Matrix metalloproteinase 3 polymorphisms as a potential marker of enhanced susceptibility to lung cancer in chronic obstructive pulmonary disease subjects

Kamil Brzóska¹, Teresa Bartłomiejczyk¹, Barbara Sochanowicz¹, Magdalena Cymerman¹, Jacek Grudny², Jacek Kołakowski³, Lucyna Kapka-Skrzypczak⁴,⁵, Marcin Kruszewski⁴,⁵, Paweł Śliwiński⁶, Kazimierz Roszkowski-Śliż⁷

¹ Institute of Nuclear Chemistry and Technology, Centre for Radiobiology and Biological Dosimetry, Warsaw, Poland
² Institute of Tuberculosis and Lung Diseases, Third Department of Lung Diseases, Warsaw, Poland
³ Institute of Tuberculosis and Lung Diseases, Department of Diagnosis and Treatment of Respiratory Insufficiency, Warsaw, Poland
⁴ Institute of Rural Health, Department of Molecular Biology and Translational Research, Lublin, Poland
⁵ University of Information Technology and Management, Faculty of Medicine, Department of Medical Biology and Translational Research, Rzeszów, Poland

Abstract

Introduction and objective. Chronic obstructive pulmonary disease (COPD) is often accompanied by lung cancer. Among the genes that may play a role in the occurrence of COPD and lung cancer are those encoding the proteolytic enzymes, such as matrix metalloproteinases (MMPs) and their tissue inhibitors. The objective of this study was to find MMPs-associated markers useful in the identification of COPD subjects with increased susceptibility to developing lung cancer.

Materials and methods. We compared the frequency of single nucleotide polymorphisms in genes coding for matrix proteases (MMP1, MMP2, MMP3, MMP9, MMP12) as well as tissue inhibitor of metalloproteinases (TIMP1) in two groups of subjects: COPD patients (54 subjects) and COPD patients diagnosed for lung cancer occurrence (53 subjects). The levels of the respective proteins in blood serum were also analyzed.

Results. The frequencies of 2 genotypes, MMP3 rs3025058 and MMP3 rs678815, were significantly different between the studied groups. In both cases, more heterozygotes and less homozygotes (both types) were observed in the COPD group than in the COPD + cancer group. A significantly higher TIMP1 level in blood serum was observed in the COPD + cancer group. There were no statistically significant differences in MMPs blood levels between the studied groups. In addition, no genotype-associated differences in TIMP1 or MMPs blood levels were observed.

Conclusions. Homozygosity for MMP3 rs3025058 and rs678815 polymorphisms is a potential marker of enhanced susceptibility to lung cancer development among COPD subjects.

Key words

COPD; matrix metalloproteinase; lung cancer, MMP, inhibitors of metalloproteinases

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by an airflow limitation that is not fully reversible, usually progressive and associated with an abnormal inflammatory response to noxious particles or gases. The main cause of COPD is cigarette smoking; other causes, such as outdoor air pollution, occupational exposure to dust and fumes, previous tuberculosis, childhood asthma and childhood respiratory infections, might increase the risk of COPD and lead to disease in nonsmokers [1]. Patients with COPD are at increased risk for both the development of primary lung cancer and poor outcome after lung cancer diagnosis and treatment. The association between COPD and lung cancer has been reported in numerous studies [reviewed in 2–6] and is independent of age or smoking habit [7].

Smokers expose their bronchial epithelial cells to oxidants and reactive oxygen species (ROS), which induce oxidative stress. The cellular response that follows depends on the activation of the NF-κB and other transcription factors, and on the subsequent transactivation of inflammation-related genes. This initiates several signaling pathways depending on both genetic and epigenetic factors which, to some extent, overlap in the COPD and lung cancer. Chronic inflammation due to cytokine release and the resulting immune response contribute to both pathogenic processes. ROS and cigarette smoke components are able to induce DNA damage. The consequence is genomic instability, a factor sufficient for the development of cancer, reinforced by aberrant airway epithelial and matrix remodeling, epithelial–mesenchymal transition (EMT) and apoptosis [reviewed in 2, 5, 6]. At a certain stage, one tendency predominates: either the aggravating COPD conditions or the pre-cancerous pathway.

It is to be expected that the fate of individual subjects depends to some extent on their genetic predispositions. Indeed, twin studies showed that lung cancer susceptibility...
is inherited in about 15–25% of cases, whereas COPD is inherited in 40–77% [6]. Hence, the recent studies seek to define the genetic susceptibility factors that contribute to the individual susceptibility to COPD and/or lung cancer. So far, the only gene that has been definitively proved to influence COPD susceptibility is SERPINA1 that encodes α1-antitrypsin [8]. Most of the candidate gene studies reporting genetic associations with COPD have not been consistently replicated, making it difficult to draw reliable conclusions. In the study performed by Castaldi et al. [9], 27 genetic variants were found to have adequate data for quantitative meta-analysis, and 4 among them were significantly associated with COPD susceptibility: GSTM1 null variant, TGFBI rs1800470, TNF rs1800629 and SOD3 rs1799896. Genome-wide association studies found regions and genes which partly overlap in both COPD and lung cancer, and correspond to the pathogenic pathways mentioned above [reviewed in 2, 5, 6]. This allows for the seeking of an association between single nucleotide polymorphisms (SNPs) in the critical genes. A list of candidate SNPs examined in 12 genes and the respective source information can be found in the paper by Young et al. [10]. In that study, the control group of 'resistant smokers' was compared with COPD and lung cancer groups.

However, since not all COPD subjects develop lung cancer, it is important to identify the factors that determine the individual cancer susceptibility/resistance in this group. Therefore, a comparison of the frequency of several SNPs in 2 groups of subjects: patients with COPD and patients with COPD + lung cancer was carried out. The SNPs under study were located in genes engaged in tissue remodeling that code the following matrix metalloproteinases (MMPs) and their inhibitors: matrix metalloproteinase 1 (MMP1), matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 3 (MMP3), matrix metalloproteinase 9 (MMP9), matrix metalloproteinase 12 (MMP12), tissue inhibitor of metalloproteinases 1 (TIMP1) [11–18]. In addition to SNPs, the blood serum levels of the respective proteins were also analyzed.

### MATERIALS AND METHOD

**DNA isolation and genotyping.** Genomic DNA was isolated from whole blood using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol. Certain polymorphisms were assessed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The PCR and RFLP conditions, together with the respective references, are presented in Table1. Other polymorphisms were assessed using TaqMan® SNP Genotyping Assays (Life Technologies) (Tab. 2). Thermal cycling was performed on the 7500 Real-Time PCR System (Life Technologies) using 100 ng DNA as a template. The initial denaturation cycle was at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The data were collected and analysed using SDS 2.4 software (Life Technologies).

### RESULTS

The COPD + lung cancer (53 patients) and COPD-only (54 patients) groups were recruited among the clinic patients normalized according to gender, age, smoking habit and

### Table 2. TaqMan SNP Genotyping Assays used in the study

| Gene     | Polymorphism | TaqMan® SNP Genotyping Assay ID |
|----------|--------------|---------------------------------|
| TIMP1    | A/G rs6609533 | C__2495015_10                   |
| MMP1     | C/T rs1938901 | C__3012119_30                   |
| MMP2     | C/T rs1038668 | C__3225976_10                   |
| MMP3     | A/G rs678815  | C__3047716_10                   |
| MMP9     | A/T rs3918241 | C__2968985_10                   |

### Estimation of MMPs and TIMP1 levels.** The levels of TIMP1, MMP1, MMP2, MMP3, MMP9 proteins in the serum samples were determined by the following ELISA tests according to the manufacturer’s protocols: TIMP1, active and pro-MMP2 (total MMP2) Quantikine ELISA Kit (R&D System Inc.); MMP1, MMP 3 and MMP9 (Merck Millipore – Calbiochem).

### Statistical analysis.** Differences in the protein levels in the serum between the subjects with COPD and those with COPD and lung cancer were analysed by Mann-Whitney U-test. The distributions of genotypes in the studied groups were compared using χ² test. In all tests significance was accepted at p<0.05. All statistical analyses were performed using Statistica 7 software (StatSoft).

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**RESULTS**

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### Table 1. PCR primers and RFLP conditions for polymorphisms assessed by PCR-RFLP technique

| Gene     | Polymorphism | PCR primers and annealing temperature (°A) | PCR product size (base pairs) | Restriction enzyme | Fragments identifying genotypes (base pairs) | Reference |
|----------|--------------|---------------------------------------------|-----------------------------|-------------------|---------------------------------------------|-----------|
| MMP1     | -1607 1G/2G rs1799750 | F 5'-GCTTCTTCCTATGGTTGCTGTA-3' R 5'-TGTTTCTGTTGCTGTA-3' Ta = 53°C | 296 | Alul (Fermentas) | 1G/1G = 241 + 28 1G/2G = 241 + 24 2G/2G = 269 | [41] |
| MMP3     | 6A/SA rs3025058 | F 5'-GGTCTTCTCTCTTTTGGTGGGAAAAGA-3' R 5'-CCCTCCTGAAATCATACTACG-3' Ta = 63°C | 129 | Psyl (Fermentas) | 6A/6A = 129 6A/SA = 129 + 97 + 32 5A/5A = 97 + 32 | [42] |
| MMP9     | -1562 C/T rs3918242 | F 5'-GGCTGGACCATAGTGAAGCCC-3' R 5'-CTCTGTCAGACGGCCGCAC-3' Ta = 65°C | 435 | Pael (Fermentas) | C/C = 435 C/T = 435 + 247 + 188 T/T = 247 + 188 | [43] |
| MMP12    | -82 A/G rs2276109 | F 5'-GGATAGTCAGCAAGGTATAGCATCCG-3' R 5'-AAGAGCTCCTGAGGAGATCGG-3' Ta = 60°C | 199 | Pvull (Fermentas) | A/A = 199 A/G = 199 + 175 + 24 G/G = 175 + 24 | [44] |
| Asn357Ser rs652438 | F 5'-GGGAAAAATTTGGCTCTCAGCTCCACCTCAAAAGA-3' R 5'-CCCATGGGAAACCATAGAAGA-3' Ta = 55°C | 204 | Muni (Fermentas) | A/G = 198 5A/5A = 204 + 180 + 24 5G/5G = 204 | [44] |
diagnosed disease. The detailed characteristics of the subject groups were presented in a previous paper [19].

**Genotype distribution.** The TIMP1 and MMPs genotypes distribution for COPD and COPD + cancer groups are shown in Table 3. The frequencies of both MMP3 genotypes were significantly different between the studied groups. In both cases, more heterozygotes and less homozygotes (both types) were observed in the COPD group than in the COPD + cancer group. In the case of MMP12 rs2276109 polymorphism, a higher frequency of G allele in the COPD + cancer group was observed, but this tendency was not statistically significant (p=0.054). There were no statistically significant differences between the groups for the other MMP or TIMP1 polymorphisms.

**TIMP1 and MMP’s levels in blood serum.** The blood serum levels of TIMP1 and MMPs were assessed using dedicated ELISA kits. A significantly higher TIMP1 level was observed in the COPD + cancer group in comparison with the COPD group (Fig. 1). There were no statistically significant differences in MMPs levels between the studied groups (Fig. 2). In addition, no genotype-associated differences in TIMP1 or MMPs levels were observed (data not shown).

**DISCUSSION**

A recent report indicates that previous lung disease (chronic bronchitis, emphysema, pneumonia, and tuberculosis) influences the lung cancer risk independently of smoking habit, and that these diseases are important for estimating individual risk of lung cancer [20]. On the other hand, Gierada et al. [21] reported a lack of association between emphysema and lung cancer. Nevertheless, the airway epithelial damage caused by exposure to cigarette smoke and the consequent inflammation, seem to be important factors in the development of tissue remodeling, dysfunction of apoptosis and promotion of angiogenesis.

Among a wide range of genes that may play a role in the occurrence of COPD and lung cancer are those encoding the proteolytic enzymes, such as matrix metalloproteinases, and their tissue inhibitors. MMPs are involved in a stromal connective tissue remodeling in inflammatory processes and necrosis, and facilitate angiogenesis and tumour growth.
Matrix metalloproteinase 3 polymorphisms [reviewed in 11, 13–15, 22, 23]; e.g. MMP9 and MMP12 belong to the markers of epithelial-mesenchymal transition [24, 25] that is involved in tumour growth [26], and COPD progression.

In the majority of studies, the significance of various MMPs polymorphisms for lung cancer risk or COPD was estimated by comparing healthy subjects with COPD or cancer patients. Overexpression of MMP1 was associated with tumour invasion and metastasis formation [27]. Su et al. suggested that the MMP1 polymorphism -1607 1G/2G rs1799750 and certain haplotypes of MMP3 and MMP12 may lead to an increased risk of lung cancer among non-smoking males [17, 18]. MMP1 -1607 1G/2G rs1799750 polymorphism in relation to lung cancer risk was also examined by other authors and found to be significantly associated [28, 29]. Associations between MMP2 C735T and C1306T polymorphisms and lung cancer risk were found in Asians but not in Caucasians [30]. In the study performed by Peng et al., MMP2 rs2285053 T allele was associated with lower risk of lung cancer, whereas for all examined cancer types, MMP2 rs243865, MMP2 rs2285053 and MMP7 rs11568818 were supposed to play allele-specific roles in cancer development [31]. According to this analysis, MMP9 rs3918242 may not be a major risk factor for most cancer types [31]. However, in another study, the same MMP9 rs3918242 polymorphism was associated with lower risk of lung cancer [32], similar to MMP8 +17C/G rs2155052 polymorphism [33] and MMP2 C1306T or C735T [34]. Also, MMP9 P574R and R279Q polymorphisms were indicated as potential biomarkers for lung cancer and metastasis [35].

Whereas these studies are warranted by a small number of subjects and require confirmation in a larger population, they also suggest the important role of MMPs as initiators of carcinogenesis in lung cancer. They also point to the important role of the tissue inhibitors of metalloproteinases, alpha 1-antitrypsin, alpha2-macroglobulin and other protease inhibitors, which modulate the proteinases activity [8, 23].

In contrast to the above mentioned studies, in the present study comparison was made between the occurrence of the MMPs and TIMP1 polymorphisms in COPD patients and COPD patients developing lung cancer. Among the SNPs examined (Tab. 3), the only difference between the subject groups was found in the distribution of the two MMP3 polymorphic alleles (MMP3 rs3025058 and MMP3 rs678815). In both cases, more heterozygotes and less homozygotes (both types) were observed in the

Figure 2. Box plots of mean MMPs levels in blood serum in the 2 groups of subjects: with COPD or COPD and lung cancer. (A) MMP1; (B) MMP2; (C) MMP3; (D) MMP9. In the box plots, the middle square represents the mean, the boxes denote standard error and the whiskers denote standard deviation. The differences are not statistically significant.
COPD group than in the COPD + cancer group. This is compatible with the notion that heterozygocity for MMP3 polymorphisms reduces the risk of cancer, a phenomenon called ‘heterozygote advantage’. According to Sellis et al., the heterozygote advantage is displayed by a substantial proportion of adaptive mutations [36]. This effect stems from the susceptibility of heterozygotes to the invasion of new adaptive mutations.

Interestingly, MMP3 (also known as stromelysin 2) belongs to proteases that are preferentially inhibited by TIMP1 [13]. A significantly higher TIMP1 level in blood serum was observed in the COPD + cancer group than in the COPD group (Fig. 1). As MMPs are involved in cancer growth and metastasis, their inhibitors seem to be suitable candidates for therapeutic factors [37, 38]. Hence, TIMP1 is typically associated with the inhibition of MMP induced cancer cell invasion. However, TIMP1 is overexpressed in many malignancies and alters the expression of approximately 600 genes in vivo, including MMP1 and MMP13 [39]. In parallel, the growth-stimulating function of TIMP1 has been described and explained in molecular terms [40]. Therefore, the high TIMP1 level found in subjects from the COPD + cancer group most probably reflects the progress of the malignancy.

CONCLUSIONS

The results of the presented study suggest that the homozygocity for MMP3 rs3025058 and rs678815 polymorphisms is a potential marker of enhanced susceptibility to lung cancer development among COPD subjects. More extensive study including a larger cohort of patients is needed to confirm this relationship.

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Declaration of Interest

The authors declare that they have no conflict of interest.

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