Clinical Study

Frequent DNA Hypermethylation at the RASSF1A and APC Gene Loci in Prostate Cancer Patients of Pakistani Origin

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DNA methylation has emerged as a potentially robust biomarker for prostate cancer (PCa). Since DNA methylomes appear to be disease as well as population specific, we have assessed the DNA methylation status of RASSF1A, APC, and p16 (potential biomarkers of PCa) in Pakistani population. Primary prostate cancer tissues were obtained from 27 formalin-fixed paraffin-embedded blocks (FFPE) of cancer patients who underwent radical prostatectomy and transurethral resection of prostate (TURP) during 2003–2008. As controls, twenty-four benign prostatic FFPE tissues were obtained from patients who underwent TURP for benign prostatic hyperplasia during 2008. DNA was extracted, and methylation-specific PCR was used to assess the methylation status for RASSF1A, APC, and p16 gene promoters. Our results revealed that the RASSF1A promoter was hypermethylated in all the tested cancer samples but was also hypermethylated in 3 out of 24 control tissues. The APC promoter was hypermethylated in 15 out of 27 cancer samples and in none of the control samples. Strikingly, none of the samples showed methylation at the p16 promoter. Our findings suggest that RASSF1A and APC gene promoters are frequently hypermethylated in the Pakistani population and therefore have the potential to develop into universally dependable biomarkers for detecting PCa.

1. Introduction

A well-characterized epigenetic mechanism is DNA methylation. DNA methylation typically occurs at CpG islands that are located in the promoter regions of about 50% of human genes. In general, hypermethylation of gene regulating regions (promoters) turns off gene expression, whereas hypomethylation has the opposite effect. Unprogrammed changes in the DNA methylome can culminate in the establishment of a disease state by activating or repressing genes related to cell cycle, growth, and apoptosis [1–3]. Aberrant DNA methylation profile (either hyper or hypo) has been linked to variety of malignancies and emerged as a potentially useful biomarker for monitoring neoplasia [4–6].

Current methods of detecting PCa including PSA and transrectal biopsy fall far short to be ideal methods for diagnosing clinically significant PCa [7, 8]. Epigenomic alterations appear to contribute significantly to PCa onset. A number of gene promoters including GSTP1, APC, RASSSF1A, COX2, MDR1, ERα, hMLH1, and p14/INK have also been found to be frequently hypermethylated in PCa [9–12]. Accumulating data on the methylation status of various genes indicates that biomarkers based on specific methylomes may serve to differentiate between cancerous and non-cancerous prostate tissues [9, 12–14].

One caveat that is beginning to emerge as more information is becoming available on alteration in DNA methylomes and PCa is that these changes are not only disease specific
but also population specific. An example of this phenomenon is illustrated by the difference in the relationship between methylation status of p16 gene in Japanese population versus Caucasian population (hypermethylation in Japanese and hypomethylation in Caucasians [10, 12]).

Further studies on different geographical populations are warranted to provide rational basis for development of both universal as well as population specific biomarkers.

Herein, we assessed the DNA methylation status of APC and RASSF1A (hypermethylated in all populations) and p16 (variable methylation status) in Pakistani PCa patients.

2. Materials and Methods

2.1. Tissues. Prostate cancer tissues were obtained from 18 paraffin-embedded blocks of cancer patients who underwent radical prostatectomy and 9 patients who underwent transurethral resection of prostate (TURP) during 2003–2008; blocks with >70% cancerous tissue were selected after histological examination of slides. Gleason score, tumor stage and serum PSA values were collected for each subject at the time of surgery. Twenty-four benign prostate tissues were obtained from paraffin-embedded blocks of patients who underwent transurethral resection of prostate (TURP) for benign enlargement of prostate gland during 2008 and used as controls. All samples were collected following the protocol approved by Ethical Review Committee of Aga Khan University Hospital, Karachi.

2.2. DNA Isolation and Bisulphite Conversion. DNA was isolated from paraffin-embedded blocks with DNA extraction kit (Qiagen) according to the manufacturer’s protocol. About 2 μg of genomic DNA was subsequently subjected to sodium bisulphite modification using Methyl-Easy DNA bisulphite modification kit (Human Genetic Signatures, Australia) using the manufacturer’s protocol.

2.3. Methylation-Specific PCR (MSP). 2 μL of the bisulphite-converted DNA was used as template for MSP. Primer sequences for amplifying RASSF1A, APC, and p16 were described previously. [15] All PCRs were carried on Mastercycler (Eppendorf) using the following cycling conditions: 95°C for 10 minutes; 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds; 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. Bisulphite-converted Ss1 methylase-treated WBC DNA was employed as a positive control, while untreated-WBC (bisulphite-converted) DNA served as negative control.

2.4. Statistical Analysis. Using CpG island methylation data for 27 primary prostate tumors and 24 controls, the optimal sensitivity and specificity of each DNA methylation marker was determined independently and in combination. A measure of differentiation of PCa was coded such that all tumors with cumulative Gleason score 7 or less were taken as differentiated, while tumors with a >7 score were considered undifferentiated aggressive cancers. Mann-Whitney U test was applied to look at the statistical significance of frequency of hypermethylation of candidate genes. A Fisher-exact test was run to determine the association between the methylation status of the candidate genes and stage of cancer. These tests were run using SPSS 16.0 (statistical software package).

3. Results

The demographics of prostate cancer patients who contributed to this study are shown in Table 1. MSP was performed to evaluate methylation status of the three genes. Our results show that RASSF1A was hypermethylated in all 27 prostate cancer tissues. Unexpectedly, the RASSF1A promoter was also hypermethylated in 3 of the 24 benign prostatic (i.e., control) tissues samples (Tables 2 and 3). Hypermethylation at the APC promoter was observed in 58% (i.e., 15 out of 27) of the cancer cases (Tables 2 and 3) but in none of the benign prostatic tissues. The promoter of p16 genes showed no hypermethylation in cancerous or control samples.

Since a collection of benign and primary prostate cancer tissues were studied, sensitivity and specificity of each of the gene markers were calculated to assess whether it is capable of distinguishing primary prostate cancer from benign prostatic hyperplasia. The sensitivity and specificity of RASSF1A and APC are given as Table 5. The sensitivity and
RASSF1A and by the Japanese group [22]. Our results showed that APC was frequently methylated at the early stage of PCa. Thus, our study showed that APC hypermethylation can be used as a biomarker to detect early stage PCAs.

5. Conclusion

Although small in size, this study is the first of its kind on a Pakistani population. Our findings that RASSF1A and APC genes are frequently methylated in Pakistani PCa patients, demonstrating that both loci could be considered as universal DNA methylation biomarkers for PCa detection. However, further verification of our findings will require a larger sample size with an evenly distributed proportion of early and advanced stage prostate cancer tissues to develop methylation markers which can predict the outcomes of PCa.

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