Biomedical Research 2017; 28 (20): 8769-8773 *ISSN 0970-938X*
www.biomedres.info

Investigation of the MMP2 and MMP9 gene polymorphisms in malignant mesothelioma and other pleural diseases.

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Abstract

Various impacts of Matrix Metalloproteinases (MMPs) were reported in many cancer types. Particularly, MMPs are involved in extracellular matrix destruction which is required cancer cells to make an invasion. Gene polymorphisms which are thought to affect expressions levels of MMPs are widely studied to determine their influence on cancer. In this study, we aimed to investigate the association of gene polymorphism frequencies of MMP2 C1306T and MMP9 C1562T in Malignant Mesothelioma (MM), other metastatic cancers to pleura and benign pleural diseases. DNA extracted from pleural tissues of 195 subjects (MM, other metastatic cancers to pleura and benign pleural diseases). MMP2 C1306T and MMP9 C1562T polymorphisms genotyped using restriction fragment length polymorphism (RFLP) method. We found no significant difference in genotype frequencies of MMP2 C1306T, MMP9 C1562T between subjects except for MMP2 C1306T CT genotypes were high in MM when compared to benign pleural diseases group. We recommend extensive mutation and polymorphism scan including whole exomes of these genes.

Keywords: Mesothelioma, Matrix metalloprotease, Metastasis, Pleura, Polymorphism.

Accepted on September 27, 2017

Introduction

Malignant Mesotheliomas (MM) are tumors originating from pleura, peritoneum, pericardium and tunica vaginalis [1]. MM has three histological subtypes; epithelial, sarcomatoid, and biphasic [2,3]. Asbestos exposure is the main known etiologic factor for MM along with Simian virus 40 (SV40), erionite and genetic susceptibility [2,3]. High prevalence of MM observed in certain geographic areas related to exposure of environmental mineral fibers like asbestos and erionite [2,3]. Mostly known exposure of mineral fibers was reported in Turkey, where MM is responsible for 50% of all deaths in three certain villages located in Cappadocia [2].

The link between asbestos exposure and the development of MM is well established. Nevertheless, some of the patients suffering from this disease do not appear exposed to asbestos fibers from any known source [4]. Only a small fraction of asbestos-exposed individuals develop MM, and disease clustering is observed in some families [5]. Linked to this finding, many researchers aimed to investigate the genetic basis of the disease and some possible genetic biomarkers related to MM susceptibility. Some certain genetic factors thought to be promote malignant transformation leading MM along with the mineral carcinogen exposure [6]. Also, there can be another genetic factor may help us to distinguish other malignant and benign pleural diseases (benign or metastasis of other cancers to pleura) from Mesothelioma.

A well-known and widely studied MM related genetic component is BAP1 gene. BAP1 identified in two unrelated families with germline mutations and elevated risk for MM and some other cancers types [6]. BAP1 (BRCA1–associated protein 1) is a member of the ubiquitin C-terminal hydrolase deubiquitinating enzymes which catalyzes the removal of ubiquitin from proteins [6,7]. A variety of researchers are trying to find out BAP1 influence in MM development. Also, other putative cancer-related genes and proteins are under a scope to enlighten their association in MM.

Along these components, Matrix Metalloproteinases (MMPs), consisting of approximately 28 enzymes, which play a significant role in tissue degradation [8,9]. Some MMPs reported with an altered expression profile in a variety of malignancies [9]. MMPs is shown to be involved in extracellular matrix (ECM) degradation. Breakdown of ECM often related with invasion and metastasis in cancer [9]. According to their enzymatic activity, MMPs may have influences on tumor, cancer prognosis and metastasis as well [10-16].

MMP2 C1306T single nucleotide polymorphism occurs as a result C (cytosine) displacement with T (thymine) in nucleotide

1306 of the MMP2 gene promoter. A dramatically decrease in MMP2 promoter activity has reported as a result of this conversion [17]. MMP9 C1562T polymorphism occurs as a result C (cytosine) displacement with T (thymine) in nucleotide 1562 of the MMP9 gene promoter. It is shown that this region of MMP promoter affects protein binding and has an influence on transcriptional activity on macrophages [16].

According to their contributions on several cancer types [10,13-15,17], especially in "metastasis," MMP2 (rs243865) and MMP9 (rs3918242) gene polymorphisms may also influence on MM as well. Besides, these polymorphisms can help us to distinguish other pleural diseases (benign or metastatic cancers to pleura) from Mesothelioma and let us to utilize them as genetic biomarkers for MM. To address these questions, we aimed to investigate the association of gene polymorphism frequencies of MMP2 C1306T and MMP9 C1562T in MM and other pleural diseases.

Methods

Subjects

The subject groups' pleural tissues consisting of 100 patients with MM, 59 other metastatic cancers to pleura and 36 benign pleural diseases who recruited from the Department of Chest Diseases and APKAM (Lung and Pleural Cancers Research and Clinical Center), Eskisehir Osmangazi University Faculty of Medicine in Eskisehir, Turkey. Informed consent by our protocol, accepted by the Ethics Committee of Eskisehir Osmangazi University Faculty of Medicine, was obtained from all patient groups. We conducted the study in by the ethical principles of the 1975 declaration of Helsinki. The study group were homogeneous Turkish population regarding ethnicity.

Genotyping

Genomic DNA was isolated from pleural tissues using the Invitrogen Genomic DNA isolation kit with tissue DNA isolation procedure. The rs243865 variant in MMP2 and the rs2066847 variant in MMP9 analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers used in the amplification of MMP2 including rs243865 polymorphism were F-5'-CTTCCTAGGCTGGTCCTTACTGA-3' and R-5'CTGAGACCTGAAGAGCTAAAGAGCT-3'and in the amplification of MMP9 including rs3918242 polymorphism was F-5'-GCCTGGCACATAGTAGGCCC-3'and R-5'-TTCCTAGCCAGCCGGCATC-3'. PCR reactions carried out on a Peltier-based Thermal Cycler (Sacem Life Technologies, Turkey). The PCRs set in a total reaction volume 25 µl consisting 12,5 µl master mix, 0,5 µl Reverse and Forward primer, 10,5 µl distilled water and 1 µl genomic DNA. Amplification protocol carried out with initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 58 °C for 60 s and extension at 72 °C for 1,5 min and final extension for 7 minutes for MMP2 (rs243865) and MMP9 (rs3918242). Amplicon quality controlled by agarose-gel electrophoresis. PCR products digested overnight with XspI (Takara Bio. Inc. Shiga, Japan) for C106T polymorphism and SphI (New England Biosystems) for C1562T polymorphism at 37 ºC and respectively separated on a 2% agarose gel for and a 4% agarose gel at 100 V for 30 min. The gel was then stained with ethidium bromide and visualized by Gene Genius Gel Light Imaging System (Syngene, Cambridge, UK). MMP2 (rs243865) C allele was characterized by an uncut fragment of 188 bp, while T allele resulted in digested the fragment of 162 bp and 26 bp. MMP9 (rs3918242) C allele was characterized by uncut fragment 435 bp, while T allele resulted in two digested the fragment 247 bp and 188 bp.

Table 1. *Genotype frequencies of the MMP2 rs243865 variant.*

Genotypes	Malignant Mesothelioma (MM) n=100	Metastasis n=59	Benign n=36
CC n (%)	66 (66.0)	38 (64.4)	26 (72.2)
Statistic comparison	MM- Metastasis: p=0.838; MM-Benign:p=0. 494; Metastasis-Benign: p=0.431		
CT n (%)	33 (33.0)	19(32.2)	10 (27.8)
Statistic comparison	MM- Metastasis: p=0.918; MM-Benign:p=0.004; Metastasis -Benign: p=0.650		
TT n (%)	1 (1.0)	2 (3.4)	0
Statistic comparison	MM- Metastasis: p=0.556; MM-Benign:p=1.00; Metastasis -Benign: p=0.524		

MM: Malignant Mesothelioma

Upon XspI digest, the MMP2 gene for the C1306T polymorphism PCR product (188 bp) resulted two fragments (162/26bp) when the T allele was present, the 26-bp fragment was not visible on the 2% agarose gel. PCR products digestion for the C1562T polymorphism of MMP9 yielded three distinct genotypes. Single 435-bp band in the CC homozygote, two bands of 247-bp and 188-bp in the TT homozygote, and all three bands in GC heterozygotes.

Statistical analysis

The comparisons genotype frequencies between groups were evaluated using chi-square analysis (Pearson and exact chi-square tests). Also, genotypes between the groups were compared using independent sample t-test. Statistical analyses were performed using (IBM SPSS Statistics 21 software). P-values less than 0.05 were considered significant.

Results

Following XspI digest and genotyping, we did not find a statistically significant difference between the MM, metastatic cancers to pleura and benign pleural diseases except for MMP2

C1306T CT genotypes between MM and benign pleural diseases (p=0.004) (Table 1). Upon SphI digest, we did not found a significant difference in the genotype distribution between MM, metastatic cancers to pleura and benign pleural diseases group (Table 2).

Table 2. *Genotype frequencies of the MMP9 rs3918242 variant.*

Genotypes	Malignant Mesothelioma (MM) n=100	Metastasis n=59	Benign n=36
CC n (%)	72 (72.0)	41 (69.5)	24 (66.7)
Statistic comparison	MM- Metastasis: p=0.736; MM-Benign:p=0.; Metastasis -Benign: p=0.774		
CT n (%)	27 (27.0)	14(23.7)	11 (30.6)
Statistic comparison	MM- Metastasis: p=0.649; MM-Benign:p=0.684; Metastasis -Benign: p=0.464		
TT n (%)	1 (1.0)	4 (6.8)	1 (2.8)
Statistic comparison	MM- Metastasis: p=0.064; MM-Benign:p=0.461; Metastasis -Benign: p=0.647		

Also, we divided MM patients into two groups according to their histologic subtype; as epithelioid and non-epithelioid. MMP2 C1306T, MMP9 C1562T genotype frequencies were compared in these two groups. However, no statistically significant difference was found (Tables 3 and 4).

Discussion

Numerous studies have shown that breakdown of basal membrane, which is built from (ECM) components may be responsible for tumor progression and invasion [18-20]. It has been reported MMPs have an altered expression profile in a variety of malignant tumors. Related to their biologic functions, their impact on degradation of extracellular matrix (ECM) specifically associated with invasion and metastasis of cancerous cells [10]. It was shown that MMPs has different levels in lung cancer and MM, particularly MMP2 and MMP9, may have an important impact on metastasis [21,22].

Table 3. *Genotype frequencies of the MMP2 rs243865 variant in Malignant Mesothelioma Histological Subtypes (Epitheloid type, Non-Epitheloid type).*

Genotypes	Epitheloid type MM n=76	Non-Epitheloid type MM n=24
CC n (%)	51(67.1)	15 (62.5)
Statistic comparison	P=0.805	
CT n (%)	24 (31.6)	9 (37.5)
Statistic comparison	P=0.591	
TT n (%)	1 (1.3)	0 (0.0)
Statistic comparison	P=1.00	
MM: Malignant Mesothelioma		

In this study, we investigated the association between Matrix Metalloproteinase 2 C1306T and Matrix Metalloproteinase 9 C1562T gene polymorphisms in MM, other metastatic cancers to pleura, benign pleural diseases group. The frequencies of MMP2 C1306T genotypes were found CC 66%, CT 33% and TT 1% in MM, CC genotype 64.4%, CT 32.2% and 3.4% TT in the metastatic cancers to pleura, CC genotype 72.2%, CT 27.8% and 0% TT in the benign pleural diseases group. Also, the frequencies of MMP9 C1562T genotypes were found as following CC 72%, CT 27% and TT 1% in the MM, CC genotype 69.5%, CT 23.7% and TT 6.8% in the metastatic cancers to pleura group, CC genotype 66.7%, CT 30.6% and 2.8% TT in the benign pleural diseases group. As a result, there is no significant difference in genotype frequencies of MMP2 C1306T, MMP9 C1562T between MM and other pleural diseases patients except for MMP2 C1306T CT genotypes between MM and benign pleural diseases.

Rollin et al. analyzed the -1306C/T MMP2 polymorphisms in 90 NSCLC patients and 90 controls. The frequencies of MMP2 C1306T genotypes was found as CC 67%, CT 32% and TT 1% in the control group, CC genotype 67%, CT 31% and TT 2% in the patient group. As a result, no difference in -1306C/T MMP-2 genotypes were found between cases and controls [23].

Zhou et al. investigated the link between -1306C/T and the risk of developing lung cancer. MMP2 genotypes were analysed in 770 patients and 777 controls. They calculated genotype associations with risk of lung cancer by logistic regression. MMP2 C1306T genotypes frequencies was CC 68.7%, CT 29.1% and TT 2.2% in the control group, CC genotype 82.5%, CT 16.3% and TT 1.2% in the patient group. As a result, they found 2-fold lung cancer risk for the -1306CC genotype in the patient group compared with noncarriers [24].

Table 4. *Genotype frequencies of the MMP9 rs3918242 variant in Malignant Mesothelioma Histological Subtypes (Epitheloid type, Non-Epitheloid type).*

Genotypes	Epitheloid type MM n=76	Non-Epitheloid type MM n=24
CC n (%)	54 (71.1)	18 (75.0)
Statistic comparison	P=0.799	
CT n (%)	21 (27.6)	6 (25.0)

Statistic comparison	P=0.800	
TT n (%)	1 (1.3)	0 (0.0)
Statistic comparison	P=1.00	
MM: Malignant Mesothelioma		

Yu et al. aimed to investigate link between -1306C/T polymorphism and esophageal cancer in 527 cases and 777 controls. They determined CC 69.4%, CT 28.3% and TT 2.3% in the control group, CC genotype 77.6%, CT 21.3% and TT 1.1% in the patient group. According to these results, 1306C allele contributes esophageal squamous cell carcinoma risk and influence on metastasis with an increased expression level of MMP2 [25]. In several other studies, It was reported that MMP2 C1306T polymorphism associated with breast, head and neck cancer development [26,27].

Bayramoglu et al. studied, gene expression profiles of MMP2 and -9, and TIMP-1, -2, -3, and -4 and also polymorphism of MMP9 C1562T and plasma MMP-9 enzyme activity in 200 Turkish lung cancer patients compared with 100 healthy controls. They found frequencies of C1562T genotypes CC 67%, CT 30%, and TT 3% in the control group and CC 75%, CT 24%, and TT 1% in the patient group respectively. In their study CC genotype were found significantly increased in patients when compared to control group [14,28].

Bueno et al. carried out a comprehensive genomic analysis including transcriptomics and exome analysis on 216 Malignant Pleural Mesotheliomas (MPMs). Using RNA-Seq, they identified four different molecular MPM subtypes. These subtypes were identified with many genetic mutations, expression profiles and gene fusions. This study demonstrates that the histological subtype differences are not a result of alteration of single a gene variation or a particular protein level but are influenced by multiple mechanisms that affect the expressions of many gene groups [29].

Ozden et al. investigated MMP9 R279Q G / G polymorphism in glial tumors. They found that this polymorphism correlated with glial tumor formation in advanced age. According to this study, they come to the conclusion that altered protein structure can promote some oncogenic mechanisms during the years. Based on their results, other polymorphisms which affect protein expression in the MMP9 gene may also be cancer-related, and they should be taken into account [30].

As a result of this study, we found no significant difference in genotype frequencies of MMP2 C1306T, MMP9 C1562T between Malignant Mesothelioma patients, other pleural diseases patients and metastatic cancers to pleura, except for MMP2 C1306T CT genotypes were significantly high in MM group when they compared to benign pleural diseases group. We recommend extensive mutation and polymorphism scan including whole exomes of these genes.

Acknowledgment

This study was carried out with the biological samples collected in the study named as “Network cooperation for the management of environmental and occupational exposure to mineral fibers induced pulmonary pathologies” which was supported by General Directorate of Health Researches, Republic of Turkey, Ministry of Health. This work was supported by the Research Foundation of Eskisehir Osmangazi University, grant number 201411D04.

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