· Advances in Medical Sciences · Vol. 58(1) · 2013 · pp 73-82 · DOI: 10.2478/v10039-012-0066-y

The value of MMP-9 for breast and non-small cell lung cancer patients' survival

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Received 31.05.2012
Accepted 29.11.2012
Advances in Medical Sciences
Vol. 58(1) 2013 · pp 73-82
DOI: 10.2478/v10039-012-0066-y

ABSTRACT

Purpose: Matrix metalloproteinases (MMPs) are implicated in cancer cells invasion and metastasis processes and have been investigated as potential cancer biomarkers. In this study MMP-9 gene expression and MMP-9 -1562 C/T polymorphism in breast and non-small cell lung cancer patients' blood and tumor samples and its correlation with clinicopathological parameters were investigated.

Material/Methods: MMP-9 gene expression was assessed by reverse transcription – polymerase chain reaction method in 108 cancer patients' blood and tumor samples. MMP-9 -1562 C/T polymorphism was determined by the polymerase chain reaction – based restriction fragment length polymorphism method.

Results: Significant relationship of MMP-9 gene expression and tumor differentiation grade was found only between groups with G1 and G3 breast tumors. Low survival rates were identified among positive MMP-9 expression in blood and ductal carcinoma of the breast ($p=0.01$) and negative progesterone receptor reaction ($p=0.04$). Significant differences in the distribution among genotypes were found between groups with stage I and stages III/IV ($p=0.005$) as well as between groups with lymph node status N0 and N1 ($p<0.001$). Breast cancer patients with tumor differentiation grade G3 and identified CC variant had a longer survival time ($p=0.014$). Shorter survival time was found among positive MMP-9 expression in tumor and stage I non-small cell lung cancer patients with negative lymph node ($p=0.012$) and squamous cell carcinoma ($p=0.019$).

Conclusions: Expression of MMP-9 in blood and tumor together indicates worse prognosis for breast cancer patients.

Key words: breast cancer, non-small cell lung cancer, matrix metalloproteinase 9

INTRODUCTION

Due to their proteolytic activity, matrix metalloproteinases (MMPs) play crucial roles in tumor invasion and metastatic processes. MMPs regulate signaling pathways that control cell growth, survival, invasion, inflammation and angiogenesis [1]. Results of many studies have demonstrated a positive correlation between the expression of MMPs and the invasive and metastatic potential of various malignant tumors including breast [2], lung [3], laryngeal [4], prostate [5], cervical [6] and gastric [7] cancer. The MMPs comprise a family of 24 zinc dependent endopeptidases with broad spectrum of enzymatic activity against all components of the extracellular matrix (ECM) and basement membrane. Based on their structure and substrate specificity, MMPs are divided into five main groups: collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs [2, 8, 9].

The expression of MMP-9 enzyme from gelatinases group correlates with the processes of tumor cell invasion and metastasis, because of its potency to degrade gelatin and type IV collagen, the major components of basement membranes. MMP-9 (gelatinase B) has been found in large quantities in the cancer tissues [1]. MMP-9 is synthesized by endothelial

cells, fibroblasts, and hematopoietic cells. In a transgenic mice study, the lack of MMP-9 has been shown to decrease the incidence of invasive tumors, while the development of cancer in MMP-9 null mice can be restored by transplanting MMP-9 expressing bone marrow cells [10].

Recent studies in breast cancer patients have shown that an increase of MMP-9 activity in patients' serum could be a strong and independent marker for aggressive breast cancer with unfavorable outcome [11]. In the study conducted by Sullu *et al.* [12] high MMP-9 expression was associated with a shorter disease-free survival and overall survival times (p=0.042 and p=0.046, respectively) for the patients with invasive ductal breast carcinoma.

The expression of MMPs in the lung is a highly regulated process, and understanding its regulation could, in part, clarify their biological function in normal developmental and in pathological processes [8]. It has been demonstrated that in non-small cell lung cancer (NSCLC) tumor tissue there is a higher MMP-7, MMP-9 and TIMP-1 genes expression in comparison with the normal non-tumor lung tissue of the same patients [13]. Shao *et al.* [3] on the basis of their study suggested that MMP-9 expression in tumor cells, vessel invasion, and pT stage together may be a significant prognostic factors on overall survival in patients with completely resected pathologic stage IA NSCLC (p=0.037, <0.001, and <0.001, respectively).

Functional polymorphisms affect the regulation of gene expression and so can contribute to individual differences in susceptibility and severity of a disease. Polymorphisms of the MMPs genes may promote cancer development or progression *via* alteration of MMPs protein expression, resulting in creation and maintenance of the microenviroment for tumor cell proliferation, migration and invasion [14]. Of the numerous polymorphisms known for MMP-9, the C to T mutation at position -1562 appears to be the most important. MMP-9 polymorphism influences the binding of a transcription repressor protein; the loss of this DNA-protein interaction is associated with higher MMP-9 promoter activity. In a study of 270 patients with breast cancer and 300 controls Przybylowska *et al.* [15] found that the T allele was associated with poor differentiation, tumor size and steroid hormone receptor status but not with lymph node involvement. Initial research study revealed no relationship between MMP-9 functional polymorphism C-1562T and decline of lung function, but more recent studies have found that this mutation appears more frequently among smokers with chronic obstructive pulmonary disease compared with healthy smokers, but the functional significance of this remains unclear [8, 16].

Evidence from research studies demonstrates that MMPs can serve as potential markers for prognosis and risk assessment, also as indicators of tumor recurrence and metastatic spread for cancer. Nevertheless, studies assessing the association between expression of MMP-9 as well as its polymorphism and survival of breast and lung cancer patients have reported inconsistent results. The difference of our study was that MMP-9 expression in blood and tumor tissue was performed for the same patient. So, the aim of this study was to identify MMP-9 gene expression and genetic variants at the position -1562 in the MMP-9 gene promoter in breast and non-small cell lung cancer patients' blood and tumor samples, and to evaluate the significance of these parameters for the overall survival rates.

MATERIALS AND METHODS

Patients

A total of 108 patients were enrolled in the study. The study included 88 patients with breast cancer (median age 58 years) and 20 patients with NSCLC (median age 61 years). All patients were operated on in the Center of Oncosurgery, Institute of Oncology, Vilnius University. The study was approved by the Lithuanian Bioethics Committee (No.16/2005). Written informed consent was obtained from all patients prior to sample collection. A histopathological assessment of tumor after tumor biopsy was done, which included histopathological grade and evaluation of lymph node status, additionally estrogen receptor/progesterone receptor status for breast tumors. Data of the breast cancer and NSCLC patients included in the study are shown in *Tab. 1-2*.

RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from fresh frozen tumor specimens using Trizol reagent (*Invitrogen*, USA) and from whole blood samples using "QIAamp RNA Blood Mini Kit" (*Qiagen*, Germany) according to the manufacturer's protocol. Additionally, residual DNA was removed by on-column DNase digestion. Complementary DNA (cDNA) was synthesized from total RNA using "RevertAid First Strand cDNA Synthesis Kit" (*Fermentas*, Lithuania). To control the quality of isolated total RNA and cDNA synthesis, the cDNA of the housekeeping gene β-actin was amplified. The primer sequences and conditions for PCR used for MMP-9 and β-actin detection are summarized in *Tab. 3*. Final reverse transcription-PCR products (540 bp for β-actin and 584 bp for MMP-9) were electrophoresed onto 1% agarose gel, stained with ethidium bromide and visualized by UV light (*Fig. 1*).

DNA extraction and single nucleotide polymorphism (SNP) analysis

Genomic DNA was extracted from fresh frozen tumor and whole blood specimens using "Genomic DNA purification kit" (*Fermentas,* Lithuania) according to the manufacturer's protocol. The concentration and the purity of DNA were determined spectrophotometrically by readings of A_{260} and

Table 1. **Baseline characteristics of breast cancer patients.**

Variable	Cases (n=88) n (%)
Pathological stage	
Stage I	21 (23.9)
pT1 (n=21)	
Stage II	57 (64.8)
pT2a (n=47)	
pT2b (n=10)	
Stage III/IV	10 (11.4)
pT3a (n=6)	
pT3b (n=3)	
pT4 (n=1)	
Lymph node status	
N0	45 (51.1)
N1	36 (40.9)
N2	7 (8.0)
Tumor differentiation grade	
G1	17 (19.3)
G2	48 (54.5)
G3	23 (26.1)
Tumor histology	
Ductal carcinoma	71 (80.7)
invasive ductal (n=50)	
infiltrating ductal (n=71)	
Lobular carcinoma	14 (15.9)
invasive lobular (n=6)	
infiltrating lobular (n=3)	
Other carcinoma	3 (3.4)
Estrogen receptor status	
Negative	34 (38.6)
Positive	54 (61.4)
Weak reaction (n=12)	
Moderate reaction (n=19)	
Strong reaction (n=23)	
Progesterone receptor status	
Negative	33 (37.5)
Positive	55 (62.5)
Weak reaction (n=21)	
Moderate reaction (n=25)	
Strong reaction (n=9)	

Table 2. **Baseline characteristics of NSCLC patients.**

Variable	Cases (n=20) n (%)
Pathological stage	
Stage I	10 (50.0)
pT1a (n=5)	
pT1b (n=5)	
Stage II	9 (45.0)
pT2a (n=2)	
pT2b (n=7)	
Stage IV	1 (5.0)
pT4 (n=1)	
Lymph node status	
N0	13 (65.0)
N1	7 (35.0)
Tumor differentiation grade	
G1	1 (5.0)
G2	10 (50.0)
G3	9 (5.0)
Tumor histology	
Squamous cell carcinoma	13 (65.0)
Other carcinoma	7 (35.0)

A_{280}. The genotypes of MMP-9 (-1562 C/T) were determined by the polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) method. The PCR cycling conditions were 2 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, and with a final step at 72 °C for 2 min to allow complete extension for PCR products. The primers and restriction enzyme are summarized in *Tab. 4*. Digested PCR products were loaded onto 2% agarose gel, stained with ethidium bromide and visualized by UV light (*Fig. 2*).

Statistical analysis

Calculations were performed using SPSS 20.0 software package (SPSS Chicago, USA). A χ^2 and Fisher's exact tests were used to analyze the MMP-9 gene expression and MMP-9 -1562 polymorphism variants in relation to clinicopathological parameters. Bonferroni correction of p values was performed to validate the significance. The follow-up interval was calculated in months, and defined as the time between surgery and date of death or last follow-up. The survival rates were calculated by the Kaplan-Meier method, and differences between the survival curves were determined by log-rank test. The prognostic significance was assessed by the Cox proportional hazards regression model. p values less than 0.05 were considered statistically significant.

RESULTS

Breast cancer

MMP-9 gene expression analysis

As it was mentioned above, 88 patients with verified breast cancer were included in this study and their clinicopathological data are presented in *Tab. 1*. Of the 88 patients with breast cancer, 21 (23.9%) had stage I, 57 (64.8%) – stage II and 10 (11.4%) patients – stage III/IV. Pathological analysis showed that 71 (80.7%) of the cases were ductal carcinoma, 14 (15.9%) of cases – lobular carcinoma and 3 (3.4%) cases – other type breast carcinoma (ductal carcinoma *in situ*, medullary carcinoma and mucinous carcinoma). Positive MMP-9 gene expression was detected in 40 blood samples (45.5 %) and 81 tumor samples (93.1 %) from breast cancer patients. The relationship between selected clinicopathological parameters and MMP-9 gene expression in patients' blood and tumor samples was analyzed and summarized in *Tab. 5*. The relationship between tumor differentiation grade and MMP-9 gene expression in blood was statistically significant (p=0.02). After correction, significant relationship of MMP-9 gene expression and tumor differentiation grade was found only between groups with G1 and G3 tumors (p=0.007) (G1

Figure 1. **RT-PCR analysis of β-actin and MMP-9.**

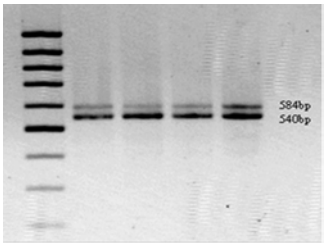


Figure 2. **PCR-RFLP analysis of the MMP-9 promoter -1562 C/T polymorphism.**

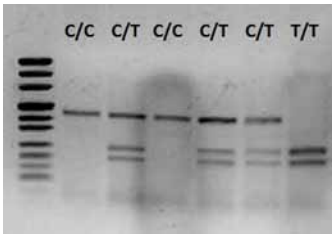


Table 3. **Characteristics of RT-PCR reaction.**

Primers	Sequence	PCR conditions			
		denaturation	annealing	extension	number of cycles
MMP-9	5'-ACCGCTATGGTTACACTCGG-3' 5'-GCAGGCAGAGTAGGAGCG-3'	94ºC 50 s	62ºC 50 s	72ºC 1 min	45
β-actin	5'-GTGGGGCGCCCCAGGGACCA-3' 5'-CTCCTTAATGTCACGCACGATTTC-3'	94ºC 50 s	62ºC 50 s	72ºC 1 min	35

Table 4. **PCR primers for MMP-9 PCR-RFLP assay.**

Polymorphism	Primers	Restriction enzyme	Fragment length
MMP-9 (-1562 C/T)	5'-GCC TGG CAC ATA GTA GGC CC-3' 5'-CTT CCT AGC CAG CCG GCA TC-3'	Pae I	436bp (C) 240+196 (T)

Table 5. **MMP-9 gene expression in breast cancer patients' blood and tumor samples according to clinicopathological parameters.**

Clinicopathological parameters	MMP-9 expression in blood		p	MMP-9 expression in tumor		p
	positive n (%)	negative n (%)		positive n (%)	negative n (%)	
Stage grouping						
Stage I	5 (12.5)	16 (33.3)		20 (23.9)	1 (16.7)	
Stage II	30 (75.0)	27 (56.3)	0.07	52 (64.8)	5 (83.3)	0.54
Stage III/IV	5 (12.5)	5 (10.4)		10 (11.4)	0	
Differentiation grade						
G1	3 (7.5)	14 (29.2)		16 (19.5)	1 (16.7)	
G2	23 (57.5)	25 (52.1)	0.02	43 (52.4)	5 (83.3)	0.26
G3	14 (35.0)	9 (18.8)		23 (28.0)	0	
Lymph node status						
N0	16 (40.0)	29 (60.4)		42 (51.2)	3 (50.0)	
N1	21 (52.5)	15 (31.3)	0.12	33 (40.2)	3 (50.0)	0.72
N2	3 (8.0)	4 (8.3)		7 (8.5)	0	
Tumor histology						
Ductal carcinoma	34 (87.2)	37 (80.4)	0.29	65 (82.3)	6 (100.0)	0.32
Lobular carcinoma	5 (12.8)	9 (19.6)		14 (17.7)	0	
Estrogen receptor						
Negative	21 (52.5)	13 (27.1)	0.13	32 (39.0)	2 (33.3)	0.57
Positive	19 (47.5)	35 (72.9)		50 (61.0)	4 (66.7)	
Progesterone receptor						
Negative	19 (47.5)	14 (29.2)	0.06	30 (36.6)	3 (50.0)	0.40
Positive	21 (52.5)	34 (70.8)		52 (63.4)	3 (50.0)	

vs G2 p=0.02; G2 *vs* G3 p=0.22)._No significant correlation was found among MMP-9 gene expression in blood as well as in tumor tissue and pathological stage, lymph node status, receptor status (*Tab. 5*).

MMP-9 -1562 C/T polymorphism analysis

Genotyping of the MMP-9 -1562 polymorphism was performed in all breast cancer patients' samples. The CC, CT and TT genotypes of MMP-9 gene were observed in 39 (44.3%), 38 (43.2%) and 11 (12.5%) of the breast cancer cases, respectively. The distributions among genotypes regarding clinicopathological parameters are shown in Tab. 6. Significant differences in the distribution among genotypes and pathological stage as well as lymph node status were found (p=0.02 and p=0.002, respectively). After Bonferroni correction, significant differences in the distribution among genotypes were found between groups with stage I and stages III/IV (p=0.005) as well as between groups with lymph node status N0 and N1 (p<0.001). Other clinicopathological parameters, such as tumor differentiation grade, estrogen or progesterone receptor status were not related to MMP-9 -1562 polymorphism variants.

MMP-9 gene expression and MMP-9 gene -1562 polymorphism variants in association with overall survival in breast cancer patients

A total of 79 breast cancer patients were included in overall survival analysis (*Tab. 7*). Survival analysis wasn't carried out for the patients with pathological stage III/IV (n=9) concerning insufficient patients' number in the group. Overall survival of the patients with histopathologically confirmed ductal carcinoma of the breast was statistically significant in relation to positive MMP-9 gene expression in blood and showed shorter survival time (p=0.01) (*Fig. 3*). A regression analysis according to a Cox proportional hazards model demonstrated that positive MMP-9 gene expression was associated with low survival rate (p=0.01, HR=0.28, 95% CI 0.09-0.81).

Similar results were found in the patients classified according to progesterone receptor status. Overall survival for the patients with negative progesterone receptor reaction was statistically significant in relation to positive MMP-9 gene expression in blood and showed shorter survival time (p=0.04) (*Fig. 4*), while the regression analysis according to a Cox proportional hazards model didn't demonstrate that positive MMP-9 gene expression could be associated with low survival rate (p=0.07, HR=0.14, 95% CI 0.01-1.24).

Positive MMP-9 expression was detected in 81 breast tumor samples from 87 samples. Overall survival analysis for the patients didn't show significant differences in survival rate among negative and positive MMP-9 expression in tumor and selected clinicopathological parameters. As for tumor differentiation grade, there was a significant difference in the presence of MMP-9 CC variant between longer overall survival time and tumor differentiation grade G3 (p=0.014, HR=0.07, 95% CI 0.009-0.59). Kaplan-Meier estimates demonstrated that patients with CC variant had a significantly longer survival time (89.4 months, 95% CI 65.7-113.0) than patients with identified TT variant (15.5 months, 95% CI 0.5-32.1). Also, survival time for the breast cancer patients with negative lymph node status and identified CC variant was longer comparing with identified TT variant (p<0.001).

NSCLC

MMP-9 gene expression analysis

As it was mentioned above, 20 patients with diagnosed NSCLC were included in this study and their clinicopathological data are presented in *Tab. 2*. Of the 20 patients with NSCLC, 10 (50.0%) had stage I, 9 (45.0%) – stage II and 1 (5.0%) patient – stage IV. Pathological analysis of these patients showed that 13 of the cases (65.0%) were squamous cell carcinoma and 7 of cases (35.0%) were other type carcinoma (large-cell carcinoma and adenocarcinoma). Positive MMP-9 gene expression was detected in 17 blood samples (85.0 %) and in 15 tumor samples (75.0 %) of NSCLC patients. The relationship between selected clinicopathological parameters and MMP-9 gene expression in patients' blood as well as in tumor tissue was analyzed and summarized in *Tab. 8*. No significant correlation was found between MMP-9 gene expression in blood and tumor samples and clinicopathological parameters of NSCLC patients.

MMP-9 -1562 C/T polymorphism analysis

Genotyping of the MMP-9 -1562 polymorphism was performed in all NSCLC patients' samples. The CC, CT and TT genotypes of MMP-9 gene were observed in 9 (45.0%), 8 (40.0%) and 3 (15.0%) of the NSCLC cases, respectively. The distributions among genotypes regarding clinicopathological parameters are shown in *Tab. 9*. Statistically significant correlation wasn't found between clinicopathological parameters and MMP-9 -1562 polymorphism variants.

MMP-9 gene expression and MMP-9 gene -1562 polymorphism variants in association with overall survival in non-small cell lung cancer patient

The overall survival analysis showed statistically significant shorter survival time for stage I NSCLC patients with negative lymph node and identified positive MMP-9 gene expression in tumor (p=0.012). Similar shorter survival time has been found for the stage I NSCLC patients with histopathologically confirmed squamous cell carcinoma and positive MMP-9 gene expression in tumor (p=0.019).

Figure 3. **Kaplan-Meier survival curves for breast cancer patients with confirmed ductal carcinoma according to identified MMP-9 gene expression in blood.**

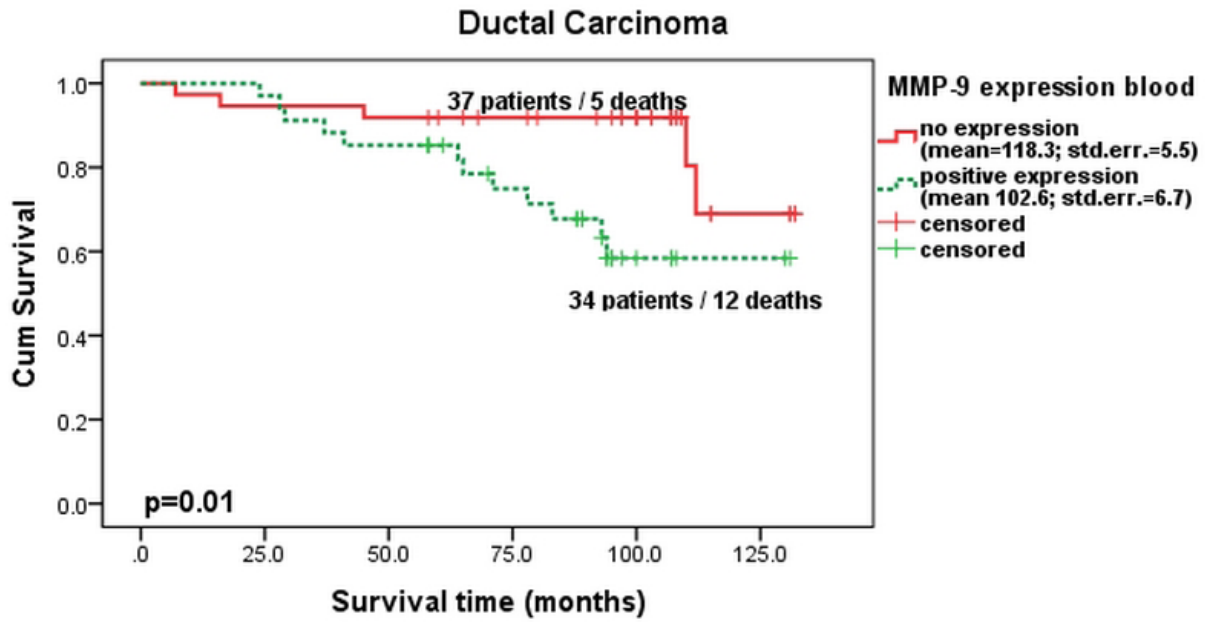
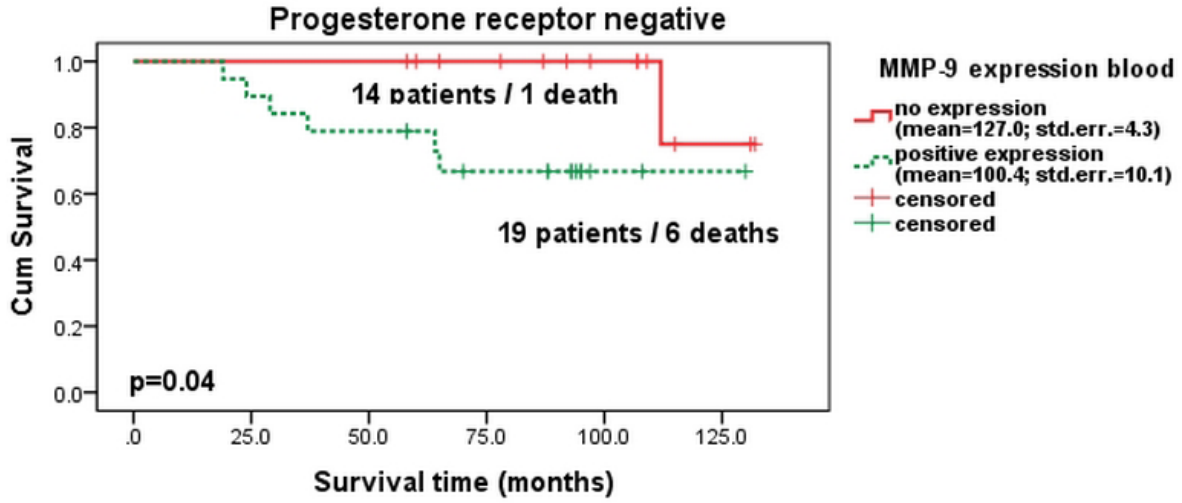


Figure 4. **Kaplan-Meier survival curves for breast cancer patients with negative progesterone receptor reaction according to identified MMP-9 gene expression in blood.**



DISCUSSION

Breast cancer is the most common cancer in women worldwide. Although well documented classic biomarkers are reliable (for example, tumor grade and stage, hormone receptor status), there is a need to search for a new molecular biomarkers for more precise prognosis of breast cancer patients.

Provatopoulou *et al.* [17] reported a significant correlation between MMP-9 serum expression and breast disease severity score, suggesting the potential application of MMP-9 measurement in the monitoring of breast cancer disease progression. Köhrmann *et al.* [18] observed significant differences between normal breast tissue and G2 breast cancer tissue concerning the mRNA levels for MMP-9 ($p = 0.0127$), MMP-11 ($p = 0.0007$) and MMP-13 ($p = 0.008$). Furthermore MMP-9, MMP-11 and MMP-28 mRNA expression was higher in breast cancer tissue G3 than in normal breast tissue. The results of our study demonstrated the statistically significant association between MMP-9 expression and breast tumor

Table 6. **Distribution of MMP-9 -1562 polymorphism variants in breast cancer patients according to clinicopathological parameters.**

Clinicopathological parameters	MMP-9 -1562 polymorphism			p
	CC, n (%)	CT, n (%)	TT, n (%)	
Stage grouping				
Stage I	12 (30.8)	9 (23.7)	0	
Stage II	25 (64.1)	25 (65.8)	7 (63.6)	0.02
Stage III/IV	2 (5.1)	4 (10.5)	4 (36.4)	
Differentiation grade				
G1	11 (28.2)	5 (13.2)	1 (9.1)	
G2	18 (46.2)	22 (57.9)	8 (72.7)	0.33
G3	10 (25.6)	11 (28.9)	2 (18.2)	
Lymph node status				
N0	24 (61.5)	19 (50.0)	2 (18.2)	
N1	14 (35.9)	17 (44.7)	5 (45.5)	0.002
N2	1 (2.6)	2 (5.3)	4 (36.4)	
Tumor histology				
Ductal carcinoma	34 (94.4)	28 (73.7)	9 (81.8)	0.05
Lobular carcinoma	2 (5.6)	10 (26.3)	2 (18.2)	
Estrogen receptor				
Negative	14 (35.9)	13 (34.2)	7 (63.6)	0.18
Positive	25 (64.1)	25 (65.8)	4 (36.4)	
Progesterone receptor				
Negative	13 (33.3)	14 (36.8)	6 (54.5)	0.43
Positive	26 (66.7)	24 (43.6)	5 (45.5)	

Table 7. **Univariate analysis of MMP-9 gene expression and MMP-9 -1562 polymorphism variants in association with overall survival of breast cancer patients.**

Parameter	MMP-9 expression in blood	MMP-9 expression in tumor	MMP-9 -1562 C/T polymorphism
	p (Log-rank test)	p (Log-rank test)	p (Log-rank test)
Stage grouping			
Stage I	0.12	--	0.32
Stage II	0.30	0.59	0.44
Differentiation grade			
G2	0.05	0.73	0.77
G3	0.60	--	0.001
Lymph node status			
N0	0.34	--	<0.001
N1	0.27	0.80	0.23
Tumor histology			
Ductal carcinoma	0.01	0.59	0.19
Lobular carcinoma	0.92	--	--
Estrogen receptor			
Negative	0.44	--	0.20
Positive	0.15	0.78	--
Progesterone receptor			
Negative	0.04	--	0.12
Positive	0.36	0.77	0.95

differentiation grade (*Tab. 5*). Regarding survival results there was a trend for favorable outcomes in patients with G2 tumors and negative MMP-9 expression in blood (p=0.05).

Hormone receptors were defined as molecular prognostic marker for the breast cancer. Immunohistochemical analysis of breast tumor tissue revealed a significant association between a strong expression of pro- and active MMP-9 in breast tumor tissue and a shortened relapse-free survival, particularly in estrogen positive tissue [19]. The results of the present study demonstrated statistically significant association of MMP-9 gene expression and lower overall survival rate for patients with negative progesterone receptor reaction (p=0.04).

The prognosis of breast cancer according to identified histological type can be good and poor. Breast cancer with

Table 8. **MMP-9 gene expression in NSCLC patients' blood and tumor samples according to clinicopathological parameters.**

Clinicopathological parameters	MMP-9 expression in blood		p	MMP-9 expression in tumor		p
	positive, n (%)	negative, n (%)		positive, n (%)	negative, n (%)	
Stage grouping						
Stage I	7 (43.8)	3 (100.0)	0.12	6 (42.9)	4 (80.0)	0.18
Stage II	9 (56.3)	0		8 (57.1)	1 (20.0)	
Differentiation grade						
G2	8 (50.0)	2 (66.7)	0.54	8 (57.1)	2 (40.0)	0.44
G3	8 (50.0)	1 (33.3)		6 (42.9)	3 (60.0)	
Lymph node status						
N0	10 (58.8)	3 (100.0)	0.25	9 (60.0)	4 (80.0)	0.40
N1	7 (41.2)	0		6 (40.0)	1 (20.0)	
Tumor histology						
Squamous cell carcinoma	10 (58.8)	3 (100.0)	0.25	9 (60.0)	4 (80.0)	0.40
Other carcinoma	7 (41.2)	0		6 (40.0)	1 (20.0)	

Table 9. **Distribution of MMP-9 -1562 polymorphism variants in NSCLC patients according to clinicopathological parameters.**

Clinicopathological parameters	MMP-9 -1562 polymorphism			p
	CC, n (%)	CT, n (%)	TT, n (%)	
Stage grouping				
Stage I	6 (66.7)	4 (50.0)	0	0.22
Stage II	3 (33.3)	4 (50.0)	2 (100.0)	
Differentiation grade				
G2	4 (50.0)	5 (62.5)	1 (33.3)	0.67
G3	4 (50.0)	3 (37.5)	2 (66.7)	
Lymph node status				
N0	7 (77.8)	5 (62.5)	1 (33.3)	0.37
N1	2 (22.2)	3 (37.5)	2 (66.7)	
Tumor histology				
Squamous cell carcinoma	6 (66.7)	6 (75.0)	1 (33.3)	0.43
Other carcinoma	3 (33.3)	2 (25.0)	2 (66.7)	

a good prognosis, such as invasive tubular and mucinous carcinoma, has shown a high 10-year survival rate (over 80%). While, infiltrating ductal carcinoma and lobular carcinoma are associated with poor 10-year survival rate (below 50%) [20]. Our results showed higher survival rates for the ductal carcinoma patients with negative MMP-9 expression in blood. Del Casar *et al.* [20] demonstrated that breast carcinomas of the ductal type have high expressions of MMPs. The remarkable finding in their study was a higher expression of MMP-7 and MMP-14 compared with other histological types of breast carcinoma.

It is important to notice the results of our study, that survival rate was lower for the patients with positive MMP-9 expression in blood and in tumor in comparison with the patients with negative MMP-9 expression in blood and positive MMP-9 expression in tumor tissue (p=0.03). It means that expression of MMP-9 in blood and tumor together indicates worse prognosis.

Tumors from breast cancer patients carrying the CT or TT genotype at the position -1562 in the MMP-9 gene are characterized by various features of good prognosis and confer a prolonged overall survival [19]. In this study MMP-9 CC variant was associated with longer overall survival time for patients with tumor differentiation grade 3 and negative lymph node status.

NSCLC is the major cancer type in developed countries. Pinto *et al.* [21] suggest that the increased levels of MMP-9 in the adenocarcinoma of NSCLC can be associated with poor prognosis. In their study relationship had been shown for the MMP-9 positive expression in tumor in presence of T4 tumor stage (p=0.0076). Rollin *et al.* [22] showed that MMP-9 -1562 CC genotype was more frequent in patients with squamous cell carcinoma than in controls. Although, MMP-9 gene expression in the tumor was not related to the -1562 C/T polymorphism and overall survival did not differ among patients of the different genotypes. Our preliminary results

suggest that a relationship between NSCLC patients' overall survival and MMP-9 expression is likely to exist.

It must be mentioned, that not only MMPs are important as proteolytic enzymes in cancerogenesis. There are other proteolytic enzymes families such as cysteine or serine, which are involved in proteolysis mechanisms. For example, cysteine protease cathepsin B is upregulated in many different tumor microenviroments and it can cleave a variety of targets in both tumor and stromal cells [23]. Cellular serine proteases such as uPA (urokinase-type plasminogen activator) directly mediate pericellular proteolysis during cell migration under physiological and pathological conditions [24].

The synergy between a plasminogen cascade and MMP-9 was indicated in the mouse model of autoimmune disease where delayed blister formation in plasminogen deficient or uPA/tPA (tissue-type plasminogen activator) knockouts was restored by applications of the active form of MMP-9. It must be noted, that this effect was not achieved with the MMP-9 zymogen (pro-enzyme), strongly implicating plasmin in functional activation of MMP-9 *in vivo* [24]. uPA and uPAR (urokinase-type plasminogen activator receptor) proteins have also been detected in the bone marrow of patients with invasive breast cancer, associating increased uPA levels with reduced disease-free interval and overall patient survival but as healthy controls were rare in the studies, the results should be interpreted with caution [25].

In lung cancer, the role of the uPA system is less clear. Overexpression of uPA and uPAR was reported in tissue extracts of lung cancer, but results on the prognosis of these components remain controversial. Chen *et al.* [26] in their study showed that serum uPA and uPAR levels in patients with lung cancer were significantly higher than those in healthy control. Although, the levels of uPA didn't correlate with clinicopathological parameters.

Taking together these findings an increased understanding of how proteolytic systems impact other enzymes, signal molecules or receptors are needed. More detailed studies of proteinases network could help to understand how tumor cells signal and interact with each other.

CONCLUSIONS

MMP-9 gene expression in blood was associated with breast tumor differentiation grade, while MMP-9 -1562 polymorphism variants were associated with pathological stage and lymph node status. Overall survival analysis of breast cancer patients showed the relationship among MMP-9 gene expression and tumor histology as well as progesterone receptor status, while MMP-9 -1562 polymorphism variants – with tumor differentiation grade and lymph node status. In cases of patients with positive expression of MMP-9 in blood and tumor survival rate was lower in comparison with cases of patients with negative expression of MMP-9 in blood and positive in tumor tissue. Shorter survival time has been found for the stage I non-small cell lung cancer patients with histopathologically confirmed squamous cell carcinoma or negative lymph node and positive MMP-9 gene expression in tumor.

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