Commentary

Flipping the Epigenetic Switch

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Just as genetic instability is a hallmark of malignant cells, so too is epigenetic instability.^{1,2} A key difference is that, in contrast to permanent genetic alterations, epigenetic changes need not be permanent in either tumor cells or normal cells. Thus, when a cell adopts a new gene expression pattern as a result of epigenetic changes, that cell and its progeny reserve the capability to switch back to previous gene expression patterns. This phenotypic plasticity would provide epigenetic metastable or unstable cancer cells the ability to alter gene expression and appropriately adapt to its environment, as it takes its destructive journey from primary lesion to metastatic colony. For example, it is reasonable to propose that cancer cells would likely directly modulate the expression of genes involved in cell adhesion and motility or the transcription factors that control their expression during cancer progression. Early in cancer development these genes remain active in the primary tumor, however, as natural selection occurs, these genes need be silenced so that the tumor has the necessary phenotypic characteristics to metastasize. Following cancer cell colonization at a distant site, the malignant tumor may require reacquisition of those gene products that suppress the properties of motility and invasion, for example. Regulation of the expression of this complement of genes, through DNA methylation and related epigenetic mechanisms, provides a unique switch mechanism in tumor cells that is not as drastic as mutation. Interestingly, some tumor suppressor genes, such as p53, are typically mutated and deleted, and rarely, if ever, are silenced in association with DNA methylation.³ In contrast, some genes with tumor suppressor function, rarely, if ever, are mutated, and often are silenced by aberrant DNA methylation. Examples of this latter class of genes include MGMT, maspin, GST pi, and as is further reported here, ER β in prostate cancer.^{4–10}

Cancer Evolution

Cancer progression is characterized by the acquisition of genetic and epigenetic changes that lead to generation of phenotypic diversity among the progeny of cancer cells. The evolution of this diversity allows for the continual selection of cells that possess the most suitable attributes for survival under any given set of conditions in the host.

As the cancer cells undergo their dispersion, it is likely that critical, but potentially transient, changes in gene expression patterns occur as cancer cells break away from a primary tumor and invade local tissues and metastasize to distant sites. One gene that may promote survival in one physiological context (eg, primary tumor site, metastatic colony site) may be deleterious in another physiological context (invasive disseminating tumor). For this reason, altering gene expression patterns epigenetically could account for loss of gene expression under one set of circumstances yet allow for the re-expression of this gene should its function provide a selective advantage later in cancer progression. The contribution by Zhu and colleagues¹¹ in this issue of *The American Journal of Pathology* provides a provocative example of how an epigenetic switch at the ER β locus may work in the case of prostate cancer progression.

DNA Methylation, Its Known Accomplishes, Epigenetic Instability, and Cancer

Ordered patterns of DNA methylation exist in the human genome that are common to all normal cell types, such as methylation of satellite sequences and an absence of methylation in all, or almost all, CpG islands. In addition, there are regions of the genome that display cell-type-specific patterns of DNA methylation and these cell-type-specific patterns can be seen from the level of the whole chromosome down to the level of the individual gene. Importantly, these DNA methylation differences are asso-

Supported by National Institutes of Health grants to F.E.D. and B.W.F.

Accepted for publication March 22, 2004.

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ciated with differences in expression of the affected genes^{9,12–23}

Epigenetic regulation is a multifaceted control system, in which CpG methylation is just one of many aspects. Methylcytosine can recruit proteins to the genomic region in which it is localized, and these proteins can act as transcriptional repressors and proceed to alter the DNA topology of the region to render it transcriptionally inert; it is not readily accessible for binding by transcriptional activating factors or RNA polymerase and is not easily transcribed. One of the more completely delineated aspects of this epigenetic regulation has demonstrated that heavily methylated DNA recruits methylcytosine binding proteins to the region, which in turn recruits histone deacetylase complexes that enzymatically deacetylate histones H3 and H4; a change in the histone modification state that has been demonstrated to be incompatible with gene transcription, whereas acetylation of multiple lysine residues on histones H3 and H4 is associated with a transcriptionally competent or active state.^{24–27} Clearly, other histone modifications are important as well in controlling the epigenetic state (eg, histone methylation), and the interplay of these histone modifications with each other and with DNA methylation is an area of intense research activity.^{28–30} The control of these epigenetic changes and their temporal relationship remain an enigmatic and controversial area.

Disruption of the ordered patterns of DNA methylation is a hallmark of the cancer phenotype. These changes can occur early during cancer evolution and are complex in nature. Overall there is genomic hypomethylation, probably due to a generalized demethylation of satellite sequences.^{31–34} In the face of overall genomic methylation, CpG island promoters often become aberrantly methylated, which is clearly associated with the transcriptional silencing of the associated gene. Targets for aberrant methylation in cancer include cell cycle regulatory genes, genes involved in maintenance of genomic integrity, and metastasis suppressor genes that encode cell adhesion molecules and motility factors. With respect to gene regulatory sequences, it is well documented that hypermethylation of CpG island regulatory sequences results in the silencing of tumor suppressor genes.^{5,7,8,35–41} Interestingly, the patterns of aberrant methylation of CpG islands have been shown to be tumor-type specific.⁴²

Unlike the genetic mutations that accumulate in a cancer cell, epigenetic mutations (or modifications) can be readily reversed. Thus, when a cell adopts a new gene expression pattern as a result of an epigenetic change, that cell and its progeny retain the capability to revert back to a previous gene expression pattern. Therefore, neoplastic progression should perhaps not be considered as a continual march toward an increasingly aggressive phenotype, but rather as a stochastic trial and error saunter toward an increasingly aggressiveness in a “two steps forward, one step backward” manner. Importantly, CpG methylation and associated epigenetic modifications appear to be causally involved in gene silencing since pharmacological inhibitors of DNA methylation and histone modification can reactivate expression.^{6,43–45} The ability to reverse these cancer-related changes in epige-

netic control provides a therapeutic opportunity to target the epigenetic machinery and induce transcriptional reprogramming.

Role of ER β in Prostate Cancer

Despite the fact that estrogens have been used historically to treat prostate cancer, their specific mechanisms of action on the prostate remain largely unknown. Since the discovery of estrogen receptor β (ER β) in 1996, the role of ER β in prostate cancer development and progression has been intensively studied.^{46,47} What is clear is that estrogens, either alone or in combination with androgens, can have potent effects on growth and carcinogenesis of the prostate. Clues to the function of ER β in prostate carcinogenesis began to emerge when it was discovered that whereas both ER α and ER β mRNA and protein were expressed in normal human prostate epithelial cells, ER β expression was lost in primary cultures of human prostate carcinoma cells and prostate cancer tissues.^{48,49} On this basis it was suggested that ER β may be a negative effector of prostate growth and that its loss could facilitate tumor development. This idea has been complicated, however, by the finding that metastatic lesions of primary prostate cancers often display high levels of ER β expression. Taken together, the results appear to indicate that loss of ER β is an important early event in prostate carcinogenesis, whereas its re-expression at a later stage of disease progression (in metastatic disease) no longer impairs prostate carcinoma growth and in fact may provide some survival advantage. In addition, the re-expression of ER β in metastatic disease also provides an attractive target for therapy in advanced disease.

Interestingly, treatment of both prostate and breast carcinoma cells with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-aza-dC) led to reactivation of silenced ER α and in some cases ER β , providing evidence that the ER β gene, similar to the ER α gene, may be subject to regulation by DNA methylation. The new study from Ho's laboratory¹¹ published in this issue of *The American Journal of Pathology* shows that ER β is a target for methylation-mediated silencing in human prostate cancer. More interestingly, their results show that the cytosine methylation patterns of the CpG island change during cancer evolution. In normal tissue the 5' CpG islands associated with exon 0N is unmethylated and the gene is expressed. As the normal tissue evolves to grade 4/5 primary prostate cancer, aberrant methylation of the ER β 5' CpG islands appears coincident with partial or complete loss of ER β expression. Later in prostate cancer evolution, Zhu et al¹¹ find re-expression of ER β in metastatic lesions and this re-expression is associated with the epigenetic switch from a methylated CpG island to an unmethylated CpG island.

An Elegant Approach

Zhu and colleagues¹¹ used immunohistochemistry to identify ER β expression status in normal human pros-

tates, prostatic intraepithelial neoplasms (PIN), primary malignant cancers, and metastatic lesions. They then used laser capture microdissection to isolate pure populations of ER β -positive or -negative epithelial cells from the various samples. From these samples they extracted DNA and performed bisulfite genomic sequencing analysis of the ER β promoter region. This method is considered by most investigators to be the most rigorous and comprehensive approach to quantitatively assess genomic methylation. Their results clearly indicate that ER β -positive normal prostate epithelia and PIN lesions maintain the ER β promoter in an unmethylated state. In contrast, malignant primary cancers that are ER β negative have adopted a distinct pattern of aberrant hypermethylation on the ER β promoter. Most interestingly, in both lymph node and bone metastases that were ER β positive, the ER β promoter methylation pattern had switched back to its unmethylated state. Using ER β -negative human prostate carcinoma cell lines *in vitro* the authors showed that 5-aza-dC caused reactivation of ER β expression and demethylation of the ER β promoter. In fact, the observed loss of methyl cytosine from the ER β promoter in LNCaP and DU145 cells treated with 5-aza-dC was remarkable, and dramatically exceeded that seen in other similar experiments.

Combining their large methylation data set with powerful statistical and mathematical tools, the authors discovered regional differences in promoter methylation that appeared to correlate with expression status. Along with another recent study, we are beginning to see more sophisticated analyses of DNA methylation patterns that promise to provide new biological insights into the role of DNA methylation in genome function.⁵⁰ These are exciting developments indeed.

In an effort to determine whether these regional differences identified by cluster analysis were causally important to ER β gene silencing they used an innovative approach to target increased *de novo* methylation to specific sequences in the unmethylated promoter of ER β -positive PC3 cells. This is one of the few references to this method that we could identify in the literature.^{51,52} If this approach is widely applicable, it represents a potentially powerful new approach to not only manipulating gene expression, but also to shed light on how aberrant *de novo* methylation during cancer progression might be targeted to specific sequences. The overriding question about this approach is how does it work? Although a mechanism has been proposed whereby transfection of methylated oligonucleotides into cells can target methylation to the genomic DNA, there is little experimental evidence to support the proposed mechanism.

Conclusion

The rigorous approaches used by Zhu and colleagues¹¹ in their analysis of the dynamic regulation of ER β expression by DNA methylation have clearly identified a primary mechanism of regulation of this gene during prostate carcinogenesis. Similar mechanisms will likely apply in other malignancies such as breast cancer as well. These

results have reinforced the concept of epigenetic instability in cancer progression, and the existence of an epigenetic switch that participates in driving the natural history of cancer progression.

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