Retinoic Acid Receptor β2 Hypermethylation: Implications for Prostate Cancer Detection, Prevention, and Therapy

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INTRODUCTION

Cancer development involves multiple steps during which cells acquire unlimited replicative potential, autonomy in growth promoting signals, resistance to growth-suppressing signals and apoptosis, effective angiogenesis, and enhanced ability to invade and metastasize (1). The acquisition of this malignant phenotype is the consequence of both genetic and epigenetic changes in the expression and function of various genes, including oncogenes and tumor suppressor genes (1). The inactivation of certain tumor suppressor genes defined as class I (2) is mediated by genetic mechanisms including loss of function mutations, gene rearrangements, or gene deletion and loss of heterozygosity. Class II tumor suppressor genes were defined as those in which the wild type gene remains intact but its loss of function results from altered regulatory events such as mutations or deletions in a different gene (2). More recent studies have demonstrated that epigenetic mechanisms can silence the expression of class II tumor suppressors. One of the major epigenetic gene silencing mechanisms is the methylation of CpG dinucleotides that appear to cluster in islands in the regulatory regions (promoter or other) of a variety of genes. DNA methylation has received considerable attention over the last few years with the increase in the number of tumor suppressor genes found to be regulated by this mechanism (3–6). For example, in prostate cancer, different degrees of hypermethylation were reported for many genes, including anaphase-promoting complex, androgen receptor, caveolin-1, CD44, cyclin D2, death-associated protein kinase, E-cadherin, endothelin B receptor, estrogen receptor β, glutathione S-transferase π, hypermethylated in cancer 1, laminin-5, O6-methylguanine-DNA methyltransferase, p16 (INK4a), retinoic acid receptor β2 (RARβ2), Ras association domain family 1A, O6-methylguanine-DNA methyltransferase, and RARβ2 has been detected already at early stages of carcinogenesis such as the premalignant lesion prostatic intraepithelial neoplasia (PIN), whereas the hypermethylation of other genes was only detected in prostate carcinoma (PCa; Ref. 9). The methylation of many of these genes has been correlated to clinicopathological features of poor prognosis.

RARβ2 mRNA was undetectable by in situ hybridization not only in malignant PCa, premalignant PIN, but also in the benign prostatic hyperplasia (BPH) and normal-appearing epithelial cells within histological sections of formalin-fixed, paraffin-embedded prostate tissue specimens (11). In this issue, Jeronimo et al. (12) have demonstrated that this observation may be, at least in part, the result of RARβ2 promoter hypermethylation, which increases during prostate cancer progression. They used quantitative methylation-specific PCR (QMSP) to examine tumor tissue from 118 patients with PCa, 38 paired high-grade PIN (HGPIN), which were obtained by radical prostatectomy, and nonneoplastic prostate tissue from 30 patients with BPH, which were derived from transurethral resection of the prostate. They found RARβ2 hypermethylation in 97.5% of PCa, 94.7% of HGPIN, and 23.3% of BPH. In addition, methylation levels were significantly higher in PCa compared with HGPIN and BPH. Thus, Jeronimo et al. (12) were able to identify differences (a) between PCa and the premalignant lesion HGPIN in terms of methylation levels, and (b) between both PCa and HGPIN and BPH in terms of the percentage of cases with methylated RARβ2. They have demonstrated that the QMSP method can discriminate between neoplastic and nonneoplastic tissue, with high sensitivity and specificity. Moreover, RARβ2 methylation levels correlated with higher pathological stage.

These findings are potentially important because they strongly suggest that RARβ2 methylation represents a novel and promising molecular marker and that the quantitative methylation assay may augment current approaches for prostate cancer detection. The authors contend that this finding may allow improved detection of prostate cancer in tissue biopsies. Although this may be true in difficult cases that cannot be diagnosed by pattern recognition, most prostate cancers are not difficult to identify on biopsy. Because Jeronimo et al. (12) had found 23% of BPH to have methylation, it would be interesting to determine whether adjacent normal-appearing tissues also manifested changes in RARβ2 methylation, as suggested by the previous in situ hybridization experiments (11). If this is the case, then one might be able to use methylation levels to identify those patients who have cancers that were missed on negative prostate biopsies. This would be of great benefit because patients with elevated prostate-specific antigen levels can have either prostate cancer or BPH, and repeat biopsies can have up to a 20% cancer detection rate after an initial negative biopsy. Because only 30–40% of patients with prostate-specific antigen levels of 4–10 ng/ml have prostate cancer, there are many

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patients with negative biopsies who would benefit from improved ability to discriminate cancer from benign such as the QMSP described by Jeronimo et al. (12).

Another question is how much tissue is required for this assay because significantly less tissue is available after prostate biopsy compared with tissue obtained from a prostatectomy specimen used by Jeronimo et al. (12). Apparently, this should not be a problem because this group has used sextant biopsies of to detect PCa by QMSP for the glutathione S-transferase π gene (13). It would also be of interest to explore the ability of the QMSP method to detect RARB2 methylation in prostate secretions used for detection as suggested by Gonzalez et al. (14). Future studies should evaluate whether methylation levels are predictive of recurrence or progression patterns and levels of expression in metastases. Because there was a correlation between methylation levels and pathological stage, one might be able to identify patients at higher risk of recurrence who may benefit from adjuvant therapies.

In addition to the potential of using the QMSP for RARB2 for early diagnosis and prognosis determinations, the findings suggest that reversal of methylation may be an approach for prostate cancer chemoprevention and therapy. RARB2 is considered to be a tumor suppressor gene based on the decrease in its expression levels at early stages of carcinogenesis in a variety of epithelial tissues (15). Furthermore, evidence from studies with cell lines indicates that sensitivity of cells to effects of retinoic acid is lost when RARB expression is suppressed and can be restored by re-expression of RARB2. Because RARB expression is silenced by methylation and the gene is intact, its expression can be awakened by demethylating agents or by agents that affect chromatin structure alone or combined with retinoic acid, the natural inducer of RARB. Indeed, the reversal of RARB2 gene silencing has been achieved by treating cells and tumors with demethylating agents [5-aza-2′-deoxycytidine or (-)-epigallocatechin-3-gallate, a green tea polyphenol] or histone deacetylase inhibitors (trichostatin A, phenylbutyrate, or suberoylanilide hydroxamic acid) alone or combined with each other and with retinoic acid (16–21). Thus, these small molecules are promising agents for cancer chemopreventive and chemotherapeutic intervention (22–25).

REFERENCES
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