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Association of Promoter Polymorphisms in MMP2 and TIMP2 with Prostate Cancer Susceptibility in North India

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Background and Aims. The importance of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in tumor progression is well documented. MMP2/TIMP2 system has a significant impact on the development and progression of cancer and genetic polymorphisms in the promoters of *MMP2* (*−1306C/T, 735C/T*) and *TIMP2* (*−418G/A*, *−303C/T*) are correlated with decreased enzyme activity. We sought to determine whether genetic polymorphisms in *MMP2* and *TIMP2* polymorphisms may be associated with varying risk of prostate cancer (PCa) in men in North India.

Methods. Genotyping was done by PCR-restriction fragment length polymorphism method in 190 histologically confirmed PCa patients and 200 unrelated, healthy, age-matched individuals of similar ethnicity.

Results. Patients with *MMP2* (*-1306*) CT genotype as well as T allele were at higher risk of PCa ($p = 0.018$; OR = 1.68 and $p = 0.015$; OR = 1.52). This effect was even more evident in the case of the T allele carrier (CT + TT) ($p = 0.011$; OR = 1.71). *MMP2* (*735*) *C>T, TIMP2* (*−418*) *G>C* and *TIMP2* (−303) C>*T* polymorphism demonstrated no association. However, *TIMP2* (*−418*) GC was found to be involved in progression of PCa but not in initiation. Haplotype results demonstrated that *MMP2* (1306T−735C) and *TIMP2* (418G-303T) were associated with a 1.5- and 1.8-fold increased risk, respectively.

Conclusions. Our data indicated that *MMP2-1306C>T* gene polymorphism contributes to PCa susceptibility. These findings suggested *MMP2* variants as a predictor of PCa progression risk among North Indian men. We assume that analysis of these gene polymorphisms can help identify patient subgroups at high risk of poor disease outcome.

Key Words: Haplotype, Matrix metalloproteinases, PCR-RFLP, Prostate cancer, Polymorphism, Tissue inhibitors of metalloproteinases.

Introduction

Prostate cancer (PCa) is the most common malignancy observed in Western men. However, its incidence in India is low and ranks as the sixth most commonly diagnosed cancer in men (1). This may account for a clear role of ethnicity in PCa pathogenesis. Lifestyle is yet another major factor that has been reported to have a significant impact (2). The pathogenesis of PCa may be influenced both by environmental as well as genetic components. It is well established that genetic factors may account for as much as 42% of the PCa risk (3). A high proportion of cancer burden is attributable to complex interactions of one or more polymorphic genetic variants of low penetrance genes, including MMPs contributing singly or in combination because of their high prevalence in the population. MMPs are extracellular matrix (ECM)-degrading enzymes that play a crucial role in inflammation and angiogenesis. MMP2 (also called type 2 collagenase or gelatinase A) is the key molecule that control invasion, tumor growth and metastasis. Tissue inhibitor of matrix metalloproteinase (TIMP)-2 is a specific inhibitor of MMP2 and plays a crucial role in regulation of MMP-2

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activation. During pathophysiological processes, MMP2/TIMP2 system may have a significant impact on the development and progression of cancer, hence genetic polymorphisms in the promoters of *MMP2* gene (rs243865: −1306 C>T; and rs2285053: −735 C>T) may be associated with varying risks of prostate cancer in North India.

Of the four members of the TIMP family, TIMP-2 is particularly interesting because of its dual functions in terms of regulating MMP-2 activity (4) and its paradoxical effects on certain cancers (5,6). A single nucleotide polymorphism (−418G>C) has also been identified in the promoter of the *TIMP2* gene (7). Although the functional significance of this germline polymorphism is currently unknown, downregulation of the transcriptional activity due to the variant has been suggested because the G>C substitution is located within the consensus sequence for the Sp1-binding site in the promoter region of *TIMP2* (8). *TIMP-2 303C>T* is located in exon 3 with no effect on the final amino-acid sequence of the protein (S101S) and no effect on the total *TIMP-2* expression between normal and tumor tissue. It is, therefore, reasonable to postulate that these polymorphisms may downregulate TIMP-2 expression and consequently cause an imbalance between the activities of TIMP-2 and MMP-2, which is believed to have a significant impact on cancer development and progression. Both MMPs and TIMPs have been characterized in prostate cancer cell lines (9,10) and clinical samples from prostate cancer patients with conflicting results.

Functional polymorphisms in the TIMP genes could lead to either increased or decreased activities, which in turn could cause an imbalance in the TIMP/MMP ratio, and thus impact cancer development and progression. The purpose of the present study was to systematically evaluate polymorphisms in *MMP2* and *TIMP2* and to characterize their association with prostate cancer susceptibility.

Materials and Methods

Study Population

This population based case-control study utilized histologically confirmed PCa cases ($n = 190$) diagnosed and recruited from 2007–2010 at Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow, India. Baseline diagnostic work-up included digital rectal examination (DRE), prostate-specific antigen level (PSA) and prostate biopsy. The pathological grades were categorized according to the Gleason grading system (11) and classified into three groups: low grade (<7), intermediate grade (7) and high grade (>7). Bone scans were performed and results were available for only 162 cases. Every effort was made to enroll control subjects that matched each case in age and ethnicity. Simultaneously, 200 healthy, age-matched controls without a history of cancer or any other chronic disease were recruited for the study. Controls were selected randomly from unrelated individuals visiting the hospital or from health awareness camp/hospital employees who were free of any chronic or urological disease or any type of cancer. Controls were free of any voiding symptoms (American Urological Association), their prostate-specific antigen (PSA) levels were within normal limits (<4 ng/mL), and they had no history of prostate surgery or clinical signs of PCa. Total PSA levels were determined in controls and PCa patients using CanAg PSA ELISA kits (Fugirebio Diagnostics, Goteburg, Sweden). The study protocol was approved by the Ethics Committee of the institute. Informed consent was obtained from each subject at time of recruitment.

DNA Extraction and Phenotyping

Genomic DNA was extracted from peripheral leukocytes by the salting out method and stored at −20°C until use (12). Genotyping of the SNPs; *MMP2* (*−1306*) *C>T;* (*735*) *C>T* and *TIMP2* (*−418*) *G>C*; (*−303*) *C>T* was done in 190 cases and 200 controls. SNPs were selected **a priori** based on minor allele frequencies of >5% in the SNP500Cancer database and plausibility of contribution to the etiology of prostate cancer, but the major criteria for selection was based on previously published reports on different cancer risks. The genotype variants were analyzed using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) methodology for all the SNPs. Primer sequences, PCR conditions and the restriction enzymes used have been described elsewhere for both *MMP2* and *TIMP2* (13–16). Positive and negative controls were used in each genotyping assay, and 5% of the samples were randomly selected and run in duplicate with 100% concordance. Some of the samples selected randomly were also subjected to validation sequencing. Results were reproducible with no discrepancy in genotyping.

Statistical Analysis

Sample size was calculated using QUANTO software, v.1.0 (http://hydra.usc.edu/gxe) for the genetic marker. Sample size achieved 80% of the statistical power. Two-tailed p value <0.05 was considered statistically significant. Distribution of age and substance use was examined using χ^2 statistics. For genotypes, the Hardy-Weinberg equilibrium tests were analyzed first. The association between disease and genotypes was assessed by calculating the OR and 95% CI. ORs were also adjusted for the confounding factors like age and cigarette smoking by binary logistic regression. Patients and controls were used as dependent variable and genotypes, age and smoking were used as covariates. Haplotypes of each individual consisting of 2 SNPs; *MMP2* and 2 SNPs: *TIMP2* were constructed, and the maximal likelihood haplotype frequencies were estimated using the SNP Analyzer, v.1.2A. All statistical