

The Role of Epigenetic DNA Methylation Changes in Aging and Prostate Cancer Disparity

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#P24-1

ABSTRACT

Background: Prostate cancer (PCa) is the most common visceral cancer in men and the second leading cause of cancer-related deaths in men in the USA. Racial differences in PCa incidence and mortality are well documented. The incidence and mortality for PCa is about twofold higher in African American (AA) versus Caucasian (Cau) men, with AA men presenting more aggressive and advanced disease worldwide. The disparity in PCa is believed to be a complex combination of socioeconomic factors, environment and genetics. Thus, in order to effectively eliminate prostate cancer disparity, we need to understand the genetics involved in the disease pathway. To date, SNPs identified on 8q24 are well documented as risk alleles for AA men with PCa. In addition to genomic defects, there is progressive acknowledgement that somatic epigenetic alterations such as DNA methylation may appear earlier during PCa development than genetic changes, as well as more commonly and consistently. Genome-damaging stresses due to both endogenous and exogenous carcinogens can cause aberrant methylation patterns resulting in tumor formation. Thus aberrant DNA methylation changes may represent the integration of environmental or lifestyle exposures and genetic predisposition to PCa. Such events may differ between individuals belonging to the same racial group or individuals belonging to different racial groups. Identifying DNA methylation changes in prostate tissues from different racial groups would contribute to our understanding of the molecular mechanisms underlying prostate cancer disparity. We know that there are different thresholds for AA versus Cau men for PSA screening. Therefore other markers such as DNA methylated genes that can clarify such ethnic sensitive screening strategies would also be helpful. Furthermore, differential methylation changes could also lead to identification of potential novel therapeutic targets for prostate cancer treatment.

OBJECTIVES

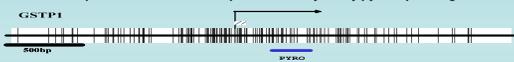
The main objective of this study is to quantitatively measure DNA methylation status in aging prostate tissues and assess the prevalence of DNA methylation levels in normal and cancer tissues from both African American and Caucasian patient samples in order to identify novel DNA methylated genes as potential biomarkers of aging and race-related prostate cancer.

METHODS

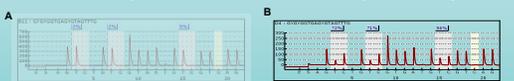
Pyrosequencing- Quantitative DNA methylation was analyzed by pyrosequencing: High molecular weight genomic DNA extracted from tissues were modified by sodium bisulfite treatment and used in PCR reaction with bisulfite designed PCR primers. The reverse primer was biotinylated. The PCR product was immobilized on streptavidin-sepharose beads, washed, denatured and the biotinylated strands released into an annealing buffer containing sequencing primer. Pyrosequencing was performed using a high throughput PSQ 96HS machine (Qiagen).

Human Tissue Samples- Samples used in the current studies includes: Prostate tumors (Ca) and matching normal (NI) surrounding tissues from AA and EA patient at the time of radical prostatectomy. Needle biopsies of normal prostate tissue samples from both AA and Cau patients with elevated serum PSA level in the presence of abnormal digital rectal exam. Normal prostate tissues from organ donors (age 17-68 years) and from patients who have undergone cystoprostatectomy for bladder cancer (age 39-84 years). Breast tumor samples and paired adjacent non-tumor tissues from AA and EA women with known hormone receptor status.

Schematic representation of GSTPI CpG island analyzed by pyrosequencing

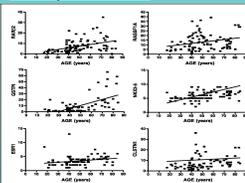


Representative pyrogram traces for GSTPI is shown below: The gray shaded bars indicate region of C-to-T polymorphic sites. The control non-CpG cytosine residue showing complete conversion to uracil is shown to the right. **A** demonstrates no methylation in WBC. **B** demonstrates methylation at all 3 CpG sites in DNA from a 58 year old male organ donor.

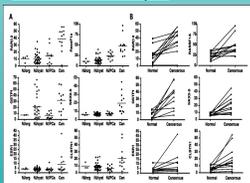


RESULTS

Age-related DNA methylation analysis in normal prostate tissues

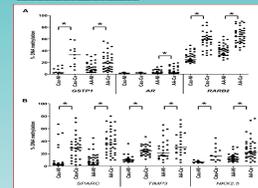


DNA methylated genes distinguishes between Prostate samples

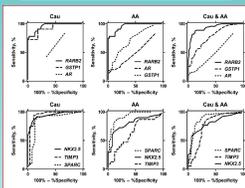


RESULTS

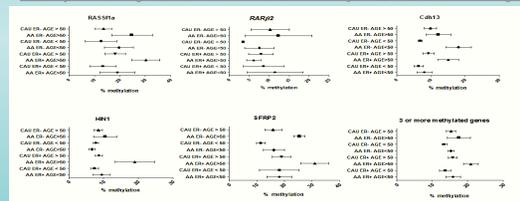
Quantitative DNA methylation analysis in AA versus Cau Pca.



ROC curves formethylation analysis in Pca tissues from AA, Cau and both.



% methylation changes in breast cancers as stratified by race, ER/PR status and age.



CONCLUSION

- We observe significantly higher prevalence of DNA methylation changes in prostate (and breast) tissue samples from African-American in comparison with European-American.
- Gene specific DNA methylation changes maybe a useful "ethnic sensitive" biomarker for cancer detection.
- Epigenetic DNA methylation changes may potentially contribute to racial disparity associated with prostate and breast cancer.

REFERENCES

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This work is supported by grant from the DoD PCRP Idea Award (PC073828/W81XWH-08-1-0372)