Methylation and Prognosis: Of Molecular Clocks and Hypermethylator Phenotypes

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Cytosine DNA methylation in CpG-rich promoters is now a well-described component of epigenetic silencing in human cells (1). Cancer cells appear to usurp this normally restricted process and use it to silence undesirable genes such as tumor suppressor genes (2). Over the past few years, aberrant DNA methylation was recognized as one of the most common molecular abnormalities in human cancers. With the rising tide of papers describing this process in a variety of malignancies, researchers are inevitably beginning to address the clinical significance of these findings. In this issue, Brock et al. (3) report on hypermethylation of seven genes in 41 patients with esophageal carcinoma treated by surgery alone. The authors found that cancers with more frequent methylation had a significantly worse survival and, in multivariate analyses, report that methylation is a better predictor of outcome than stage or age.

How can methylation and associated epigenetic changes impact prognosis in human cancers? In a manner analogous to genetic changes, investigators early on studied the impact of methylation of single genes on disease-free and overall survival. In these studies, the implicit assumption was that methylation affects silencing and, therefore, function of the gene in question. Thus, the impact on survival would be related to the altered biology of the tumor as a result of this change. Indeed, several well-powered studies reported that methylation of individual genes could have prognostic and/or predictive importance in multivariate analysis. These included favorable outcome of ER methylation in patients with acute myelogenous leukemia (4), favorable outcome of MGMT methylation in patients with non-Hodgkin’s lymphoma (5), and unfavorable outcome of DAPK methylation in early stage non-small cell lung cancer (6). However tempting it is to attribute such statistically significant results to the function of a single gene, one needs to exercise caution before rushing to target the offending molecule. Another implicit assumption of prognostic (and predictive) studies is that the disease is fairly uniform and that the two groups of tumors (methylated/unmethylated at a single gene) are otherwise very similar. This, of course, is a vast oversimplification as marked heterogeneity between different tumors of the same histological type is a dominant theme of modern tumor biology (7).

A new generation of studies, including the one reported in this issue, is beginning to interrogate the prognostic significance of methylation of multiple genes in various malignancies. This is a statistically treacherous route, and reported positive results need confirmation in separate groups of patients. Nevertheless, a pattern is emerging whereby methylation of single genes have no or weak prognostic significance, whereas methylation of multiple genes is often associated with shortened survival after standard therapy. In addition to esophageal cancers described here (3), this phenomenon has been described in bladder cancer (8), in head and neck cancer (9), in ovarian cancer (10), and in acute lymphocytic leukemia (11). Moreover, in early stage non-small cell lung cancer, methylation of each of DAPK (6), P16 (12), MGMT (13), and IGFBP3 (14) have been separately reported as having negative prognostic significance. This remarkable set of findings most likely points to convergence toward a single group of lung cancers with a high degree of methylation (of multiple genes) and associated poor prognosis.

Why would methylation of multiple genes portend an unfavorable outcome? Methylation requires cellular replication (15). In proliferative tissues such as colon epithelium, methylation of some genes increases linearly as function of age (16, 17). Increased proliferation related to injury/inflammation is associated with increased methylation in normal appearing tissues, such as in nondysplastic colon epithelium in patients with ulcerative colitis (18) or nondysplastic esophageal epithelium in patients with Barrett’s esophagus (19). Thus, in some situations, methylation may reflect a molecular clock of past replication. Brock et al. (3) suggest that tumors with methylation of multiple genes may have a worse outcome because this molecular clock reflects how long the cancer has been present, assuming that (undefined) molecular lesions associated with a worse outcome accumulate as a result of this longer incubation period. However, in the clearest example of methylation as a molecular clock, that is hypermethylation of the translocated ABL promoter in the Philadelphia chromosome characteristic of chronic myelogenous leukemia (20), methylation was shown to increase progressively with advancing stage of the disease and to have minimal prognostic importance in early stage disease (21). Thus, if methylation of multiple genes simply reflects a molecular clock, one should see a clear-cut increase in methylation with increasing size of the tumor or stage. In this (3) and other (22) studies, this has generally not been observed. Indeed, methylation precedes neoplasia in many cases, a finding that could be related to aging as well.

If not (solely) a molecular clock, then what? Simultaneous and intense hypermethylation of multiple genes has been described as a distinct phenotype in colorectal cancer, and was
termed descriptively CIMP\(^3\) for lack of a better (mechanistic) explanation (22). CIMP has characteristic clinical, pathological, and molecular features, being more common in women, in the proximal colon and in poorly differentiated tumors (23–25). CIMP\(^+\) cases also have a distinct profile of genetic abnormalities (23, 25). The presence of this phenotype in colon cancer has been confirmed (24, 25), and it was also described in multiple other malignancies (26–28). In esophageal cancer, a previous study was unable to discern a CIMP\(^+\) group (19). However, whereas not formally analyzed, scrutiny of Figure 1 in the paper by Brock et al. (3) reveals significant correlations (by the Fisher’s exact test) among methylation of \(P16, MGMT, ECAD,\) and \(ER\) a quatum of genes that defines best the poor prognostic subset in this study. The other three genes studied were either too infrequently (\(DAPK\) and \(TIMP3\)) or too frequently (\(APC\)) methylated to factor in a methylation-based classification. These results can be interpreted as revealing the presence of a distinct group of esophageal cancers marked by the simultaneous methylation of multiple genes (\(i.e., \) CIMP\(^+\)). By analogy to colorectal cancer, CIMP\(^+\) esophageal cancer cases could represent a markedly distinct group, a hypothesis that will need confirmation. Nevertheless, by proposing that CIMP\(^+\) cancers arise through vastly different mechanisms (and indeed may have different triggering environmental factors, as shown in hepatocellular carcinomas; Ref. 27), it becomes easier to understand why they might have such a different clinical course. Presumably, a few disease progression genes affected by CIMP are inactivated preferentially in this group of tumors, leading to the adverse clinical outcome. It would also be of particular interest to relate this subset of cancers to other genetic changes and to global gene expression profiles in esophageal cancer and other malignancies.

A huge attraction of research into the epigenetics of cancer is the prospect of intervening therapeutically to reverse these changes (29). Drugs that modulate DNA methylation are in clinical trials (30) and have been shown to potentially affect gene expression in vivo (31). Such approaches could be particularly useful in the subset of patients with poor prognosis as a result of hypermethylation of multiple genes.

References


\(^3\) The abbreviations used are: CIMP, CpG island methylator phenotype.
Clinical Cancer Research

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