Aberrant Methylation of Gene Promoters in Cancer—Concepts, Misconcepts, and Promise

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In this issue of the Journal, Tang et al. (1) report that the presence of abnormal methylation in the 5′ region of the death-associated protein (DAP) gene in tumor DNA predicts shorter survival in patients who had undergone surgery for non-small-cell lung cancer (NSCLC). This change in methylation is an example of an epigenetic process that is attracting increasing attention, both because of its potential significance to our basic understanding of cancer and because of its possible use for improved cancer diagnosis and treatment. However, the study by Tang et al. also raises many of the questions that constitute an ongoing vigorous, but constructive, debate as to the true biologic significance of these postreplicative DNA changes (2).

“CpG islands” surround the transcription start regions of almost half of the genes in the human genome and are normally unmethylated. Hypermethylation of CpG islands in cancers is associated with transcriptional silencing of the genes in which this change occurs. “Association” is the operative word here because central to this debate is whether methylation initiates, or is even essential for, gene silencing. However, the main point of the study by Tang et al. involves CpG methylation as a molecular marker that may be independent of its functional implications. Indeed, as the authors imply, promoter hypermethylation is emerging as one of the most promising molecular strategies for early detection of cancer, independent of its role in tumor development.

Methylation of CpG islands may act as a relatively simple “yes–no” signal for the presence of tumor when examined for under optimal assay conditions by sensitive polymerase chain reaction (PCR) techniques such as the one (3) used by Tang et al. When cancers, even of the same histologic types, are compared between individual patients, DNA mutations in genes such as p53 or mitochondrial genes (4) often involve myriad different base changes at many locations within the gene. In contrast, aberrant promoter hypermethylation always occurs in virtually the same location within an affected gene, allowing a single PCR primer to be applicable to all patients for examination of the methylation status of a specific gene. Most important, sites distant from primary tumors can then be studied without first examining tumor DNA from each individual patient. Further evidence of a useful diagnostic role for methylation is the finding that the hypermethylation changes for a given gene can be extremely frequent in a cancer type (5), as was found by Tang et al. for DAP in NSCLC. The number of genes known to be hypermethylated in cancer is also growing, and the ability to detect most tumor types with a relatively small panel of these markers is a distinct possibility. Proof of principle for these above possibilities is apparent in the recent finding (6) that hypermethylation markers are detected with high frequency and sensitivity in sputum DNA from patients with squamous cell lung carcinoma up to 3 years before clinical diagnosis (6).

Tang et al. (1) suggest that DAP hypermethylation may be a marker for monitoring prognosis in patients with NSCLC. Certainly, as the authors point out, this possibility must be validated by additional larger studies. In this regard, it will be of interest to see how DAP hypermethylation may relate to other molecular alterations, such as p53 mutations, that might influence prognosis for patients with NSCLC. Why should methylation changes in the DAP gene be capable of providing this type of marker? It is possible that distinct patterns of epigenetic changes in this gene (or other genes) could simply accompany pathways to the evolution of certain tumor types that predict for their metastatic behavior. Alternatively, as the authors suggest, loss of function of a given hypermethylated gene may constitute the specific factor dictating tumor behavior. Indeed, DAP has been implicated in the metastatic potential of lung cancer in an experimental model (7). Moreover, a recent study (8) found an association between the presence of DAP hypermethylation and lymph node involvement in patients with head and neck cancer. Finally, the DAP gene encodes a protein that mediates apoptotic response, and reactivation of a hypermethylated DAP gene can restore interferon-induced apoptosis in tumor cells (9).

The possibility that DAP function determines tumor behavior points to the critical questions about the significance of promoter hypermethylation in cancer (2). Do such changes really signify biologically important events? In properly demanding more concrete data to answer this question, participants in the debate often fail to examine the selective advantage of hypermethylation (2). This is a most fundamental biologic principle for suggesting the functional significance of any given DNA change and, for the patterns of hypermethylation of bona fide tumor suppressor genes in cancer, such selective advantage tracks exceedingly well with predictions of biologic importance (5). Indeed, the hypermethylation changes often mimic the patterns for mutations in these same genes. For example, both genetic and epigenetic alterations of the VHL, BRCA1, and STK11 genes are restricted to the same specific tumor types (5,10). Also, there is an absolute inverse relationship, in tumors of multiple types, between genetic disruption of the Rb gene and both genetic or hypermethylation changes for the p16INK4a gene (5). In addition, loss of gene function in the presence of promoter methylation can be associated with the predicted phenotypic consequence, i.e., MLH1 and the microsatellite instability phenotype in colon
carcinoma (11). Thus, whether or not the methylation is fully responsible for transcription silencing of a hypermethylated gene, this change seems hard to refute as a signature for the mechanisms responsible for loss of gene function when selective advantage is carefully examined.

Does every promoter hypermethylation change in tumors signify a biologically important event? The answer is almost certainly “no.” However, one might best view the problem with the same eye as one views the consequences of a change, such as germline or somatic mutations, in the mismatch repair genes. These latter events as biologic processes certainly contribute directly to tumorigenesis, yet only occasionally is the coding region of a key gene involved. Instead, the clonal presence of mutations in the repair genes reflects the global importance of the defective repair process, rather than the contribution of the majority of the resultant mutations, to tumor development.

If promoter hypermethylation can disrupt the function of critical genes, what explains, at a molecular level, the associated gene silencing? This question has plagued the field of DNA methylation since its inception. Recent research suggests intriguing possibilities. Through methylcytosine-binding proteins, DNA methylation may recruit transcriptional corepressors and histone deacetylases that are critical for the composition of transcriptionally repressive chromatin (12). For hypermethylated cancer genes, the methylation appears dominant over the histone deacetylation activity in the process of repression (13). Furthermore, enzymes that establish DNA methylation are now appreciated as complex proteins that, independent of their methylating properties, have intrinsic transcriptional repression activity, the ability to target transcriptional corepressors, and the potential to regulate histone deacetylation in newly forming chromatin (14–16). Recent evidence (17) suggests that, in some cancer types, more than one such enzyme may be important for tumor gene hypermethylation. It will thus be important to define the precise complexes that repress the transcription of specific genes hypermethylated in cancer, with the possibility that they could have a role in actually causing the abnormal methylation patterns.

In summary, epigenetic events may be comediators with genetic alterations to drive tumorigenesis. Hypermethylation changes may be excellent tumor markers—and, as Tang et al. (1) note, the reversible nature of epigenetic gene silencing makes this process an attractive target for cancer therapy. Much has been learned, much remains unanswered, and only future results in both basic and clinical research will reveal the ultimate impact of findings to date for the understanding and control of cancer.

REFERENCES


