

■ TUMOR BIOLOGY

Methylation of Genes in Prostate Tumor–Associated Stromal Cells

Hanson JA, Gillespie JW, Grover A, Tangrea MA, Chuaqui RF, Emmert-Buck MR, Tangrea JA, Libutti SK, Linehan WM, and Woodson KG. Gene promoter methylation in prostate tumor–associated stromal cells. *J Natl Cancer Inst* 98: 255–61, 2006.

There is currently much interest in characterizing changes in the cells that compose the tumor microenvironment because it is now thought to be as important as the tumor itself in tumor progression. The prostate gland is composed of glandular epithelial tissue that is supported by stromal compartments predominantly comprising fibroblasts and smooth muscle cells. The stromal compartment of the tissue microenvironment associated with cancer epithelia is fundamentally different from that of normal tissue. Unique characteristics of the tumor microenvironment include an activated cellular phenotype that more readily supports tumor growth, presence of modified extracellular matrix proteins, and increased micro-vessel density. Several studies have noted alterations in gene and protein expression in stromal cells associated with tumors. Genetic modifications such as loss of heterozygosity, p53 mutation, and mutation of the phosphatase and tensin homolog (*PTEN*) gene have been described in stromal cells adjacent to breast carcinomas. Although most DNA-methylation studies have focused on tumor epithelial cells, we and others have more recently shown aberrant DNA-methylation patterns in tumor-associated stromal cells.

Epigenetic alterations such as promoter DNA hypermethylation are one of the hallmarks of carcinogenesis; hypermethylation is one of the most common alterations in human prostate cancer with more than 90% of tumors having aberrantly methylated genes. DNA methylation refers to the covalent bonding of a methyl group to the dinucleotide CpG, catalyzed by a group of enzymes called DNA methyltransferases. The majority of CpG dinucleotides in the genome, which are methylated in normal cells, are dispersed across retrotransposons or are found within the coding regions and introns of genes. About 15% of these dinucleotides are clustered in what are called CpG islands in the promoter regions of genes and are normally unmethylated. In tumors, promoter CpG islands are often methylated (or hypermethylated), a state that facilitates tumorigenesis by the silencing of tumor suppressor or other regulatory genes.

To investigate the presence of epigenetic changes in the tumor microenvironment, we evaluated the methylation of three genes important in prostate carcinogenesis in the tumor

epithelium and stromal cells from prostate specimens of prostate cancer patients. For this analysis, we used two separate microdissection techniques (laser capture and expression microdissection) to validate the selective isolation of epithelium from the stromal compartment. This was important because cross-contamination of cell types (in particular, the presence of tumor epithelium in stromal samples) could produce spurious methylation results. Gene-methylation status was analyzed using quantitative methylation-specific PCR and was confirmed in some samples by a second technique shown to be accurate for quantitative methylation analysis (pyrosequencing). We found that glutathione S-transferase pi 1 (*GSTP1*) and retinoic acid receptor β 2 (*RAR β 2*) were methylated in the tumor epithelia of all patients (similar to other reports) and in the tumor-associated stroma of 80% of the samples. Methylation of *CD44* was observed in 80% of prostate tumor epithelia samples but not in any of the tumor-associated stroma (Figure 1). The biological significance of the presence of *GSTP1/RAR β 2* methylation and the absence of *CD44* methylation in stroma is unclear. Methylation of *GSTP1* and *RAR β 2* has been shown to be prevalent in prostate tumors (in more than 90%), and their methylation occurs early in prostate carcinogenesis (seen in approximately 50% of prostate intraepithelial neoplasia, a prostate cancer precursor lesion). Methylation of *CD44*, however, is more likely to be associated with aggressive cancer (more prevalent in high-grade tumors) and is not observed in early pre-neoplastic lesions.

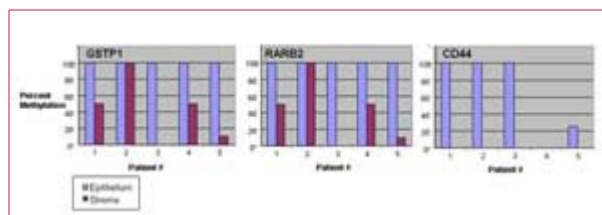


Figure 1. Methylation of *GSTP1*, *RAR β 2*, and *CD44* in epithelial and stromal tissue taken from microdissected whole-mount prostate sections.

These findings raise several questions regarding the mechanism of aberrant promoter methylation in neoplastic and associated stromal cells. At present, it is not known whether tumor and stromal cell methylation are interdependent or if they are independent responses to the microenvironment. Although epigenetic changes in tumor cells have been very well characterized, the cause of changes in DNA methylation are unknown. Some likely sources include response to inflammation and/or infection.

Our findings of gene-specific hypermethylation in prostate tumor epithelia and its associated microenvironment may have implications for cancer prevention, treatment, and diagnosis. Identification of changes in stroma that contribute to tumor progression may provide more effective treatment modalities by altering the “soil” for tumor growth. We are currently pursuing methylation screening techniques to investigate the patterns of gene methylation specific to the stromal compartment of prostate tumors.

Karen G. Woodson, PhD, MPH

Investigator

Genetics Branch

NCI-Bethesda, 6116 Executive Blvd./Ste. 705

Tel: 301-496-0651
 Fax: 301-402-3134
woodsonk@mail.nih.gov

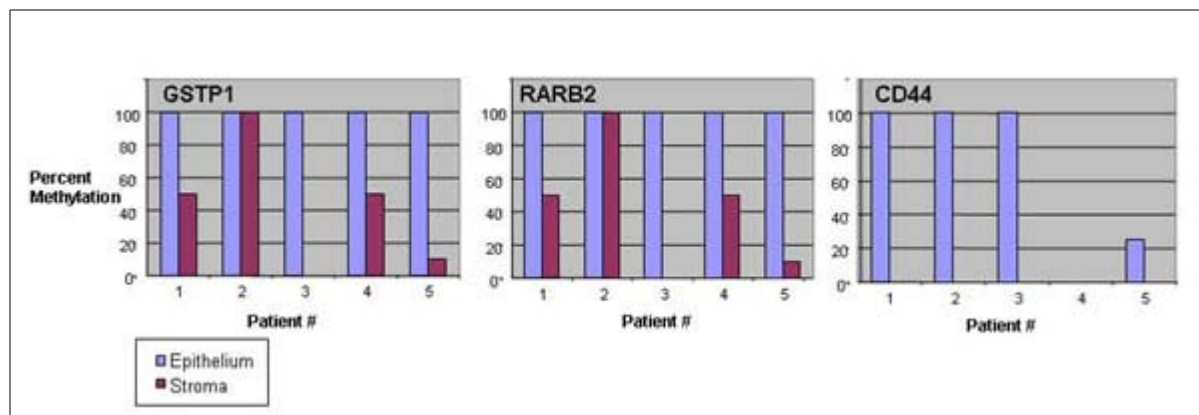


Figure 1. Methylation of *GSTP1*, *RARβ2*, and *CD44* in epithelial and stromal tissue taken from microdissected whole-mount prostate sections.