Molecular Biology Reports (2020) 47:9373–9383
https://doi.org/10.1007/s11033-020-05968-5

ORIGINAL ARTICLE

Activities and polymorphisms of MMP-2 and MMP-9, smoking, diabetes and risk of prostate cancer

Amir Kiani[1] · Marjan Kamankesh[2] · Asad Vaisi-Raygani[2] · Mahmoud-Reza Moradi[1] · Maryam Tanhapour[2] · Zohreh Rahimi[3] · Saeed Elahi-Rad[1] · Fariborz Bahrehmand[3] · Mahdieh Aliyari[2] · Faranak Aghaz[2] · Hadi Mozafari[2] · Nayebali Rezvani[2] · Lida Haghnazari[2] · Tayebeh Pourmotabbed[4]

Received: 6 April 2020 / Accepted: 31 October 2020 / Published online: 9 November 2020

Abstract
Matrix metalloproteinases (MMPs) are a group of zinc dependent enzymes that are involved in tumor cell invasion and metastasis. The role of MMP-2 and -9 genetic polymorphism in different malignancies has been the subject of numerous studies. The present research has attempted to discover any positive correlation between MMP-2 and MMP-9 SNPs and prostate cancer (PCa) in patients with a history of either diabetes or smoking habits. 112 PCa-patients and 150 unrelated healthy-controls that matched for age and sex were selected for present case–control study. MMP-2 -1575G/A and MMP-9 -1562 C/T polymorphisms detected by PCR–RFLP, serum tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2), testosterone, prostate-specific antigen (PSA), free-prostate-specific-antigen (fPSA), and fPSA/PSA levels were detected by ELISA and enzyme assay, respectively. MMP-2 and MMP-9 activities were measured by gelatin-zymography. Covariates were considered as age, status of cigarette smoking, and a possible history of diabetes mellitus (DM). The frequency of -1575 MMP-2 A/A + A/G and -1562 MMP-9 C/T + T/T genotypes were higher in PCa-patients with DM (74.3%,p = 0.003) and with smoking habits (72.5%,p = 0.005). These genotypes were associated with the increased risk of prostate cancer in smokers (3.52-folds) and in individuals with history of DM (4.34-folds). A significant positive association was found between level of TIMPs (TIMP -1 and TIMP-2) and BMI in PCa-patients and also between testosterone levels and MMP-9 activity in healthy control subjects. For the first time, this study demonstrated that activities of MMP-2 -1575G/A and MMP-9 -1562C/T variants in association with smoking and diabetes are considered significant risk factors for PCa.

Keywords Matrix metalloproteinase (MMP) · Tissue inhibitors of metalloproteinases (TIMPs) · Prostate cancer · Diabetes · Prostate-specific antigen and testosterone

Introduction

Prostate cancer (PCa) is the second most prevalent cancer among males [1, 2]. While some types of prostate cancer grow slowly and may need minimal or even no treatment, other types are aggressive and can spread quickly. The most common sites of metastasis are liver, bladder, bones, lungs, brain and lymph nodes [3]. Although the specific mechanisms facilitating the invasive behaviors of PCa are unclear, tumor cell invasion and metastasis are known to be mediated, at least in part, through MMPs secreted by tumor cells or stroma cells.

Matrix metalloproteinases (MMPs) as cancer biomarker are one of the members of zinc-dependent family of enzymes that are involved in different physiologic processes, such as embryogenesis, angiogenesis, and tissues remodeling. MMPs degrade extracellular matrix components, such as interstitial collagen, fibronectin, and proteoglycans [4]. MMP's activity is regulated by tissue inhibitor of matrix metalloproteinase (TIMP)-1-4 and a balance between TIMPs and MMPs is essential for ECM remodeling and degradation [5]. Thus, an imbalance in MMP enzymes activity plays a pivotal role in tumor growth and metastasis [6, 7]. The key enzymes involved in the breakdown of collagen type IV and gelatin are MMP-2 and MMP-9 [8]. These enzymes are upregulated in PCa and their high abundance in cancer cells

✉ Asad Vaisi-Raygani
avaisiraygani@gmail.com

✉ Tayebeh Pourmotabbed
tpourmotabbe@gmail.com; Tpourmot@uthsc.edu

Extended author information available on the last page of the article

may indicate a poor prognosis [9, 10]. Thus, it is believed that MMPs-2&-9 and their inhibitors (TIMPs) play major roles in the PCa development and progression [6]. Previous studies have reported that MMPs overexpression and TIMPs suppression lead to dysregulation of extracellular matrix (ECM) remodeling causing numerous pathologic conditions such as cancer, neurodegenerative disease, arthritis and cardiovascular disease [11].

In addition, imbalance between the MMP-TIMP levels in vessel walls has been observed in diabetes patients which may correlated with microvascular complications [12].

A few reports indicate that MMP-2 -1575G/A (rs243866) and MMP-9 -1562 C/T (rs3918242) genotypes are associated with the risk of several types of cancer such as lung [13], gastric [14], esophageal [15], breast carcinomas [16] and prostate cancer [9, 17]. The -1575G/A is located in the promoter of MMP-2 gene and has been shown to be associated with higher MMP-2 expression and activity [18–20]. MMP-9 -1562C/T gene polymorphism is involved in tumor progression and metastasis [21]. Sfar et al. have suggested that the presence of the -1562 T allele may contribute to augmenting intra-cellular MMP-9 protein production, which then generates growth-promoting signals and increases the pathogenesis, invasion, and severity of PCa [22].

Other factors that increase the risk of PCa including age, race, family history, obesity, and smoking [23, 24]. Evidence has indicated that obese individuals may have an increased chance of being diagnosed with high-grade PCa [25]. In addition, obesity is correlated with increased risk of metabolic disorders and Type 2 diabetes mellitus (T2DM), which are characterized by insulin resistance and increased serum level of insulin-like growth factor-1 (IGF-1) [26]. Insulin and IGF-1, both can promote cell growth directly or indirectly and may be tumor promoters. However, a few studies demonstrated that the T2DM reduces the risk of prostate cancer. It is assumed that hyperinsulinemia not only induces cellular proliferation, but also decreases testosterone level, resulting in a lower risk for PCa [27]. Crawley and coworkers have reported that although PCa and T2DM might happen concurrently, T2DM has a protective effect against PCa risk. Moreover, impact of T2DM on grade and stage of PCa is not clear [28].

Cigarette smoking increases the risk of cancer. There is a direct relationship between number of pack of cigarette smoked per year and risk of developing cancer [29]. It has been reported that the effect of smoking on progression and increased risk of PCa development is associated with higher levels of circulating aldosterone and testosterone [30] that are linked to development of aggressive prostate cancer [31, 32].

In this study, we investigated the association between activities and genotypes of MMP-2 -1575G/A, MMP-9 -1562C/T enzymes and their inhibitors (TIMP-1 and TIMP-2) with risk of PCa in Iranian population. In addition, we explored the association of the mentioned polymorphisms with increased risk of PCa in diabetics and cigarettes smokers.

Materials and methods

Participants

The Ethics Committee of Kermanshah University of Medical Sciences, Iran approved the present study (1.Research involving Human Participants 2. Informed consent ethical legal cod KUMS.Rec.1396.136)) (Grant #96430). All participants gave informed written consent for use of their samples and clinical data in research.

We selected participants based on availability. In addition, the association between the frequencies of MMP9 and 2 genotypes with PCa was considered in sample size calculation. Since the frequencies of MMP2 and 9 genotypes in Iranian population are not clear, we referred to previous studies [33, 34] and with respect to the MMP2 genotypes frequencies sample size in both patients and control group was calculated (with 95% confidence intervals (CI) at 90% test power) by STATA software as follow;

p2 = 0.76, p1 = 0.575, power = 0.90, alpha = 0.05, n2/n1 = 1.00.

Estimated required sample sizes: n1 = 145, n2 = 145

$$n = \frac{(Z1 - \alpha/2 + Z1 - \beta)^2[P1(1 - P1) + P2(1 - P2)]}{(P1 - P2)^2}$$

$$= (1.96 + 1.280[0.58(1–0.575) + 0.76(1–0.76)] = 145.$$

As is observed from above calculation the sample size in both patients and control groups is 145. Therefore, 111 PCa patients and 150 gender and age-matched unrelated healthy controls were recruited for this study during one year.

In addition, this calculation was performed for MMP9 as follow;

alpha = 0.05, power = 0.90, p1 = 0.57, p2 = 0.754, sampsi 0.754.

Estimated sample size for two-sample comparison of proportions.

Test Ho: p1 = p2, where p1 is the proportion in population 1and p2 is the proportion in population 2.

Assumptions: alpha = 0.0500 (two-sided), power = 0.9000, p1 = 0.7540 and p2 = 0.5700.

n2/n1 = 1.00.

Estimated required sample sizes: n1 = 148 and n2 = 148.

As is observed from above calculation the sample size in both patients and control groups is 148. Therefore, 111 PCa

patients and 150 gender and age-matched unrelated healthy controls were recruited for this study during one year.

The total sample size for the serum concentration of TIMP1 was calculated as follow;

Z1-α /2 + Z1- β) (δ21 + δ22) (n = (μ1-μ2)2.

n = (1.96 + 1.28) (0.07 2 + 0.1 2).

(122. 7–25.2)2.

n = 15.

The total sample size for the serum concentration of TIMP1 was calculated as follow;

Z1 − α/2 + Z1 − β) (δ21 + δ22)(n = (μ1 − μ2)2.

n = (1.96 + 1.28) (0.07 2 + 0.1 2).

(61–24)2

n = 17.

All of the PCa patients from Hospitals of the Kermanshah University of Medical Sciences with the mean age of 35.3 ± 10.9 years participated in this case control study.

According to campbell-walsh urology, patients with lower urinary tract symptoms (LUTS) referred to urologist. The investigation of patient's history, digital rectal exam (DRE) and PSA tests were performed. A biopsy (6 part) was taken through transrectal ultrasound guided (TRUS) from patients who have abnormal DRE and elevated levels of PSATZ (prostate specific antigen adjusted for the transition zone volume) [35]. According to the prostate biopsy results BPH was distinguished from PCa. Tumor volume and invasion were determined by radical prostatectomy.

After biopsy procedures patients were classified in five groups according to Gleason score range as follow; Gleason ≤ 6: grade group 1, Gleason 3 + 4 = 7: grade group 2, Gleason 4 + 3 = 7: grade group 3, Gleason 4 + 4 = 8, 3 + 5 = 8, 5 + 3 = 8: grade group 4, Gleason 9–10: grade group 5 [36].

The most of the patients in this study were in groups 1, 2 and 3 and under the radiotherapy and hormone therapy. Moreover, patients with high-grade prostate cancer (8–10) were recruited after radical prostatectomy (RP).

The clinical features including Gleason score, PSA density, family history, type of treatment and also age, history of tobacco smoking, diabetes, height, weight, and pretreatment testosterone levels have obtained from their medical records.

Since we aimed to detect the influence of smoking and diabetes mellitus in susceptibility to PCa, the cases and controls with smoking habit or with diabetes were included in the study.

Diagnosis and classification criteria for diabetes was based on reports has been published by the World Health Organization (WHO) in 2006. Diagnosis criteria for diabetes included fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or 2–h plasma glucose ≥ 11.1 mmol/l (200 mg/dl) [37].

During the same period, 150 healthy controls volunteers (average age of 35.7 ± 13.2 years) with normal DRE results and PSA levels and without any history of malignancy or BPH and also unrelated to one another were recruited from Hospitals of the Kermanshah University of Medical Sciences. Exclusion criteria for control subjects was including serum levels > 2.5 ng/ml of prostate specific antigens (PSA), the free to total PSA ratio ≤ 0.1 and positive digital rectal examination (DRE).

Chemical analyses

Serum concentration of TIMP-1 and TIMP-2 were measured by ELISA according to the manufacturer protocol (Quantikine R&D Systems, USAs). Serum testosterone, PSA, fPSA and fPSA/PSA levels were determined by the standard methods (Pars Azmoon kit, Iran), using the automated Erba XL-600 (Mannheim, Germany).

DNA extraction, MMP-2 and MMP-9 genotyping

Method used to extract genomic DNA from peripheral blood samples has been described previously [39]. The MMP-2 -1575G/A and MMP-9 -1562C/T polymorphisms were identified by PCR–RFLP [18, 40].

The forward; 5′- GCC TGG CAC ATA GTA GGC CC-3′, and reverse; 5′-CTT CCT AGC CAG CCG GCA TC-3′, primers were used to determined MMP-9 -1562 SNP. Thermocycling condition for PCR reaction was 95˚C for 7 min, followed by 35 cycles at 95 °C for 30 s, 67.7˚C for 30 s, 72 °C for 30 s, with a 10 min final extension at 72 °C. SphI restriction endonuclease was used to digest the 435 bp PCR products at 37 °C overnight and the digested products were separated on a 1.5% agarose gel containing ethidium bromide. The homozygote mutant TT fragments were 244 bp and 191 bp, heterozygote mutant CT fragments were 435 bp, 244 bp, and 191 bp and homozygote wild CC was 435 bp as shown in (Fig. 1a) [40].

The forward 5′- CACACCCACCAGACAAGCCT–3′ and reverse 5′- CCT AGG AAG GGG GCA GAT AGG AC-3′ primers were used to identify MMP-2 -1575G/A polymorphism.

The thermocycling condition for PCR reaction was 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 58.2 °C for 1 min, 72 °C for 1.5 min, with a final extension at 72 °C for 10 min. The amplified PCR products (301 bp) were treated with the restriction enzyme PagI (RcaI). The length of digested fragments were: the GG genotype (wild type), 301 bp; the AA genotype (homozygous mutant) 189 and 112 bps; heterozygous AG genotype, 301, 189, and 112 bps (Fig. 1b) [18].

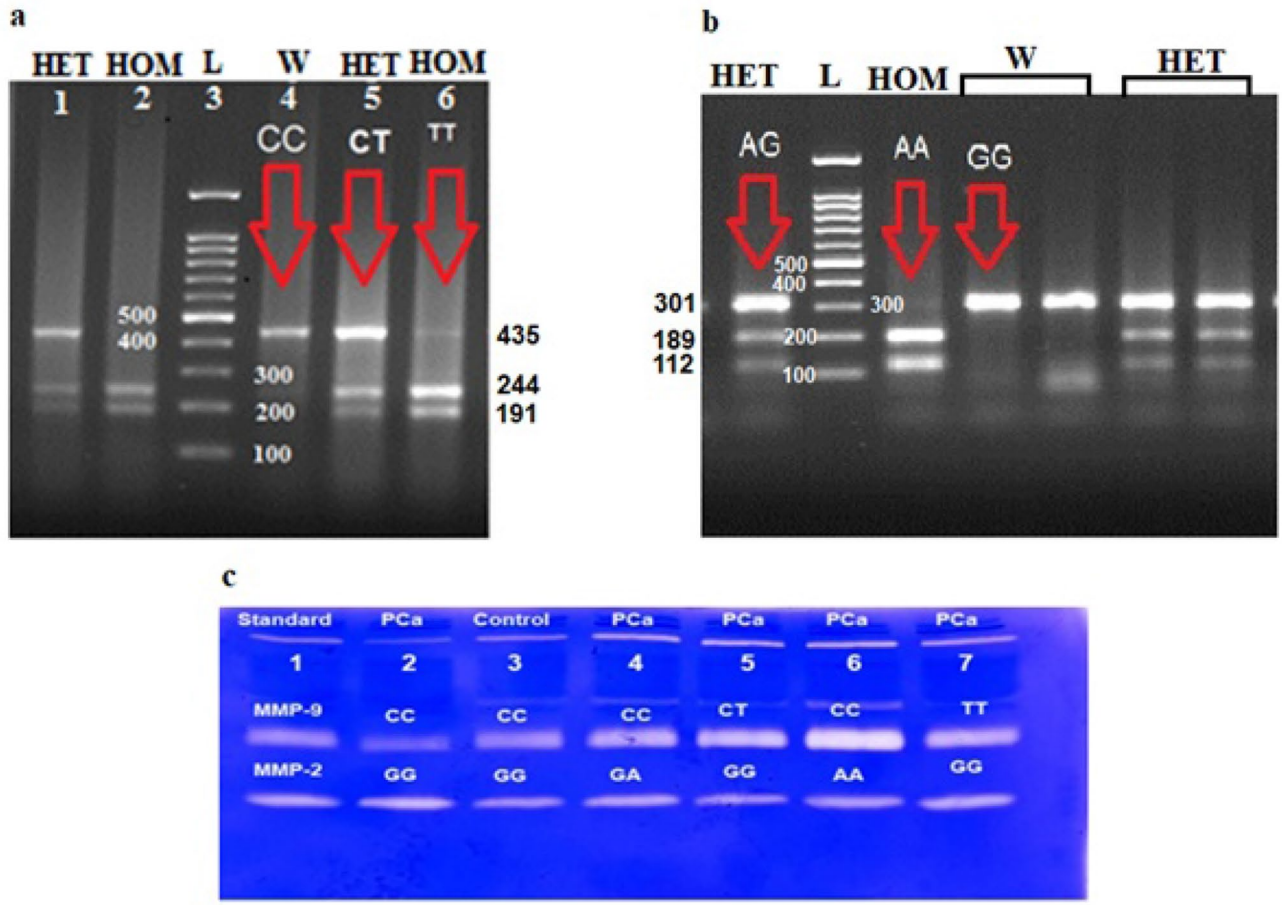

Fig. 1 Agarose gel electrophoresis (1.5%) pattern of MMP-9, C1562T digested PCR products with SphI enzyme. From left to right lanes 2 and 6 indicate HOM: homozygous mutant (TT: 244, 191 bp), lanes 1 and 5 demonstrate HET: heterozygous mutant (CT: 435, 244, 191 bp), lanes 4 indicates W: homozygous wild-type (CC: 435 bp) and lane 3 shows L: 1000 bp ladder (**a**). Agarose gel electrophoresis (1.5%) pattern of MMP-2, G1575A digested PCR products with pagI enzyme. From left to right lanes 1, 6 and 7 demonstrate HET: heterozygous mutant (AG: 301, 189, 112 bp), lane 2 shows L: 1000 bp ladder, lane 3 indicates HOM: homozygous mutant (AA: 189, 112 bp), lanes 4 and 5 indicate W: homozygous wild-type (GG: 301 bp) (**b**). SDS-PAGE Zymogram of plasma MMP-2 and MMP-9 activities of three different genotypes of MMP-9 and MMP-2 in samples of patients and control subjects, lane 1 standard (The Human MMP-9 and MMP-2 Standard recombinant in a buffered protein base (R&D Systems®, Catalog # PDMP900 and Catalog # PMMP200, respectively)), lane 2 wild types of MMP-2 (CC) and MMP-9 (GG) in PCa patients, lane 3 wild types of MMP-2 (CC) and MMP-9 (GG) in control group, lane 4 and 6 wild types of MMM-9 and hetero and mutant of MMP-2. Lanes 5 and 7 wild type of MMP-2 and hetero and mutant of MMP-9 in patient samples (**c**)

Gelatin zymography

The activities of MMP-9 and MMP-2 in the serum were detected by gelatin zymography as described previously [38]. The MMP-9 and MMP-2 activities were then quantified by Image J software, using a high-resolution digital image of gel against the amount of MMPs standard in each gel (Fig. 1c).

Statistical analyses

We calculated the allelic frequencies by gene counting method. Pearson's χ^2 test was used to test the difference in the distribution of the haplotypes in patients and controls. Statistical significance was assumed at the $p \leq 0.05$. The genotypes and allele frequencies of MMP-2 -1575G/A and MMP-9 -1562C/T in PCa patients were compared to control group using χ^2 test in three different genotype models of co-dominant, the dominant/recessive, and the heterozygous.

The Odds ratios (OR) and 95% confidence intervals (CI) were obtained by SPSS logistic regression. The correlation of MMP-2 and MMP-9 activities, levels of NO, PSA (mg/dl), fPSA (mg/dl), fPSA/PSA, and TIMP-1, TIMP-2 in serum, BMI, and age between two groups were calculated by linear regression and an unpaired t-test. The t-test, ANOVA and nonparametric independent sample Mann–Whitney

analysis were used to compare the quantitative data. The SPSS statistical software (version 16) was used for the statistical analysis.

Results

Demographic characteristics and test results of patients and control group are demonstrated in Table 1. The levels of PSA [61 (18–102.2) vs 0.7 (0.3–1.4) (mg/dl), $p < 0.001$], fPSA [8.6 (1.7–20) vs. 0.3(0.1–0.6) (mg/dl), $p < 0.001$], MMP-2 activity (15,850 vs. 13,017, $p = 0.011$), TIMP-1 (13 vs. 7.5 pg/ml $p < 0.001$) and TIMP-2 (67 vs. 38 pg/ml $p < 0.001$) were significantly higher in the serum of PCa patients than control groups. While, serum levels of testosterone (2.9 vs. 0.6 µmol/l $p < 0.001$) and NO (49.1 vs. 5.4, $p < 0.001$) were significantly higher in healthy control compared to PCa patients. In addition, significant positive association was found between smoking and risk of PCa ($p < 0.001$).

The odd ratio (95% confidential interval) and frequency of MMP-2 -1575 and MMP-9 -1562 genotypes and alleles are presented in Table 2. The overall distribution of MMP-2 -1575 and MMP-9 -1562 alleles and genotypes in PCa patients were similar to control group.

The influence of dominant model of -1575 MMP-2 (G/G, A/G + A/A) and -1562 MMP-9 (C/C, C/T + T/T) genotypes on testosterone, PSA, fPSA, NO and fPSA/PSA concentration and BMI between PCa patients and control group is presented in Table 3. For MMP-2, the presence of G/G genotype in PCa patients was strongly associated with higher concentration of PSA (60.4 vs. 0.8 mg/dl, $p < 0.001$), fPSA (7.9 vs.0.3 mg/dl $p < 0.001$), TIMP-1 (10 vs. 7 pg/ml, $p > 0.009$), TIMP-2 (59 vs. 41.5 ng/ml, $p = 0.021$) and MMP-2 activity (17,377 vs. 13,198, $p = 0.013$) compared to control subjects. Similar results were observed for MMP- 9 genotypes -1562 C/C and C/T + T/T (Table 3).

We found a significant positive correlation between concentration of both TIMP-1 and TIMP-2 and BMI in PCa patients ($r = 0.233$, $p = 0.027$ and $r = 0.222$, $P = 0.035$) (Table 4). But, TIMP-2 level was negatively correlated with age in PCa patients ($r = -0.215$, $p = 0.041$) and in control subjects ($r = -0.213$, $p = 0.044$). In healthy control subjects, MMP-9 activity was found to be negatively correlated with testosterone concentration ($r = -0.137$, $P = 0.018$), but it was positively correlated with fPSA/PSA ($r = 0.118$, $p = 0.043$) (Table 4).

The impact of diabetes, smoking, and/or dominant model of -1575 MMP-2 and -1562 MMP-9 genotypes on the prostate cancer risk was investigated and results indicated that smoking and diabetes had significant effect on the incidence of PCa. -1575 MMP-2 A/A + A/G genotypes and -1562 MMP-9 C/T + T/T genotypes significantly increased the risk of PCa in diabetics and smokers by 4.34 ($p = 0.004$) and 3.52 ($p = 0.002$) folds, respectively.

Table 1 The demographic characteristic and distribution of risk factors in prostatic cancer patients and control subjects in a population from west of Iran

Parameters	Prostatic cancer patients N = 112	Control subjects N = 150	p# values
Age	35.3 ± 10.9	35.7 ± 13.2	0.82
Testosterone (µmol/L)	*0.6 (0.1–2.18)	*2.9 (2.2–4.03)	< 0.001
NO	*5.4 (3.6–8.6)	*49.1 (42.2–82.1)	< 0.001
PSA (mg/dl)	*61 (18–102.2)	*0.7 (0.3–1.4)	< 0.001
fPSA (mg/dl)	*8.6 (1.7–20)	*0.3(0.1–0.6)	< 0.001
fPSA/PSA	*0.19 (0.1–0.27)	*0.42(0.22–0.67)	< 0.001
Diabetes			
No	85 (75.9%)	127 (84.7%)	0.068
Yes	27 (24.1%)	23 (15.3%)	
Smoking			
No	53 (47.3%)	No 109 (72.2%)	< 0.001
Yes	59 (52.7%)	Yes 41 (27.3%)	
MMP2 zymography activity	*15,850 (9663–21,987)	*13,017 (9173–15,837)	0.011
MMP9 zymography activity	*22,016 (13,459–37,747)	*28,194 (15,299–41,161)	0.037
TIMP1 (pg/ml)	*13 (8–25)	*7.5 (6–9)	< 0.001
TIMP2(ng/ml)	*67 (41.5 -206.5)	*38 (28–58.5)	< 0.001
Prostate volume (cm^3)	39.3 ± 9.6	24.9 ± 3.5	< 0.001
PSA/ Prostate volume	2.1 ± 2.5	0.037 ± 0.028	< 0.001

Compared serum MMP2 and MMP9 zymography activities, TIMP1 and 2, testosterone, PSA, fPSA, NO concentration, age, diabetes, smoking and fSPS/SPA between patients and controls were used*non-parametric 2 independent samples test Mann–Whitney (p# values), two-tailed Student's t test and χ^2 test

Table 2 Odd ratio (95% confidential interval) and distribution of MMP-2 and MMP9 genotypes and alleles in patients with PCa patients and control subjects

	PCa patients (n = 112)	Control subjects (n = 150)
MMP2genotypes		
G/G	77 (68.8%)	109 (72.7%)
A/G	32 (28.6%)	41(27.3.7%)
A/A	3(2.6%)	0(0%)
	($\chi^2 = 4.2$, df = 2, p = 0.12)	
Dominant model of MMP2 genotypes		
G/G	77 (68.8%)	109 (72.7)
A/G + A/A	35 (31.2%)	41 (27.3%)
	($\chi^2 = 0.34$, df = 1, p = 0.56) 1.083 (0.83–1.42, p = 0.56)	
MMP2alleles		
G	186 (83%)	259 (86.3%)
A	38 (17%)	41 (13.7%)
	($\chi^2 = 0.51$, df = 1, p = 0.48) 1.093 (0.86–1.4, p = 0.47)	
MMP9		
C/C	72 (64.3%)	100 (66.7%)
C/T	36 (32.1%)	49(32.7%)
T/T	4(3.6%)	1(0.7%)
	($\chi^2 = 2.9$, df = 1, p = 0.28) 2.36 (0.78- 7.1, p = 0.281)	
	($\chi^2 = 2.93$, df = 2, p = 0.23)	
Dominant model of MMP9 genotypes		
C/C	72 (64.3%)	100 (66.7%)
C/T + T/T	40 (35.7%)	50 33.3%)
	($\chi^2 = 0.1$, df = 1, p = 0.76) 1.041 (0.804–1.348, p = 0.761)	
MMP9alleles		
C	180 (80.4)	249 (83%)
T	44 (19.6%)	51 (17.3%)
	($\chi 2 = 0.49$, df = 1, p = 0.48) 1.083 (0.87–1.36, p = 0.48)	

In order to, assessment the TIMP as a risk factor in PCa binary regression analysis was calculated as follow;

According to the TIMP normal range reported by one study entitled "Significant Increases in Serum and Plasma Concentrations of Matrix Metalloproteinases 3 and 9 in Patients with Rapidly Destructive Osteoarthritis of the Hip" zero and 1 was defined for the normal range of TIMP and for the values > normal range respectively [41].

This analysis was performed for TIMP2 only. Because all of the patients and control subjects had the normal range for TIMP1 concentration except for two individuals. (normal range of TIMP2: 26-110 ng/ml and normal range of TIMP1: 108–223 ng/ml). The TIMP2 plasma levels had significant difference between the patients and control groups thus binary logistic regression was assessed to evaluate the TIMP2 as a risk factor in PCa. OR = 8.2, 95%CI (2.5–26,8, p < 0.001).

All of the methods used in evaluation of the association between the high and low concentration of TIMP with both MMP9 and 2 genotypes had not significant difference.

Discussion

Matrix metalloproteinases are associated with various cancer types but the prognostic value of MMP-2 -1575G/A and MMP-9 -1562C/T polymorphisms in PCa remains controversial [42]. The expression of MMP-9 and its gene variants at position -1562 are shown to be associated with prostate cancer. It has been reported that presence of MMP-9 -1562C/C genotype is correlated with prostate cancer development [43] while, the expression of MMP-2 is associated with the progression and metastasis of prostate cancer [44].

Table 3 Concentration of testosterone, PSA, fPSA, NO, fPSA/PSA and BMI with dominant model of MMP2 genotypes (G/G, A/G + A/A) and MMP9 genotypes (C/C, C/T + T/T) comprised between PCa patients and control subjects

Dominant model of MMP2 genotypes (G/G A/G + A/A)	PCa patients	Control subjects	P values	Dominant model of MMP9 genotypes (C/C, C/T + T/T)	PCa patients	Control subjects	P values
G/G	N = 77	N = 109		C/C	N = 72	N = 100	
Testosterone (µmol/L)	0.5 (0.1–2.1)	2.7 (2.2–4)	< 0.001	Testosterone (µmol/L)	0.9 (0.2–2.37)	2.9 (2.2–3.9)	< 0.001
NO	4.6 (3.98–8.59)	48.9 (40.45–62.15)	< 0.001	NO	5.6 (3.42–8.44)	48 (41.7–60.1)	< 0.001
PSA (mg/dl)	60.4 (15.8–151.3)	0.8 (0.3–1.4)	< 0.001	PSA (mg/dl)	59.05 (15.15–104)	0.736 (0.36–1.42)	< 0.001
fPSA (mg/dl)	7.9 (1.65–20)	0.3 (0.1–0.6)	< 0.001	fPSA (mg/dl)	8.55 (1.65–20)	0.3 (0.1–0.61)	< 0.001
BMI	24.5 ± 4.4	26 ± 4.4	0.017	BMI	23.8 ± 4.1	25.4 ± 4.7	0.18
MMP2 zymography activity	17,377(9669–21,933)	13,198(8704–15,925)	0.013	MMP2 zymography activity	14,936 (9775–22,598)	12,865 (8789–15,448)	0.004
MMP9 zymography activity	22,481(1,218,836,028)	27,841(15,854–40,648)	0.031	MMP9 zymography activity	22,248 (14,479–37,654)	29,178 (15,299–42,062)	0.06
TIMP1 (pg/ml)	10 (7–24)	7 (7–9)	0.009	TIMP1 (pg/ml)	15.5 (45- 196)	7 (6–8)	< 0.001
TIMP2(ng/ml)	59 (37–176)	41.5 (28.7- 68.2)	0.021	TIMP2(ng/ml)	81.5 (45–196.7)	43 (27.8- 51.3)	0.001
AG + A/A	N = 35	N = 41		**C/T + T/T**	N = 40	N = 50	
Testosterone (µmol/L)	0.8 (0.17–2.32)	2.9 (2.2–4)	< 0.001	Testosterone (µmol/L)	0.4 (0.1- 1.91)	2.6 (2.1–4.32)	< 0.001
NO	5.41 (2.5–11.37)	55.1 (42.6–63.2)	< 0.001	NO	4.7 (3.6- 10.72)	54.4 (43.6–65.4)	< 0.001
PSA (mg/dl)	65.5 (18.7–109.2)	0.611 (0.273–1.25)	< 0.001	PSA (mg/dl)	68.1 (19- 100.5)	0.7 (0.2- 1.25)	< 0.001
fPSA (mg/dl)	10 (1.95–19)	0.2 (0.1–0.8)	< 0.001	fPSA (mg/dl)	10 (1.6- 18)	0.22 (0.1–0.6)	< 0.001
BMI	24.67 (20.49–27.64)	23.76 (21.33–25.78)	0.038	BMI	23.8 ± 4.1	25.5 ± 4.7	98
MMP2 zymography activity	13,384(9365–22,977)	12,840 (10,243–16,622)	0.38	MMP2 zymography activity	14,936 ± 8221	13,997 ± 14,949	0.51
MMP9 zymography activity	21,497.5(10,187.5–44,480.5)	29,839 (14,559–43,440)	0.77	MMP9 zymography activity	25,160 ± 18,000	27,966 ± 14,047	0.41
TIMP1 (pg/ml)	18 (8.7–25.7)	8 (6–9)	0.014	TIMP1 (pg/ml)	9 (7–25)	8 (7–15)	0.2
TIMP2(ng/ml)	123 (49–313)	34 (25.5–53.5)	0.005	TIMP2(ng/ml)	59 (37–206)	34 (28–90)	0.034

Both MMP-2 and MMP-9 genes consist of 13 exons and their SNPs are located on chromosomes 16q12.2 and 20q11.2-q13.1, respectively [45]. MMP-2 and MMP-9, also known as gelatinases, degrade collagen types I, II, III and IV, the important structural component of the basement membrane and ECM [46]. TIMPs and α2 macroglobulin block the proteinase activity of MMPs and inhibit the extracellular matrix degradation [45].

We hypothesized that MMP-2 and/or MMP-9 activities and polymorphisms and their inhibitors, TIMPs, in association with smoking and obesity may have a crucial role in PCa pathogenesis. Current study is the first study to show that MMP-2 -1575G/A and MMP-9 -1562C/T gene polymorphisms and serum levels of TIMP-1 and TIMP-2 in association with smoking and diabetes increase the risk of PCa in population from west of Iran.

It is known that level of pro-MMP-9 in PCa is inversely proportional to TIMPs concentration [6]. Our results clearly demonstrated a high incidence of PCa was observed in individuals with -1575 MMP-2 AG + A/A and -1562 MMP9 C/T + T/T genotypes and with high levels of PSA and TIMP-2. Results of an investigation performed by Wood et al. indicated that expression of TIMP-1 and TIMP-2 were high in organ-confined samples, lower in samples with capsular penetration, and low or negative in PCa samples with surgical margin/seminal vesicle and lymph node involvement [47]. The association of MMP-2 -1575G/A and MMP-9 -1562C/T polymorphisms and their correlation with serum concentration of PSA, TIMP and other parameters, which were studied in the current paper, is not sufficient to assess the risk of prostate cancer accurately.

Table 4 Correlation of zymography MMP2 MMP9 activities and TIMP1 and TIMP2 with parameters studies in PCa patients and control group

	MMP2 zymography activity(mU/l)	MMP9 zymography activity(mU/l)	TIMP1	TIMP2	testosterone (µmol/l)	NO	PSA (mg/dl)	fPSA (mg/dl)	fPSA/PSA	BMI	AGE
PCa patients											
MMP2 zymography activity(mU/L)	r = 1	r = 0.19 p = 0.012	r = 0.04 P = 0.74	r = 0.11 P = 0.29	r = 0.05 P = 0.5	r = − 0.12 P = 0.09	r = 0.07 P = 0.27	r = 0.05 P = 0.5	r = − 0.06 p = 0.46	r = − 0.07 p = 0.33	r = 0.1 p = 0.15
MMP9 zymography activity(mU/L)	r = 0.19 p = 0.012	r = 1	r = 0.16 P = 0.14	r = 0.21 *P* = 0.052	r = − 0.04 *P* = 0.56	r = − 0.13 P = 0.077	r = − 0.044 *P* = 0.51	r = − 0.004 P = 0.96	r = − 0.02 p = 0.74	r = − 0.05 p = 0.46	r = 0.1 p = 0.14
TIMP1 (pg/ml)	r = 0.04 P = 0.75	r = 0.16 P = 0.13	r = 1	r = 0.878 P < 0.001	r = 0.006 P = 0.96	r = 0.07 P = 0.56	r = − 0.07 P = 0.49	r = − 0.17 P = 0.15	r = − 0.03 p = 0.8	r = 0.233 p = 0.027	r = − 0.28 P = 0.009
TIMP2 (ng/ml)	r = 0.113 P = 0.287	r = − 0.2 P = 0.05	r = 0.89 P < 0.001	r = 1	r = − 0. 11 P = 0.325	r = 0.003 p = 0.976	r = − 0.03 p = 0.763	r = − 0.12 p = 0. 3	r = − 0.028 P = 0.82	r = 0.222 P = 0.035	r = − 0.215 p = 0. 041
Control group											
MMP2 zymography activity(mU/L)	r = 1	r = 0.318 p = 000	r = − 0.041 P = 0.704	r = − 0.064 P = 0.546	r = − 0.156 P = 0.007	r = 0.050 P = 443	r = 0.011 p = 0.853	r = 0.017 p = 0.776	r = 0.073 p = 0.215	r = 0.069 p = 0.231	r = − 0.123 p = 0.033
MMP9 zymography activity(mU/L)	r = 0.318 p = 0.000	r = 1	r = 0.064 P = 0.556	r = 0.001 P = 0.994	r = − 0.137 P = 0.018	r = 0.079 p = 0.222	r = 0.087 P = 0.132	r = 0.076 p = 0.193	r = 0.118 p = 0.043	r = 0.081 p = 0.164	r = − 0.005 p = 0.926
TIMP1 (pg/ml)	r = − 0.41 P = 0.704	r = 0.064 P = 0.556	r = 1	r = 0.697 P = 0.000	r = 0.087 P = 0.420	r = − 0.152 p = 0.325	r = − 0.057 p = 0.595	r = − 0.039 p = 0.720	r = 0.031 p = 0.775	r = 0.150 p = 0.162	r = − 0.110 P = 0.306
TIMP2 (ng/ml)	r = − 0.064 P = 0.546	r = 0.001 P = 0.994	r = 0.697 P = 0.000	r = 1	r = 0. 156 P = 0.143	r = − 0.143 p = 0.342	r = − 0.045 p = 0.677	r = − 0.057 p = 0. 597	r = − 0.034 P = 0.753	r = − 0.122 p = 0.253	r = − 0.213 p = 0. 044

Our results clearly show that -1575 MMP-2 A/A + A/G genotypes increase the risk of PCa in smokers and in diabetics by 4.34 folds (p = 0.004). In addition, the presence of -1562 MMP-9 C/T + T/T genotypes significantly increase the susceptibility of smokers and diabetics patients to PCa by 3.52 times (p = 0.002). However, the overall frequencies of MMP-2 -1575G/A and MMP9 -1562G/T alleles and genotypes in PCa patients were not significantly different from the control group. Dos et al. reported that in Brazilian population MMP-1 and MMP-2 polymorphisms might have a protective role against prostate cancer development, while MMP-9 may increase the risk of PCa [48]. In Tunisia MMP-9 -1562 T allele increases the incidence of PCa by threefolds (OR = 2.86; P = 0.004) [22], while in North India MMP-2-1306C/T gene polymorphism apparently increases the risk of PCa [49]. These results suggest that in addition to genetic variation of MMP-2 and MMP-9 other environmental factors such as age, smoking and disease affect PCa incidence in population from Western Iran.

We found a significant positive association between testosterone levels and MMP-9 activity in healthy control subjects and between TIMPs level (TIMP-1 and TIMP-2) and BMI in PCa patients. We also found that in both PCa patients and in control subjects, TIMP-2 level is negatively associated with age.

Conclusion

For the first time, we found that smoking and/or diabetes in the presence of -1575 MMP-2 A/A + A/G or -1562 MMP-9 C/T + T/T genotypes significantly increase the risk of PCa. In addition, the levels of PSA, fPSA, TIMP1 and TIMP2 were found to be significantly higher in PCa carrying homozygous or heterozygous mutation at these MMPs' loci. Current study suggests that -1575 MMP-2 A/A + A/G or -1562 MMP-9 C/T + T/T genotypes and high plasma concentration of PSA, fPSA, TIMP1 and TIMP2 in patients with diabetes or in individual smokers may be considered as risk factors for PCa.

Acknowledgements This study was performed in partial fulfillment of requirements for an MSc degree in the Clinical Biochemistry, Kermanshah University of Medical Sciences, Kermanshah Iran (M. Kamankash). This study was funded by Kermanshah University of Medical Sciences, Kermanshah, Iran (Grant #96430). The Ethics Committee of Kermanshah University of Medical Sciences, Iran approved the present study (1.Research involving Human Participants 2. Informed consent ethical legal cod KUMS.Rec.1396.136)) (Grant #96430). All participants gave informed written consent for use of their samples and clinical data in research.

Compliance with ethical standards

Conflict of interest There are no conflicts of interest.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M et al (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5):E359–E386
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer J Clin 68(6):394–424
3. Gandaglia G, Abdollah F, Schiffmann J, Trudeau V, Shariat SF, Kim SP, Perrotte P, Montorsi F, Briganti A, Trinh QD, Karakiewicz PI, Sun M (2014) Distribution of metastatic sites in patients with prostate cancer: a population-based analysis. Prostate 74(2):210–216
4. Deryugina EI, Quigley JP (2006) Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 25(1):9–34
5. Batra J, Soares AS, Mehner C, Radisky ES (2013) Matrix metalloproteinase-10/TIMP-2 structure and analyses define conserved core interactions and diverse exosite interactions in MMP/TIMP complexes. PLoS ONE 8(9):e75836
6. Patterson ML, Atkinson SJ, Knauper V, Murphy G (2001) Specific collagenolysis by gelatinase A, MMP-2, is determined by the hemopexin domain and not the fibronectin-like domain. FEBS Lett 503(2–3):158–162
7. Cicco CD, Ravasi L, Zorzino L, Sandri MT, Botteri E, Verweij F et al (2008) Circulating levels of VCAM and MMP-2 may help identify patients with more aggressive prostate cancer. Curr Cancer Drug Targets 8(3):199–206
8. El-Chaer WK, Moraes CF, Nóbrega OT (2018) Diagnosis and prognosis of prostate cancer from circulating matrix metalloproteinases and inhibitors. J Aging Res. https://doi.org/10.1155/2018/7681039
9. dos Reis ST, Villanova FE, Andrade PM, Pontes J Jr, de Sousa-Canavez JM, Sañudo A et al (2010) Matrix metalloproteinase-2 polymorphism is associated with prognosis in prostate cancer. UrolOncol 28:624–627
10. Schveigert D, Valuckas KP, Kovalcis V, Ulys A, Chvatovic G, Didziapetriene J (2013a) Significance of MMP-9 expression and MMP-9 polymorphism in prostate cancer. Tumori 99(4):523–529
11. Raeeszadeh-Sarmazdeh M, Do LD, Hritz BG (2020) Metalloproteinases and their inhibitors: potential for the development of new therapeutics. Cells 9(5):1313
12. Munhoz F, Godoy-Santos A, Santos M (2010) MMP-3 polymorphism: genetic marker in pathological processes. Mol Med Rep 3(5):735–740
13. Yu C, Pan K, Xing D, Liang G, Tan W, Zhang L et al (2002) Correlation between a single nucleotide polymorphism in the matrix metalloproteinase-2 promoter and risk of lung cancer. Cancer Res 62(22):6430–6433
14. Peng Z, Jia J, Gong W, Gao X, Ma P, Jin Z et al (2017) The association of matrix metalloproteinase-9 promoter polymorphisms with gastric cancer risk: a meta-analysis. Oncotarget 8(58):99024
15. Zhou Y, Yu C, Miao X, Wang Y, Tan W, Sun T et al (2005) Functional haplotypes in the promoter of matrix metalloproteinase-2 and lung cancer susceptibility. Carcinogenesis 26(6):1117–1121
16. Grieu F, Li WQ, Iacopetta B (2004) Genetic polymorphisms in the MMP-2 and MMP-9 genes and breast cancer phenotype. Breast Cancer Res Treat 88(3):197–204
17. Weng H, Zeng XT, Wang XH, Liu TZ, He DL (2017) Genetic association between matrix metalloproteinases gene polymorphisms and risk of prostate cancer: a meta-analysis. Front Physiol 8:975
18. Bahrehmand F, Vaisi-Raygani A, Kiani A, Rahimi Z, Tavilani H, Navabi S et al (2012) Matrix metalloproteinase-2 functional

promoter polymorphism G1575A is associated with elevated circulatory MMP-2 levels and increased risk of cardiovascular disease in systemic lupus erythematosus patients. Lupus 21(6):616–624
19. Zhang K, Chen X, Zhou J, Yang C, Zhang M, Chao M et al (2017) Association between MMP2-1306 C/T polymorphism and prostate cancer susceptibility: a meta-analysis based on 3906 subjects. Oncotarget 8(27):45020
20. Medina-González A, Eiró-Díaz N, Fernández-Gómez JM, Ovidio-González L, Jalón-Monzón A, Casas-Nebra J, Escaf-Barmadah S (2020) Comparative analysis of the expression of metalloproteases (MMP-2, MMP-9, MMP-11 and MMP-13) and the tissue inhibitor of metalloprotease 3 (TIMP-3) between previous negative biopsies and radical prostatectomies. ActasUrolEsp 44(2):78–85
21. Zhang X, Miao X, Xiong P, Yu C, Tan W, Qu S et al (2004) Association of functional polymorphisms in matrix metalloproteinase-2 (MMP-2) and MMP-9 genes with risk of gastric cancer in a Chinese population. Chin J Cancer 23(11):1233–1237
22. Sfar S, Saad H, Mosbah F, Gabbouj S, Chouchane L (2007) TSP1 and MMP9 genetic variants in sporadic prostate cancer. Cancer Genet Cytogenet 172(1):38–44
23. Gann PH (2002) Risk factors for prostate cancer. Rev Urol 4(Suppl 5):S3–S10
24. Brawley OW (2012) Trends in Prostate Cancer in the United States. J Nat Cancer Inst 2012(45):152–156
25. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl J Med 348(17):1625–1638
26. McGreevy KM, Hoel BD, Lipsitz SR, Hoel DG (2007) Impact ofnutrients on insulin-like growth factor-I, insulin-like growth-factor binding protein-3 and their ratio in African American and white males. Public Health Nutr 10(1):97–105
27. Chen CB, Eskin M, Eurich DT, Majumdar SR, Johnson JA (2018) Metformin, Asian ethnicity and risk of prostate cancer in type 2 diabetes: a systematic review and meta-analysis. BMC Cancer 18(1):65
28. Crawley D, Chamberlain F, Garmo H, Rudman S, Zethelius B, Holmberg L et al (2018) A systematic review of the literature exploring the interplay between prostate cancer and type two diabetes mellitus. Ecancermedicalscience 12:802
29. Plaskon DF, Penson TL, Vaughan JL (2003) Stanford;cigarette smoking and risk of prostate cancer in middle-aged men. Cancer EpidemiolPrevBiomark 12(7):604–609
30. Lu X, Yamano Y, Takahashi H, Koda M, Fujiwara Y, Hisada A et al (2015) Associations between estrogen receptor genetic polymorphisms, smoking status, and prostate cancer risk: a case-control study in Japanese men. Environ Health Prev Med 20(5):332–337
31. Fowler JE Jr., Whitmore WF Jr. (1981) The response of metastatic adenocarcinoma of the prostate to exogenous testosterone. J Urol 126(3):372–375
32 Schweizer MT, Antonarakis ES, Wang H, Ajiboye AS, Spitz A, Cao H et al (2015) Effect of bipolar androgen therapy for asymptomatic men with castration-resistant prostate cancer: results from a pilot clinical study. SciTransl Med 7(269):269ra2
33. dos Reis ST et al (2010) Matrix metalloproteinase-2 polymorphism is associated with prognosis in prostate cancer. UrolOncol 28(6):624–627
34. Schveigert D et al (2013) Significance of MMP-9 expression and MMP-9 polymorphism in prostate cancer. Tumori J 99(4):523–529
35 Bultitude MF (2012) Campbell-walsh urology tenth edition. BjuInt 109(3):E10
36 Barakzai MA (2019) Prostatic adenocarcinoma: A grading from Gleason to the new grade-group system: a historical and critical review. Asian Pac J Cancer P: APJCP 20(3):661
37 World Health Organization (2006) Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. World Health Organisation, Geneva
38. Saedi M, Vaisi-Raygani A, Khaghani S, Shariftabrizi A, Rezaie M, Pasalar P, Rahimi Z, Pourmotabbed T (2012) Matrix metalloproteinase-9 functional promoter polymorphism 1562C>T increased risk of early-onset coronary artery disease. MolBiol Rep 39(1):555–562
39. Vaisi-Raygani A, Rahimi Z, Entezami H, Kharrazi H, Bahrhemand F, Tavilani H et al (2008) Butyrylcholinesterase K variants increase the risk of coronary artery disease in the population of western Iran. Scand J Clin Lab Inv 68(2):123–129
40. Bahrehmand F, Vaisi-Raygani A, Kiani A, Rahimi Z, Tavilani H, Ardalan M, Vaisi-Raygani H, Shakiba E, Pourmotabbed T (2015) Matrix metalloproteinase 9 polymorphisms and systemic lupus erythematosus: correlation with systemic inflammatory markers and oxidative stress. Lupus 24(6):597–605
41. Masuhara K, Nakai T, Yamaguchi K, Yamasaki S, Sasaguri Y (2002) Significant increases in serum and plasma concentrations of matrix metalloproteinases 3 and 9 in patients with rapidly destructive osteoarthritis of the hip. Arthritis Rheum 46(10):2625–2631
42. Xie T, Dong B, Yan Y, Hu G, Xu Y (2016) Association between MMP-2 expression and prostate cancer: a meta-analysis. Biomed Rep 4(2):241–245
43. Schveigert D, Valuckas KP, Kovalcis V, Ulys A, Chvatovic G, Didziapetriene J (2013b) Significance of MMP-9 expression and MMP-9 polymorphism in prostate cancer. Tumori J 99(4):523–529
44. Murray NP, Reyes E, Tapia P, Badínez L, Orellana N (2012) Differential expression of matrix metalloproteinase-2 expression in disseminated tumor cells and micrometastasis in bone marrow of patients with nonmetastatic and metastatic prostate cancer: theoretical considerations and clinical implications—an immunocytochemical study. Bone Marrow Res. https://doi.org/10.1155/2012/259351
45. Maral S, Acar M, Balcik OS, Uctepe E, Hatipoglu OF, Akdeniz D et al (2015) Matrix metalloproteinases 2 and 9 polymorphism in patients with myeloproliferative diseases: a STROBE-compliant observational study. Medicine (Baltimore) 94(1):e732
46. Dong Z, Nemeth JA, Cher ML, Palmer KC, Bright RC, Fridman R (2001) Differential regulation of matrix metalloproteinase-9, tissue inhibitor of metalloproteinase 1 (TIMP 1) and TIMP 2 expression in cocultures of prostate cancer and stromal cells. Int J Cancer 93(4):507–515
47. Wood M, Fudge K, Mohler J, Frost A, Garcia F, Wang M et al (1997) In situ hybridization studies of metalloproteinases 2 and 9 and TIMP-1 and TIMP-2 expression in human prostate cancer. ClinExp Metastasis 15(3):246–258
48. Dos Reis ST, Pontes J Jr, Villanova FE, de Andrade Borra PM, Antunes AA, Dall'oglio MF et al (2009) Genetic polymorphisms of matrix metalloproteinases: susceptibility and prognostic implications for prostate cancer. J Urol 181(5):2320–2325
49. Srivastava P, Lone TA, Kapoor R, Mittal RD (2012) Association of Promoter Polymorphisms in MMP2 and TIMP2 with Prostate Cancer Susceptibility in North India. Arch Med Res 43(2):117–124

Affiliations

Amir Kiani[1] · Marjan Kamankesh[2] · Asad Vaisi-Raygani[2] · Mahmoud-Reza Moradi[1] · Maryam Tanhapour[2] · Zohreh Rahimi[3] · Saeed Elahi-Rad[1] · Fariborz Bahrehmand[3] · Mahdieh Aliyari[2] · Faranak Aghaz[2] · Hadi Mozafari[2] · Nayebali Rezvani[2] · Lida Haghnazari[2] · Tayebeh Pourmotabbed[4]

Amir Kiani
amir1kiani@yahoo.com

Marjan Kamankesh
marjan.89.kam@gmail.com

Mahmoud-Reza Moradi
dr-mmoradi@yahoo.com

Maryam Tanhapour
maryam.tanhapoor2@gmail.com

Zohreh Rahimi
rahimizus@yahoo.com

Saeed Elahi-Rad
saeedelahirad@gmail.com

Fariborz Bahrehmand
fariborzbahrehmand@gmail.com

Mahdieh Aliyari
mahdiehaliyari@gmail.com

Faranak Aghaz
faranak_aghaz@yahoo.com

Hadi Mozafari
hmozafari2003@yahoo.com

Nayebali Rezvani
arezvani51@gmail.com

Lida Haghnazari
lida_haghnazari@yahoo.com

[1] Regenerative Medicine Research Center (RMRC), Kermanshah University of Medical Sciences, Kermanshah, Iran

[2] Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

[3] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

[4] Department of Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, 858 Madison Ave, Memphis, TN 48163, USA