

Polymorphisms in MMP-2 and TIMP-2 in Turkish patients with prostate cancer

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Received: 14.05.2013 • Accepted: 29.06.2013 • Published Online: 15.08.2014 • Printed: 12.09.2014

Aim: Prostate cancer is the most commonly diagnosed malignancy and the second most common cause of cancer deaths in the Western male population. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) modulate the remodeling of the extracellular matrix (ECM). The imbalance between MMPs and TIMPs may lead to an emergence of pathological processes such as cancer. In this study, the association between TIMP-2 (-418 G/C) and MMP-2 (-1306 C/T) polymorphisms and prostate cancer in the Turkish population was investigated.

Materials and methods: Sixty-one prostate cancer patients and 46 healthy subjects were included in the study. DNA was isolated from 2 mL of peripheral blood taken from subjects, and genotypes were analyzed by the polymerase chain reaction-restriction fragment length polymorphism method.

Results: The TIMP-2 -418 (GC) genotype was found in 15 cases (32.6%) in the control group and in 9 cases (14.8%) in the patients group, and statistical significance was determined ($P = 0.037$, $OR = 0.346$). The MMP-2 -1306 (CT) genotype was found 2.17 times more in the patient group than in the control group ($P = 0.149$, $OR = 2.17$).

Conclusion: Our results show that the TIMP-2 -418 (GC) genotype had a putative protective effect against prostate cancer.

Key words: Matrix metalloproteinases, prostate cancer, polymorphism, tissue inhibitors of metalloproteinases

1. Introduction

Cancer is one of the leading causes of death worldwide, and it is estimated that 12.7 million cancer cases and 7.6 million cancer deaths occurred in 2008. Among them, prostate cancer is the second most frequent cancer type after lung cancer and the sixth leading cause of death, accounting for 14% (903,500) of new cases and 6% (258,400) of deaths in males in 2008 (1). Prostate cancer, a multifactorial disease, is the most commonly diagnosed malignancy, and the early diagnosis of prostate cancer is very important due to its increasing incidence worldwide (2).

Matrix metalloproteinases (MMPs) are a group of 24 zinc-dependent endopeptidases capable of degrading all kinds of extracellular matrix (ECM) and nonmatrix substrates (receptors, apoptotic ligands, chemokines, growth factors, etc.). The ECM is involved in vital biological

processes, such as cell proliferation, differentiation, morphogenesis, tissue repair, and remodeling; it acts as a reservoir of biological active molecules. Many MMPs are associated with pathological destruction of ECM components (3–6). The activity of MMPs is controlled by specific endogenous inhibitors, called tissue inhibitors of metalloproteinases (TIMPs). The group consists of 4 members and their involvement in cell signaling is well established (7,8). The imbalance between MMPs and TIMPs may lead to the emergence of pathological processes such as arthritis and cancer. Several studies have shown that MMP expression is elevated and the MMP/TIMP physiological equilibrium is shifted in almost all human cancer types. These findings lead to the idea of potential involvement of the MMPs/TIMPs in cancer progression and tumor-cell adhesion (9,10).

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The association of several MMP and TIMP polymorphisms and cancer types has been previously investigated. A vast amount of clinical data have shown that among MMPs, the gelatinases (MMP-2 and MMP-9), in particular, are associated with several types of human neoplasms (11). The aim of this study was to evaluate the potential role of the MMP-2 -1306 C/T (rs243865) and TIMP-2 -418 G/C (rs8179090) gene polymorphisms in the pathogenesis of prostate cancer in the Turkish population.

2. Materials and methods

2.1. Study subjects

Sixty-one patients with prostate cancer and 46 healthy controls without cancer history admitted to the Urology Departments of the Medical Faculty of Düzce University and Abant İzzet Baysal University were included in the study. The study was approved by the local ethics committee and informed consent was obtained from the subjects according to the Declaration of Helsinki. The demographic characteristics (age and prostate-specific antigen) of the participants were recorded.

2.2. DNA extraction and genotyping

The genotyping of MMP-2 -1306 C/T and TIMP-2 -418 G/C polymorphisms was performed as previously described (12). Briefly, blood samples were obtained from the study population and were collected in 2-mL tubes with ethylenediaminetetraacetic acid. Genomic DNA was obtained from peripheral blood leukocytes by using a nucleic acid extraction kit (Vivantis, Malaysia) according to the manufacturer's instructions, and the samples were stored at -20 °C until analysis by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The oligonucleotide primers to amplify the promoter region of MMP-2 and TIMP-2 were: 5'-CTTCCTAGGCTGGTCCCTACTGA-3' (forward), 5'-CTGAGACCTGAAGAGCTAAAGAGCT-3' (reverse) and 5'-CGTCTCTTGTGGCTGGTCA-3' (forward), 5'-CCTTCAGCTCGACTCTGGAG-3' (reverse), respectively. PCR was performed in a total volume of 25 µL with 0.5 µL of Taq DNA Polymerase (Fermentas, Lithuania). The PCR mix was incubated for 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C and 45 s at 58 °C for MMP-2 -1306 C/T and 30 s at 94 °C and 45 s at 59 °C for TIMP-2 -418 G/C, 45 s at 72 °C, and a final step at 72 °C for 10 min.

The PCR products for MMP-2 -1306 C/T sites were digested by BfaI (XspI) restriction enzymes, while TIMP-2 -418 G/C sites were digested by AvaI (BsoBI) restriction enzymes (NEB, UK). Digestion was accomplished by incubation with 10 U of enzyme at 37 °C in a 25-µL total reaction volume. The digested products were separated on 4% agarose gel stained with ethidium bromide.

The used primers produced a 193-bp fragment containing the MMP-2 -1306 C/T site. The C allele

recognizes 1 restriction site (188 + 5 bp), and the T allele recognizes 2 restriction sites (162 + 26 + 5 bp). The used primers produced a 304-bp fragment containing the TIMP-2 -418 G/C site. The G allele recognizes 2 restriction sites (230 + 51 + 23 bp) and the C allele recognizes 1 restriction site (253 + 51 bp).

2.3. Statistical analysis

Statistical analyses were performed using PASW 18 statistical software (ver. 18.0 for Windows; SPSS Inc., Chicago, IL, USA). Categorical variables were compared in 2 groups by using the Pearson and Fisher χ^2 tests, and the results were expressed as frequency and percent. Binary logistic regression analyses were performed to identify independent predictors for prostate cancer. Age and genotype were cover rates. An independent-sample t-test was performed to compare normally distributed continuous variables in the 2 groups and these variables were expressed as mean \pm standard deviation. If continuous variables were not normally distributed, the Mann-Whitney U test was used and data were expressed as medians (25th-75th percentiles); P-values of less than 0.05 were accepted as statistically significant.

3. Results

The age and prostate-specific antigen (PSA) values of the study population are summarized in Table 1. The study population consisted of 107 patients with a mean age of 70 ± 9.5 years. Among them, 61 patients with a mean age of 71.25 ± 10.5 years formed the patient group and 46 patients with a mean age of 68.17 ± 7.6 years formed the control group. No statistical significance was determined ($P = 0.092$) in terms of age. However, the level of PSA in the patient group was found to be significantly higher compared to the control group ($P = 0.000$).

The MMP-2 -1306 C/T SNP genotype distribution is shown in Table 2. The CC, CT, and TT genotypes were found in 51 (83.6%), 7 (11.5%), and 3 (4.9%) cases in the patient group, respectively. In the control group, the CC and CT genotypes were found in 42 (91.3%) and 4 (8.7%) cases, respectively. The TT genotype was not detected in the control group. There was no association of genotype frequency and prostate cancer. However, the genotype of CT was found 2.17 times more in the patient group than in the control group ($P = 0.149$, OR = 2.17).

The TIMP-2 -418 G/C SNP genotype distribution is shown in Table 3. The GG and GC genotypes were found in 52 (85.2%) and 9 (14.8%) cases in the patient group, respectively. In the control group, the GG and GC genotypes were found in 31 (67.4%) and 15 (32.6%) cases, respectively. The CC genotype was not detected in either patient or control groups. A statistical significance was determined between the GC genotype and prostate cancer ($P = 0.037$, OR = 0.346).

Table 1. The characteristics of the patient and control groups.

	Total (107)	Control (46)	Patient (61)	P
Age (years)	70 ± 9.5	68.17 ± 7.6	71.25 ± 10.5	0.092
PSA (ng/mL)	3 (1.2–10)	1.6 (0.94–2.0)	9 (6–19.8)	0.000

Table 2. MMP-2 –1306 C/T SNP genotype distributions in the patient and control groups.

Genotype	Total (107)	Control (46)	Patient (61)	P (χ^2)	OR, CI*	P*
CC n (%)	93 (87)	42 (91.3)	51 (83.6)	Ref.		
CT n (%)	11 (10.2)	4 (8.7)	7 (11.5)	0.267	2.17 (0.75–6.2)	0.149
TT n (%)	3 (2.8)	0	3 (4.9)	-	-	-
CT+TT n (%)	14 (13)	4 (8.7)	10 (83.6)	0.242		

OR: odds ratio, CI: confidence interval, *: for logistic regression.

Table 3. TIMP-2 –418 G/C SNP genotypes distributions in the patient and control groups.

Genotype	Total (107)	Control (46)	Patient (61)	P (χ^2)	OR*	P*
GG n (%)	83 (77.6)	31 (67.4)	52 (85.2)	Ref.		
GC n (%)	24 (22.4)	15 (32.6)	9 (14.8)	0.028	0.346 (0.12–0.93)	0.037
CC n (%)	-	-	-	-	-	-
GC+CC n (%)	24 (22.4)	15 (32.6)	9 (14.8)	0.028	0.346 (0.12–0.93)	0.037

OR: odds ratio, CI: confidence interval, *: for logistic regression.

4. Discussion

Cancer, the plague of the 20th century, is a multifactorial physical disease and decreases the patient's quality of life. Early diagnostic methods could help to overcome this disease and therefore considerable effort has been made in this respect. The genetic tendencies of individuals have been studied for early diagnosis; the development and progression of cancer is affected by inherited or somatic genetic mutations (13,14). Despite the fact that prostate cancer is the second leading cause of cancer-related deaths in the Western world, its prevalence differs markedly in various ethnic groups, suggesting the potential role of genetic predisposition to prostate cancer (15).

MMPs and their inhibitors, TIMPs, are putative gene families involved in the pathogenesis of cancer. The MMP/TIMP balance is a critical biological process in the maintenance of ECM components; a balance shift towards MMPs promotes ECM degradation in cancer development (16). To date, changed expression levels of several MMPs and TIMPs in prostate cancer have been reported. The involvement of MMP-9 in prostate cancer pathogenesis was reported by Huang et al. (17). Membrane type 1-MMP

(MT1-MMP) with the capacity for pro-MMP-2 activation is a vital agent and involved in the remodeling of the ECM. The central role of MT1-MMP in inducing oxidative stress in prostate cancer was proven by Nguyen et al. (18). Reis et al. investigated the expression levels of MMPs and TIMPs in 2 independent studies (19,20). They examined the expression levels of MMP-2 and its regulators (TIMP-2, MT1-MMP, IL-8, and MMP-9) and its inhibitor, TIMP-1, in prostate cancer. The changed expression level of these genes in prostate cancer was found in both studies. Similarly, the differential expression of MMP-2 and MMP-9 was investigated by several groups (21,22).

After elucidation of the potential roles of these genes in the development of tumorigenesis, they have become putative therapeutic and diagnostic targets, and several studies have been conducted to clarify the relationship between the polymorphisms of these genes and cancer (23). Most of these studies succeeded in showing a relationship between MMP and TIMP polymorphisms and several cancer types, including colorectal (24), gastric (25,26), head and neck (27), lung (28), and ovarian (29) cancers.

The aim of this study was to investigate the possible impact of the MMP-2 -1306 C/T and TIMP-2 -418 G/C polymorphisms in the pathogenesis of prostate cancer in the Turkish population. The transitions of C to T and G to C at the promoter region of the MMP-2 and TIMP-2 genes, respectively, are very important in terms of gene expression level, due to them being situated at the consensus sequence for the Sp1-binding site (30). In addition, our study is the first to investigate the relationship between prostate cancer and MMP/TIMP polymorphisms.

Our results demonstrated that a statistically significant difference was not determined between the CT and TT genotypes of MMP-2 and prostate cancer, but the CT genotype was found 2.17 times more often in the patient group compared to the control group ($P = 0.149$, $OR = 2.17$). It could be speculated that the MMP-2 (CT) genotype might create a genetic tendency towards prostate cancer. However, the GC genotype of the TIMP-2 gene was

found to be statistically significant ($P = 0.037$, $OR = 0.346$). Similar results have been obtained by a study performed in the North Indian population (31), which found that the genotypes MMP-2 (CT) and TIMP-2 (GC) increased genetic tendency towards prostate cancer by 1.68 and 0.79 times, respectively.

In conclusion, it might be speculated that MMP-2 -1306 C/T and TIMP-2 -418 G/C polymorphisms are involved in the pathogenesis of prostate cancer, and TIMP-2 -418 G/C more so. However, these results should be supported by larger study groups. To elucidate the putative roles in prostate cancer, the remaining MMP and TIMP polymorphisms should be investigated.

Acknowledgement

This project was supported by the Düzce University Research Fund, Project Number 2012.04.02.107.

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