patients are expected to provide sensitive biomarkers for rapid, unaggressive, and cost-effective assessment of risk of recurrence or metastatic development.

The past decade has witnessed major advances in our knowledge of the clinical and molecular aspects of colon cancer. However, the gaps remaining in our understanding of cancer are large and significant. Further efforts need to be made to design informative panels of biomarkers that are able to anticipate the malignant phenotype effectively. The authors whose work is discussed here have designed an integrative approach that provides encouraging data and places 2 clinically relevant issues, the prediction of cancer cell behavior and patient stratification, firmly in the spotlight.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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