

MMP7 and MMP8 genetic polymorphisms in bladder cancer patients

Edyta Wieczorek¹, Edyta Reszka¹, Wojciech Wasowicz¹, Adam Grzegorzczak², Tomasz Konecki², Marek Sosnowski², Zbigniew Jablonowski²

¹Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Łódź, Poland

²1st Department of Urology, Medical University, Łódź, Poland

Article history

Submitted: Jun. 16, 2013

Accepted: Oct. 24, 2013

Correspondence

Edyta Wieczorek
Nofer Institute of
Occupational Medicine
8, Św. Teresy od Dzieciątka
Jezus Street
91–348 Łódź, Poland
phone: +48 42 631 46 25
edytaw@imp.lodz.pl

Introduction. Breakdown of the extracellular matrix by matrix metalloproteinases (MMPs), as we know, is one of mechanisms involved and required in tumor invasion. *MMP7* is a negative prognostic factor of various malignances, while *MMP8* exhibits an inhibitory effect on tumorigenesis and metastasis. We evaluated the potential association of functional polymorphisms in the promoter of the *MMP7* (*rs11568818*) and *MMP8* (*rs11225395*) genes and bladder cancer (BCa) risk.

Materials and methods. The study included 241 BCa cases and 199 healthy population controls that were collected at the First Department of Urology, Medical University (Łódź, Poland) and at the Nofer Institute of Occupational Medicine (Łódź, Poland). Genomic DNA samples were isolated from venous blood and genetic polymorphisms were analyzed by real-time polymerase chain reaction using TaqMan fluorescent probes. Associations between genotype and allele status were estimated by logistic regression models adjusted for classic risk factors (e.g. age, gender and cigarette smoking).

Results. *MMP7* and *MMP8* genotypes were distributed similarly in BCa patients and in controls and at least one variant allele was not associated with BCa cancer risk (OR, 0.91; 95% CI, 0.60–1.39; $p = 0.662$ for *MMP7* and OR, 0.96; 95% CI, 0.63–1.46; $p = 0.836$ for *MMP8*). We observed higher prevalence of *MMP7* GG genotypes among BCa patients than in controls (OR, 1.54; 95% CI, 0.93–2.55; $p = 0.093$). Additionally, genetic polymorphisms in the *MMP7* and *MMP8* were not associated with the tumor grade or stage.

Conclusions. Our results suggest that genetic variations in two genes encoding members of the *MMP7* and *MMP8* are not associated with a risk of BCa in the Caucasian population.

Key Words: MMP ◊ genetic polymorphism ◊ case–control study ◊ bladder cancer

INTRODUCTION

Bladder cancer (BCa) was estimated to be the seventh most common type of cancer worldwide in 2009 [1]. In Poland, BCa was the third most frequent malignant tumor in men and thirteenth in women in 2009 [2]. Etiology of BCa includes environmental factors such as exposure to tobacco smoke, occupational exposure, and geography of selected infectious diseases like schistosomiasis [3]. The group of patients with non-muscle-invasive disease (NMIBC) covers 75% and muscle invasive disease (MIBC) covers 25% of BCa patients. About 20% of NMIBC occur as re-

currences of MIBC and are related with an increased risk of metastases and low survival [4]. Therefore, more extensive studies are currently in progress to elucidate the importance of the various tumor characteristics or genetic factors in the areas of BCa risk, recurrence or progression [5, 6].

MMPs are implicated in various stages of physiological processes such as embryonic development, ovulation, wound healing, and pathophysiological processes like heart failure, arthritis, atherosclerosis, periodontal disease, bone remodeling, and carcinogenesis. MMPs can hydrolyze most of the extracellular matrix (ECM) components, a range of non-

matrix proteins, matrix substrates, and bioactive substrates. MMPs can also release from the ECM the signaling molecules important in tumor progression and metastasis [7]. MMPs are the enzymes that are able to organize the tissue architecture required in the process of carcinogenesis and contribute to cell adhesion, epithelial to mesenchymal transition, and tumor angiogenesis [8].

MMP7 also known as matrilysin-1 or PUMP-1, possesses broad substrate specificity, and can degrade collagen types: IV, V, IX, X, XI, aggrecan, entactin, laminin, vitronectin, fibrin/fibrinogen, tenascin, gelatin, fibronectin, and other substrates of the ECM components [7]. MMP7 expression can promote cancer cell growth and thus can be associated with tumor invasion, recurrence and poorer survival for various malignancies [9–16]. MMP8, commonly known as collagenase-2 or neutrophil collagenase, hydrolyzes collagen types: I, II, III, VII, X, gelatin, entactin, tenascin, aggrecan, and other ECM components [7]. MMP8 is one of MMPs that has been found to produce anticancer effects due to its ability to inhibit tumorigenesis and metastasis [17, 18]. Cancer studies conducted over eight years have found an association between the development of specific cancer sites and functional polymorphisms that can alter gene expression located in the promoter regions of *MMP7* (*rs11568818*) and *MMP8* (*rs11225395*) [19–23]. However, there have been no association studies of *MMP7* and *MMP8* polymorphisms in BCa risk in the Caucasian population. Therefore, the objective of this association study was to evaluate polymorphisms in *MMP7* and *MMP8* and characterize their associations with BCa susceptibility in a population of patients from Łódź.

MATERIALS AND METHODS

Study population

BCa patients were recruited from the First Department of Urology, Medical University of Łódź and Nofer Institute of Occupational Medicine in Łódź from 2007 to 2013. The 241 BCa patients and 199 healthy population controls were recruited from an ethnically homogeneous Polish population. Data on histological cancer grades diagnosed at the First Department of Urology were not accessible for all cases. All of the BCa patients underwent transurethral resection and had histopathologically confirmed NMIBC or MIBC at various tumor (T) stage and grade (G) of neoplasm. The tumor stage (87.1% of diagnosed BCa patients), and tumor grade (89.2% of diagnosed patients) were included in the association analyses. To evaluate differenc-

es between *MMP7* or *MMP8* genotypes and tumor grade or stage, patients with BCa were divided into categories: group 1) with G1, and group 2) with G2 and G3 or group 1) with T1, and group 2) with T2–T4, respectively. All patients with missing data were excluded from these analyses. Additionally, to examine the joint effects of *MMP7* or *MMP8* genotypes and tobacco smoking status on BCa risk, we stratified cases and controls into three categories: 1) never smokers – persons who had never smoked in their lifetime, 2) ex-smokers – individuals who were abstinent for at least 1 year before the interview, 3) smokers – persons who stated they currently smoked cigarettes or who were abstinent for up to 1 year before the interview. The study was approved by the Local Ethical Committee of the Nofer Institute of Occupational Medicine.

DNA isolation and genotyping

The genomic DNA was isolated from the peripheral blood samples of the study subjects using commercial DNA kits QIAamp DNA Mini Kits (Qiagen) following the manufacturer's protocol. The promoter single nucleotide polymorphisms (SNPs) in the *MMP7* (*rs11568818*) and *MMP8* (*rs11225395*) genes were genotyped using TaqMan fluorescent probes, assays on demand (Assay ID: C_27852953_10 and C_1366493_10, respectively) (Life Technologies). All patients and controls were genotyped using Real-Time PCR CFX96 System (BioRad).

Statistical analysis

Group characteristics were determined by the χ^2 (chi-square) test and Student's *t*-test. Hardy-Weinberg equilibrium (HWE) was tested by comparing the observed and expected genotype frequencies in cases and controls at equilibrium based on the χ^2 test at a significant level of $p < 0.05$. The strength of the association between genetic polymorphisms in the *MMP* and BCa susceptibility was measured by odds ratios (ORs) corresponding to 95% confidence interval (95% CI). ORs and 95% CI were determined by logistic regression analyses using additive models that included adjustment for age, gender, and cigarette smoking status. Major allele homozygotes served as the reference group, and heterozygotes and minor allele homozygotes were separately compared. The association between the *MMPs* genotype frequencies and characteristics was estimated by Fisher's exact test using the Stata 11 (StataCorp LP, USA) software. All statistical tests presented in this paper are two-sided and p values were considered to be statistically significant when $p \leq 0.05$.

RESULTS

Cohort characteristics

We analyzed genetic polymorphisms in the promoter region of *MMP7* and *MMP8* in 241 BCa patients and 199 controls from Poland. In Table 1 we summarized demographics, cigarette smoking status, and clinical characteristics of the patients. Statistically significant differences were observed between BCa cases and controls in terms of the distribution of gender ($p = 0.001$) and tobacco smoking status ($p = 0.001$). At the time of BCa diagnosis, patients were between ages 64.9 and 67.7 years (mean 66.1 ± 10.4) and were at the same age as the controls (mean 66.3 ± 10.6) ($p = 0.872$). The genotype distributions of both chosen polymorphisms were consistent with Hardy–Weinberg equilibrium (Table 2).

Analysis of association

For the genetic polymorphisms in the *MMP7* (*rs11568818*) and *MMP8* (*rs11225395*) genes, significant differences between genotyped BCa patients and controls were not observed. Prevalence of *MMP7*

Table 1. Selected characteristics of the BCa patients and healthy controls at the time of diagnosis

Variable	Controls n (%)	Cases n (%)	p -value ^b
Gender			
Female	23 (11.6)	59 (24.5)	0.001
Male	176 (88.4)	182 (75.5)	
Mean age (years) \pm SD	66.1 \pm 10.4	66.3 \pm 10.6	0.872 ^c
Smoking status ^a			
Never–smokers ^d	70 (35.4)	64 (27.0)	0.001
Ex–smokers ^e	91 (45.9)	92 (38.8)	
Smokers ^f	32 (18.7)	81 (34.2)	
Tumor grade ^a			
G1	–	113 (46.9)	–
G2	–	64 (26.6)	
G3	–	38 (15.8)	
G2 and G3	–	102 (42.3)	
Unknown	–	26 (10.7)	
Tumor stage ^a			
NMIBC (T1)	–	169 (70.1)	–
MIBC (T2–T4)	–	41 (17.0)	
Unknown	–	31 (12.9)	

^athe number of cases and controls may vary because of some missing data; ^bchi-square test was used to determine p -value; ^ct-test was used to determine p -value; ^dnever smokers – persons who had never smoked; ^eex-smokers – individuals who were abstinent for at least 1 year; ^fsmokers – individuals who currently smoke or who were abstinent for up to 1 years before the interview; NMIBC – non-muscle-invasive bladder cancer; MIBC – muscle-invasive bladder cancer

Table 2. Summary of the genetic polymorphisms in the *MMP* analyzed in this study

Gene	<i>MMP7</i>	<i>MMP8</i>	
MMPs exert cancer effects	Supporting	Inhibiting	
dbSNP ID	<i>rs11568818</i>	<i>rs11225395</i>	
Nucleotide position ^a	–181	–799	
Type of region	Promoter	Promoter	
Chromosome	11q22.2a	11q22.2b	
RefSNP Alleles ^b	A/G	C/T	
Allele with greater transcriptional activity	G allele	T allele	
Alleles frequency of BCa patients ^c	A	0.55	0.56
	B	0.45	0.44
	HWE ^d	1.06	0.64
Alleles frequency of controls ^c	A	0.58	0.56
	B	0.42	0.44
	HWE ^d	1.01	0.15

^aminus indicates promoter region; ^bmajor/minor alleles and frequencies, as determined by the distribution among the case and controls; ^cA major allele, B minor allele; ^dHardy–Weinberg equilibrium p -value

G/G genotype was only slightly different among patients (22.1%) and controls (16.1%), and BCa risk among *MMP7 G/G* was estimated to OR, 1.54; 95% CI, 0.93–2.55; $p = 0.093$ (Table 3). Association of single *MMP7* and *MMP8* genotypes with BCa risk in groups stratified by tobacco smoking status was assessed. The statistical significance of additive effects was not observed among stratified groups (data not shown). We also studied *MMP7* and *MMP8* polymorphisms and their risk associated with different histology grade of BCa. None of the G and T-categorized groups of patients were associated with individual *MMP7* or *MMP8* polymorphisms (data not shown). In order to examine whether the effect of gene–gene interactions in *MMP7* and *MMP8* polymorphisms might change the BCa risk, we analyzed various combinations of genotypes. We observed that the combined effect of *MMP7* and *MMP8* genotypes did not provide statistically significant differences between cases and controls (Table 4), nor in any tobacco smoking status (data not shown).

DISCUSSION

It is generally believed that the development of the invasive phenotype (advanced metastatic stage of cancer) and further poor prognosis may be associated with increased expression of MMPs [22, 24, 25]. Furthermore, we know that MMPs regulatory effect may be related to *MMP* promoter SNPs. It is

Table 3. Distribution of the genetic polymorphisms in the MMP7 and MMP8 among controls and BCa patients

Gene dbSNP	Genotypes ^a	Controls ^b		Cases ^b		BCa risk OR (95% CI) ^c , <i>p</i> -value	
		n	(%)	n	(%)	Crude	Adjusted ^d
MMP7 (rs11568818)	A/A	63	(31.6)	76	(31.7)	Ref.	Ref.
	A/G	104	(52.3)	111	(46.3)	0.79 (0.54–1.14), 0.210	0.72 (0.49–1.08), 0.112
	G/G	32	(16.1)	53	(22.1)	1.48 (0.91–2.40), 0.114	1.54 (0.93–2.55), 0.093
	A/G+G/G	136	(68.3)	164	(68.3)	1.00 (0.67–1.50), 0.999	0.91 (0.60–1.39), 0.662
MMP8 (rs11225395)	C/C	60	(30.2)	72	(29.9)	Ref.	Ref.
	C/T	101	(50.8)	125	(51.9)	1.04 (0.72–1.52), 0.816	0.97 (0.65–1.44), 0.880
	T/T	38	(19.1)	44	(18.2)	0.94 (0.58–1.53), 0.822	1.00 (0.60–1.66), 0.997
	C/T+T/T	139	(69.9)	169	(70.1)	1.01 (0.67–1.53), 0.950	0.96 (0.63–1.46), 0.836

^agenotypes and frequencies, as determined by the distribution among the cases and controls; ^bthe number of cases and control may vary because of some missing data; ^cOR, odds ratio, CI, 95% confidence interval; ^dodds ratio adjusted for age, gender and cigarette smoking status

Table 4. Risk of BCa associated with the gene-gene interactions in MMP7 and MMP8 polymorphisms

Genotype MMP7	Genotype MMP8	Controls n (%)	Cases n (%)	OR (95% CI) ^a , <i>p</i> -value Crude
A/A	C/C	16 (25.4)	15 (19.7)	Ref. ^b
	C/T+T/T	47 (74.6)	61 (80.3)	1.38 (0.62–3.08), 0.540
A/G+G/G	C/C	44 (32.4)	57 (34.8)	1.38 (0.62–3.10), 0.537
	C/T +T/T	92 (67.6)	107 (65.2)	1.24 (0.58–2.65), 0.699

^aOR, odds ratio, CI, 95% confidence interval; ^bmajor allele homozygotes (reference group)

considered that the analysis of SNPs in MMPs as prognostic biomarkers, might identify BCa patients at an increased risk of lymph node metastasis even before surgery. MMP gene variants may influence their transcriptional activity and protein expression, function, and activity. Therefore, they may be associated with cancer risk, prognosis, and responses to treatment.

MMP7 and MMP8 may play different roles in cancer risk and cancer progression. MMP7 over-expression is often observed in various malignancies, which supports action on carcinogenesis, while MMP8 possesses inhibitory effect [16, 17, 26]. Genetic polymorphism in the MMP7 –181 A/G (rs11568818) promoter have been described in several association studies on lung, breast, colorectal, gastric, and prostate cancer, indicating significant association between at least one MMP7 G allele and gastric cancer risk (OR, 1.90; 95% CI, 1.43–2.51), but no consistent results for other types of cancer [27]. MMP7 genetic polymorphism affects the transcriptional activity of that gene and leads to changes in its expression. Namely, the G allele compared to the A allele is transcriptionally more active [28]. Expression of MMP7 has been detected in various cancers [16, 26]. Studies which investi-

gated serum and plasma levels of MMP7, identified high levels of MMP7 as predictors of BCa outcomes. Moreover, MMP7 protein expression can correlate with pathologic parameters like tumor stage, tumor grade, and metastasis in BCa [29, 30, 31].

Recent studies on metastatic potential of breast cancer cells show that over-expression of MMP8 protein provides a protective effect in the metastatic process [32]. Also, in studies of the squamous cell carcinoma of the tongue it has been found that MMP8 protein expression is correlated with improved survival of patients [34]. However, there are only a few case-control studies regarding MMP8 –799 C/T (rs11225395) impact on cancer development. *In vitro* data has suggested that promoter MMP8 polymorphism has putative functional significance and higher transcription activity for minor MMP8 T allele compared with MMP8 C allele [35]. Indeed, breast cancer risk (OR, 0.7; 95% CI, 0.5–0.9) and survival (OR, 0.4; 95% CI, 0.2–0.8) was significantly reduced for women carrying the MMP8 allele T [35, 36]. However, a study of Polish individuals reported that allele T was associated with risk of developing malignant melanoma [37]. In analyzed individuals from Central Poland, the frequency of MMP7 G alleles was 0.42, while for MMP8 T it was 0.44. To compare, in control individuals from Łódź, the frequency of MMP7 G alleles was 0.45 [38], while in individuals from Szczecin, the frequency was 0.39 [37].

In the present association study we tested the hypothesis whether the functional genetic polymorphisms in the MMP7 and MMP8 genes contributed to BCa risk. To our knowledge, this is the first study investigating the relationship between MMP7 (rs11568818) and MMP8 (rs11225395) polymorphisms and BCa risk in Caucasian population. Two studies conducted by Srivastava et al. in North India population showed significantly higher risk of BCa among MMP7 G/G (rs11568818) individuals (OR, 2.38; 95%

CI, 1.30–4.34), while *MMP8 C/T+T/T (rs11225395)* genotype carriers possessed reduced BCa risk (OR, 0.27; 95% CI, 0.10–0.69) [19, 23]. Nevertheless, we found that these genetic polymorphisms do not contribute to risk of BCa and cancer grade. However, we observed that individuals with *MMP7 G/G* genotype were at statistically borderline risk of BCa (OR, 1.54; 95% CI, 0.93–2.55; $p = 0.093$). We found no additive effect of smoking and genetic polymorphisms in the *MMP7* and *MMP8* among never-smokers, ex-smokers, and smokers. Additionally, our investigation of the potential combined effect of gene–gene interaction between genetic polymorphisms, reported no correlation with BCa risk and cancer grade.

CONCLUSIONS

In this study, the results show no association between the genetic polymorphisms in biologically rel-

evant candidate genes such as *MMP7 (rs11568818)* and *MMP8 (rs11225395)* and BCa risk in the Caucasian population. It should be noted that our study was encumbered with a limitation. Due to the small number of cases studied, some associations were not evident. The search for new biomarkers of cancer is still required and more extensive research on genetic polymorphisms of *MMP* in BCa should be undertaken in the future.

ACKNOWLEDGEMENTS

This work was supported by Polish Society of Urology, Internal Grant IMP 1.22 and the Ministry of Science and Higher Education (2012/05/B/NZ5/01406).

References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. *CA Cancer J Clin.* 2009; 59: 225–249.
- Didkowska J, Wojciechowska U, Zatonski W. Cancer incidence – tables and figures, Cancer in Poland in 2009, Warszawa: Institute of Oncology, 2011; Chapt 5, pp. 51–80.
- Parkin DM. The global burden of urinary bladder cancer. *Scand J Urol Nephrol Suppl.* 2008; 218: 12–20.
- Raghavan D, Shipley WU, Garnick MB, Russell PJ, Richie JP. Biology and management of bladder–cancer. *N Engl J Med.* 1990; 322: 1129–1138.
- Borkowska EM, Jędrzejczyk A, Marks P, Catto JWF, Kałużewski B. EORTC risk tables – their usefulness in the assessment of recurrence and progression risk in non–muscle–invasive bladder cancer in Polish patients. *Cent Eur J Urol.* 2013; 66: 14–20.
- Banaszkiewicz M, Constantinou M, Pietrusiński M, Kępczyński Ł, Jędrzejczyk A, Roźniński M, et al. Concomitance of oncogenic HPV types, CHEK2 gene mutations, and CYP1B1 gene polymorphism as an increased risk factor for malignancy. *Cent Eur J Urol.* 2013; 66: 23–29.
- Overall CM. Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites. *Mol Biotechnol.* 2002; 22: 51–86.
- Nabeshima K, Inoue T, Shimao Y, Sameshima T. Matrix metalloproteinases in tumor invasion: Role for cell migration. *Pathol Int.* 2002; 52: 255–264.
- Fingleton B, Vargo–Gogola T, Crawford HC, Matrisian LM. Matrilysin MMP–7 expression selects for cells with reduced sensitivity to apoptosis. *Neoplasia.* 2001; 3: 459–468.
- Noe V, Fingleton B, Jacobs K, Crawford HC, Vermeulen S, Steelant W, et al. Release of an invasion promoter E–cadherin fragment by matrilysin and stromelysin–1. *J Cell Sci.* 2001; 114: 111–118.
- Mitsiades N, Yu WH, Poulaki V, Tsokos M, Stamenkovic I. Matrix metalloproteinase–7–mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res.* 2001; 61: 577–581.
- Strand S, Vollmer P, van den Abeelen L, Gottfried D, Alla V, Heid H, et al. Cleavage of CD95 by matrix metalloproteinase–7 induces apoptosis resistance in tumour cells. *Oncogene.* 2004; 23: 3732–3736.
- Miyamoto S, Yano K, Sugimoto S, Ishii G, Hasebe T, Endoh Y, et al. Matrix metalloproteinase–7 facilitates insulin–like growth factor bioavailability through its proteinase activity on insulin–like growth factor binding protein 3. *Cancer Res.* 2004; 64: 665–671.
- Hemers E, Duval C, McCaig C, Handley M, Dockray GJ, Varro A. Insulin–like growth factor binding protein–5 is a target of matrix metalloproteinase–7: Implications for epithelial–mesenchymal signaling. *Cancer Res.* 2005; 65: 7363–7369.
- Nakamura M, Miyamoto S, Maeda H, Ishii G, Hasebe T, Chiba T, et al. Matrix metalloproteinase–7 degrades all insulin–like growth factor binding proteins and facilitates insulin–like growth factor bioavailability. *Biochem Biophys Res Commun.* 2005; 333: 1011–1016.
- Cardillo MR, Di Silverio F, Gentile V. Quantitative immunohistochemical and in situ hybridization analysis of metalloproteinases in prostate cancer. *Anticancer Res.* 2006; 26: 973–982.
- Konstantinopoulos PA, Karamouzis MV, Papatsoris AG, Papavassiliou AG. Matrix metalloproteinase inhibitors as anticancer agents. *Int J Biochem Cell Biol.* 2008; 40: 1156–1168.
- Dejonckheere E, Vandenbroucke RE, Libert C. Matrix metalloproteinase8 has a central role in inflammatory disorders and cancer progression. *Cytokine Growth Factor Rev.* 2011; 22: 73–81.
- Srivastava P, Gangwar R, Kapoor R, Mittal RD. Bladder cancer risk associated with genotypic polymorphism of the matrix metalloproteinase–1 and 7 in North Indian population. *Dis Markers.* 2010; 29: 37–46.

20. Kader AK, Shao L, Dinney CP, Schabath MB, Wang Y, Liu J, et al. Matrix metalloproteinase polymorphisms and bladder cancer risk. *Cancer Res.* 2006; 66: 11644–11648.
21. Kader AK, Liu J, Shao L, Dinney CP, Lin J, Wang YF, et al. Matrix metalloproteinase polymorphisms are associated with bladder cancer invasiveness. *Clin Cancer Res.* 2007; 13: 2614–2620.
22. Szarvas T, vom Dorp F, Erguen S, Ruebben H. Matrix metalloproteinases and their clinical relevance in urinary bladder cancer. *Nat Rev Urol.* 2011; 8: 241–254.
23. Srivastava P, Kapoor R, Mittal RD. Association of single nucleotide polymorphisms in promoter of matrix metalloproteinase–2, 8 genes with bladder cancer risk in Northern India. *Urol Oncol.* 2013; 31: 247–254.
24. O'Mara TA, Clements JA, Spurdle AB. The use of predictive or prognostic genetic biomarkers in endometrial and other hormone-related cancers: Justification for extensive candidate gene single nucleotide polymorphism studies of the matrix metalloproteinase family and their inhibitors. *Cancer Epidemiol Biomarkers Prev.* 2009; 18: 2352–2365.
25. Davies B, Waxman J, Wasan H, Abel P, Williams G, Krausz T, et al. Levels of matrix metalloproteinases in bladder cancer correlate with tumor grade and invasion. *Cancer Res.* 1993; 53: 5365–5369.
26. Hashimoto K, Kihira Y, Matuo Y, Usui T. Expression of matrix metalloproteinase–7 and tissue inhibitor of metalloproteinase–1 in human prostate. *J Urol.* 1998; 160: 1872–1876.
27. Peng B, Cao LH, Ma XP, Wang WZ, Wang D, Yu L. Meta-analysis of association between matrix metalloproteinases 2, 7 and 9 promoter polymorphisms and cancer risk. *Mutagenesis.* 2010; 25: 371–379.
28. Zhang JH, Jin X, Fang SM, Wang R, Li Y, Wang N, et al. The functional polymorphism in the matrix metalloproteinase–7 promoter increases susceptibility to esophageal squamous cell carcinoma, gastric cardiac adenocarcinoma and non-small cell lung carcinoma. *Carcinogenesis.* 2005; 26: 1748–1753.
29. Szarvas T, Jaeger T, Becker M, Tschirdewahn S, Niedworok C, Kovalszky I, et al. Validation of circulating MMP–7 level as an independent prognostic marker of poor survival in urinary bladder cancer. *Pathol Oncol Res.* 2011; 17: 325–332.
30. Szarvas T, Becker M, vom Dorp F, Gethmann C, Toetsch M, Bankfalvi A, et al. Matrix metalloproteinase–7 as a marker of metastasis and predictor of poor survival in bladder cancer. *Cancer Sci.* 2010; 101: 1300–1308.
31. Szarvas T, Singer BB, Becker M, Dorp FV, Jaeger T, Szendroi A, et al. Urinary matrix metalloproteinase–7 level is associated with the presence of metastasis in bladder cancer. *BJU Int.* 2011; 107: 1069–1073.
32. Decock J, Hendrickx W, Vanleeuw U, Van Belle V, Van Huffel S, Christiaens MR, et al. Plasma MMP1 and MMP8 expression in breast cancer: protective role of MMP8 against lymph node metastasis. *BMC Cancer.* 2008; 8: 77.
33. Korpi JT, Kervinen V, Maklin H, Vaananen A, Lahtinen M, Laara E, et al. Collagenase–2 (matrix metalloproteinase–8) plays a protective role in tongue cancer. *Br J Cancer.* 2008; 98: 766–775.
34. Wang H, Parry S, Macones G, Sammel MD, Ferrand PE, Kuivaniemi H, et al. Functionally significant SNP MMP8 promoter haplotypes and preterm premature rupture of membranes (PPROM). *Hum Mol Genet.* 2004; 13: 2659–2669.
35. Decock J, Long JR, Laxton RC, Shu XO, Hodgkinson C, Hendrickx W, et al. Association of matrix metalloproteinase–8 gene variation with breast cancer prognosis. *Cancer Res.* 2007; 67: 10214–10221.
36. Beeghly–Fadiel A, Zheng W, Lu W, Long J, Zheng Y, Cai H, et al. Replication study for reported SNP associations with breast cancer survival. *J Cancer Res Clin Oncol.* 2012; 138: 1019–1026.
37. Debniak T, Jakubowska A, Serrano–Fernandez P, Kurzawski G, Cybulski C, Chauhan SR, et al. Association of MMP8 gene variation with an increased risk of malignant melanoma. *Melanoma Res.* 2011; 21: 464–468.
38. Dziki L, Przybylowska K, Majsterek I, Trzcinski R, Mik M, Sygut A. A/G Polymorphism of the MMP–7 Gene Promoter Region in Colorectal Cancer. *Pol Przegl Chir.* 2011; 83: 622–626. ■