

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

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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 proteinases (*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 than in the COPD 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- κ B 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

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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 $\alpha 1$ -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 Table 1. 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).

Table 2. TaqMan SNP Genotyping Assays used in the study

Gene	Polymorphism	TaqMan® SNP Genotyping Assay ID
<i>TIMP1</i>	A/G rs6609533	C__2459015_10
<i>MMP1</i>	C/T rs1938901	C__3012119_30
<i>MMP2</i>	C/T rs1030868	C__3225949_1_
	A/C rs7201	C__3225976_10
<i>MMP3</i>	C/G rs678815	C__3047716_10
<i>MMP9</i>	A/T rs3918241	C__29689865_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 χ^2 test. In all tests significance was accepted at $p < 0.05$. All statistical analyses were performed using Statistica 7 software (StatSoft).

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

Gene	Polymorphism	PCR primers and annealing temperature (Ta)	PCR product size	Restriction enzyme	Fragments identifying genotypes (base pairs)	Reference
<i>MMP1</i>	-1607 1G/2G rs1799750	F 5'-TGACTTTTAAAACATAGTCTATGTTCA-3' R 5'-TCTTGGATTGATTGAGATAAGTCATAGC-3' Ta = 53°C	269 bp	AluI (Fermentas)	1G/1G = 241 + 28 1G/2G = 269 + 241 + 28 2G/2G = 269	[41]
<i>MMP3</i>	6A/5A rs3025058	F 5'-GGTTCCTCATTCTTTGATGGGGGAAAGA-3' R 5'-CTTCGGGAATTCACATCACTGCCACCACT-3' Ta = 63°C	129 bp	PvuII (Fermentas)	6A/6A = 129 6A/5A = 129 + 97 + 32 5A/5A = 97 + 32	[42]
<i>MMP9</i>	-1562 C/T rs3918242	F 5'-GCCTGGCCACATAGTAGGCC-3' R 5'-CTTCTAGCCAGCCGGCATC-3' Ta = 65°C	435 bp	PaeI (Fermentas)	C/C = 435 C/T = 435 + 247 + 188 T/T = 247 + 188	[43]
<i>MMP12</i>	-82 A/G rs2276109	F 5'-GAGATAGTCAAGGGATGATATCAGC-3' R 5'-AAGAGCTCCAGAAGCAGTGG-3' Ta = 60°C	199 bp	PvuII (Fermentas)	A/A = 199 A/G = 199 + 175 + 24 G/G = 175 + 24	[44]
	Asn357Ser rs652438	F 5'-GGGATAATTTGGCTCTGGCTCTCAA-3' R 5'-CCATGGGAACCATAGAAAAGA-3' Ta = 55°C	204 bp	MunI (Fermentas)	Asn/Asn = 180 + 24 Asn/Ser = 204 + 180 + 24 Ser/Ser = 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 *MMPs*' 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).

Table 3. Distribution of genotypes in the studied groups of patients. The results for the polymorphisms marked with asterisks were published in the previous paper [19]

Genotype	COPD + cancer n=53 (%)	COPD n=54 (%)	χ^2 test
<i>TIMP1</i> rs6609533			
G/G	28 (52.83)	27 (50.00)	p=0.770
A/G	25 (47.17)	27 (50.00)	
<i>MMP1</i> rs1799750*			
1G/1G	12 (22.64)	17 (31.48)	p=0.492
1G/2G	26 (49.06)	21 (38.89)	
2G/2G	15 (28.30)	16 (29.63)	
<i>MMP1</i> rs1938901			
C/C	27 (50.94)	28 (51.85)	p=0.741
C/T	21 (39.62)	23 (42.59)	
T/T	5 (9.43)	3 (5.56)	
<i>MMP2</i> rs1030868			
C/C	23 (43.40)	24 (44.44)	p=0.432
C/T	22 (41.51)	26 (48.15)	
T/T	8 (15.09)	4 (7.41)	
<i>MMP2</i> rs7201			
A/A	18 (33.96)	21 (38.89)	p=0.859
A/C	25 (47.17)	23 (42.59)	
C/C	10 (18.87)	10 (18.52)	
<i>MMP3</i> rs3025058*			
5A/5A	16 (30.19)	9 (16.67)	p=0.006
5A/6A	19 (35.85)	36 (66.67)	
6A/6A	18 (33.96)	9 (16.67)	
<i>MMP3</i> rs678815			
G/G	16 (30.19)	11 (20.37)	p=0.017
C/G	19 (35.85)	34 (62.96)	
C/C	18 (33.96)	9 (16.67)	
<i>MMP9</i> rs3918242*			
C/C	42 (79.25)	40 (74.07)	p=0.547
C/T	11 (20.75)	13 (24.07)	
T/T	0 (0)	1 (1.85)	
<i>MMP9</i> rs3918241			
T/T	42 (79.25)	36 (66.67)	p=0.254
A/T	11 (20.75)	17 (31.48)	
A/A	0 (0)	1 (1.85)	
<i>MMP12</i> rs652438*			
Asn/Asn	51 (96.23)	49 (90.74)	p=0.251
Asn/Ser	2 (3.77)	5 (9.26)	
<i>MMP12</i> rs2276109*			
A/A	36 (67.92)	46 (85.19)	p=0.054
A/G	14 (26.42)	8 (14.81)	
G/G	3 (5.66)	0 (0)	

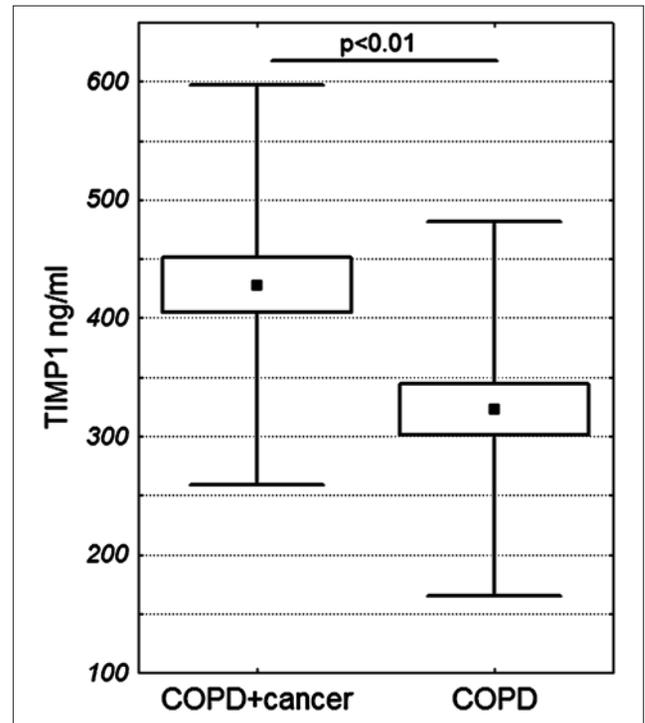


Figure 1. Box plots of mean *TIMP1* levels in blood serum of the 2 groups of subjects: with COPD or COPD and lung cancer. In the box plots, the middle square represents the mean, the boxes denote standard error and the whiskers denote standard deviation. Significantly different in Mann-Whitney U-test, $p < 0.01$

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

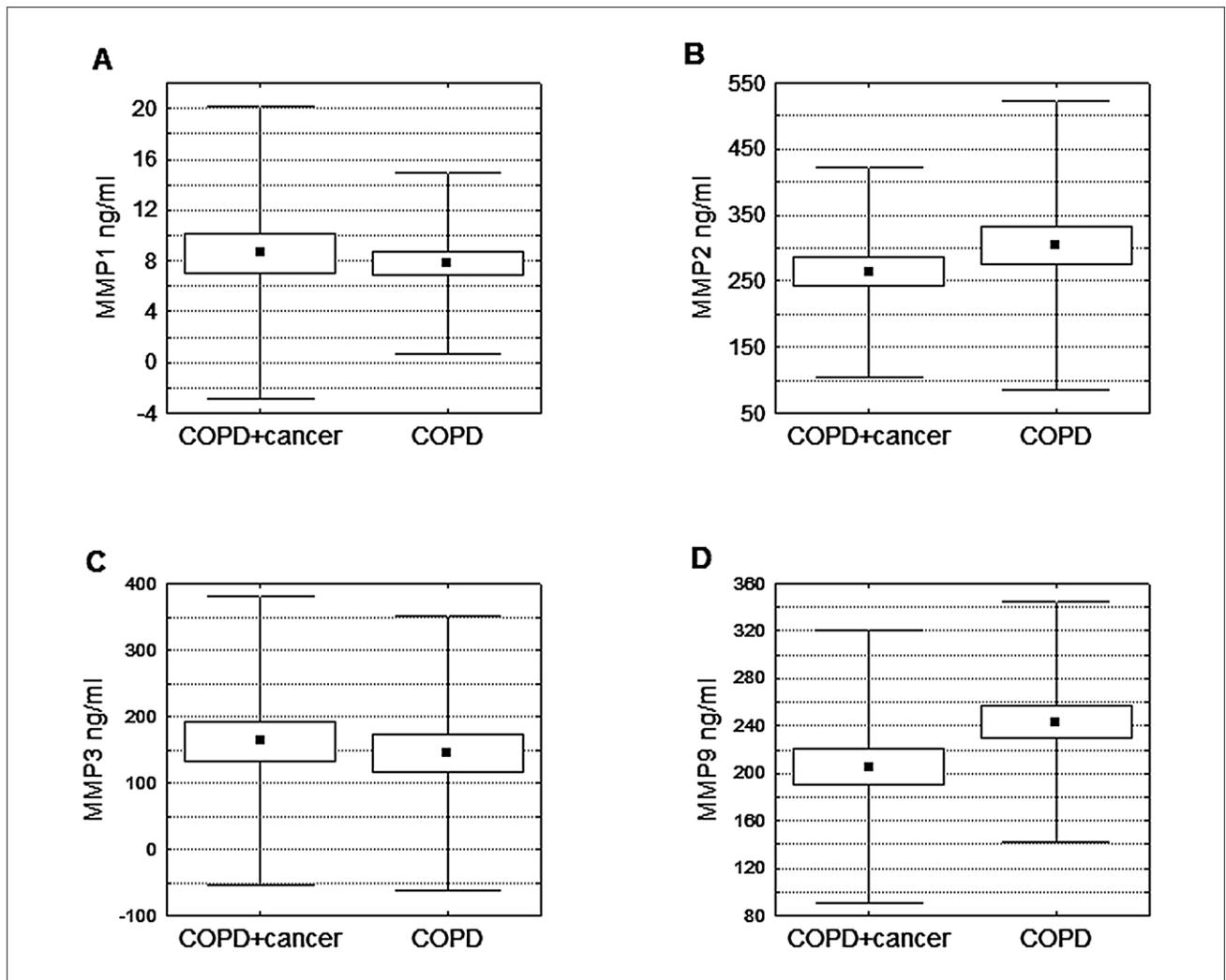


Figure 2. Box plots of mean MMPs levels in blood serum in the 2 groups of subjects: with COPD or COPD and lung cancer. (A) MMP1; (B) MMP2; (C) MMP3; (D) MMP9. In the box plots, the middle square represents the mean, the boxes denote standard error and the whiskers denote standard deviation. The differences are not statistically significant

[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, alpha 2-macroglobulin and other protease inhibitors, which modulate the proteinases activity [8, 23].

In contrast to the above mentioned studies, in the presented 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

COPD group than in the COPD + cancer group. This is compatible with the notion that heterozygosity 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 stromelysin2) 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 *MMPI* 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 homozygosity 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.

REFERENCES

- Eisner MD, Anthonisen N, Coultas D, Kuenzli N, Perez-Padilla R, Postma D, et al. An official American Thoracic Society public policy statement: Novel risk factors and the global burden of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2010; 182(5): 693–718.
- Adcock IM, Caramori G, Barnes PJ. Chronic obstructive pulmonary disease and lung cancer: new molecular insights. *Respiration*. 2011; 81(4): 265–284.
- Decramer M, Rennard S, Troosters T, Mapel DW, Giardino N, Mannino D, et al. COPD as a lung disease with systemic consequences--clinical impact, mechanisms, and potential for early intervention. *COPD*. 2008; 5(4): 235–256.
- Decramer M, Janssens W, Miravittles M. Chronic obstructive pulmonary disease. *Lancet*. 2012; 379(9823): 1341–1351.
- Yang IA, Relan V, Wright CM, Davidson MR, Sriram KB, Savarimuthu Francis SM, et al. Common pathogenic mechanisms and pathways in the development of COPD and lung cancer. *Expert Opin Ther Targets*. 2011; 15(4): 439–456.
- Young RP, Hopkins RJ. How the genetics of lung cancer may overlap with COPD. *Respirology*. 2011; 16(7): 1047–1055.
- Skullrud DM, Offord KP, Miller RD. Higher risk of lung cancer in chronic obstructive pulmonary disease. A prospective, matched, controlled study. *Ann Intern Med*. 1986; 105(4): 503–507.
- Stoller JK, Aboussouan LS. Alpha1-antitrypsin deficiency. *Lancet*. 2005; 365(9478): 2225–2236.
- Castaldi PJ, Cho MH, Cohn M, Langerman F, Moran S, Tarragona N, et al. The COPD genetic association compendium: a comprehensive online database of COPD genetic associations. *Hum Mol Genet*. 2010; 19(3): 526–534.
- Young RP, Hopkins RJ, Whittington CF, Hay BA, Epton MJ, Gamble GD. Individual and cumulative effects of GWAS susceptibility loci in lung cancer: associations after sub-phenotyping for COPD. *PLoS One*. 2011; 6(2): e16476.
- Churg A, Zhou S, Wright JL. Series "matrix metalloproteinases in lung health and disease": Matrix metalloproteinases in COPD. *Eur Respir J*. 2012; 39(1): 197–209.
- Elkington PT, Friedland JS. Matrix metalloproteinases in destructive pulmonary pathology. *Thorax*. 2006; 61(3): 259–266.
- Hua H, Li M, Luo T, Yin Y, Jiang Y. Matrix metalloproteinases in tumorigenesis: an evolving paradigm. *Cell Mol Life Sci*. 2011; 68(23): 3853–3868.
- Loffek S, Schilling O, Franzke CW. Series "matrix metalloproteinases in lung health and disease": Biological role of matrix metalloproteinases: a critical balance. *Eur Respir J*. 2011; 38(1): 191–208.
- Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. *Semin Cell Dev Biol*. 2008; 19(1): 34–41.
- Oikonomidi S, Kostikas K, Tsilioni I, Tanou K, Gourgoulis KI, Kiriopoulou TS. Matrix metalloproteinases in respiratory diseases: from pathogenesis to potential clinical implications. *Curr Med Chem*. 2009; 16(10): 1214–1228.
- Su L, Zhou W, Park S, Wain JC, Lynch TJ, Liu G, et al. Matrix metalloproteinase-1 promoter polymorphism and lung cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(3): 567–570.
- Su L, Zhou W, Asomaning K, Lin X, Wain JC, Lynch TJ, et al. Genotypes and haplotypes of matrix metalloproteinase 1, 3 and 12 genes and the risk of lung cancer. *Carcinogenesis*. 2006; 27(5): 1024–1029.
- Grudny J, Kolakowski J, Kruszewski M, Szopinski J, Sliwinski P, Wiatr E, et al. Association of genetic dependences between lung cancer and chronic obstructive pulmonary disease. *Pneumonol Alergol Pol*. 2013; 81(4): 308–318.
- Brenner DR, Boffetta P, Duell EJ, Bickeboller H, Rosenberger A, McCormack V, et al. Previous lung diseases and lung cancer risk: a pooled analysis from the International Lung Cancer Consortium. *Am J Epidemiol*. 2012; 176(7): 573–585.
- Gierada DS, Guniganti P, Newman BJ, Dransfield MT, Kvale PA, Lynch DA, et al. Quantitative CT assessment of emphysema and airways in relation to lung cancer risk. *Radiology*. 2011; 261(3): 950–959.
- Dejonckheere E, Vandenbroucke RE, Libert C. Matrix metalloproteinase8 has a central role in inflammatory disorders and cancer progression. *Cytokine Growth Factor Rev*. 2011; 22(2): 73–81.
- Mocchegiani E, Giacconi R, Costarelli L. Metalloproteases/anti-metalloproteases imbalance in chronic obstructive pulmonary disease: genetic factors and treatment implications. *Curr Opin Pulm Med*. 2011; 17 (Suppl. 1): S11–S19.
- Yao H, Rahman I. Current concepts on the role of inflammation in COPD and lung cancer. *Curr Opin Pharmacol*. 2009; 9(4): 375–383.
- Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest*. 2009; 119(6): 1429–1437.
- Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene*. 2010; 29(34): 4741–4751.
- Feng X, Wu Z, Wu Y, Hankey W, Prior TW, Li L, et al. Cdc25A regulates matrix metalloprotease 1 through Foxo1 and mediates metastasis of breast cancer cells. *Mol Cell Biol*. 2011; 31(16): 3457–3471.
- Liu L, Wu J, Wu C, Wang Y, Zhong R, Zhang X, et al. A functional polymorphism (-1607 1G-->2G) in the matrix metalloproteinase-1 promoter is associated with development and progression of lung cancer. *Cancer*. 2011; 117(22): 5172–5181.
- Xiao XY, Wang XD, Zang DY. MMP1-1607 1G/2G polymorphism and lung cancer risk: a meta-analysis. *Tumour Biol*. 2012; 33(6): 2385–2392.
- Guo XT, Wang JF, Zhang LY, Xu GQ. Quantitative assessment of the effects of MMP-2 polymorphisms on lung carcinoma risk. *Asian Pac J Cancer Prev*. 2012; 13(6): 2853–2856.
- Peng B, Cao L, Ma X, Wang W, Wang D, Yu L. Meta-analysis of association between matrix metalloproteinases 2, 7 and 9 promoter polymorphisms and cancer risk. *Mutagenesis*. 2010; 25(4): 371–379.

32. Zhang LF, Mi YY, Cao Q, Wang W, Qin C, Wei JF, et al. Update analysis of studies on the MMP-9 -1562 C>T polymorphism and cancer risk. *Mol Biol Rep.* 2012; 39(4): 3435–3441.
33. Gonzalez-Arriaga P, Lopez-Cima MF, Fernandez-Somoano A, Pascual T, Marron MG, Puente XS, et al. Polymorphism +17 C/G in matrix metalloprotease MMP8 decreases lung cancer risk. *BMC Cancer.* 2008; 8: 378.
34. McColgan P, Sharma P. Polymorphisms of matrix metalloproteinases 1, 2, 3 and 9 and susceptibility to lung, breast and colorectal cancer in over 30,000 subjects. *Int J Cancer.* 2009; 125(6): 1473–1478.
35. Hu Z, Huo X, Lu D, Qian J, Zhou J, Chen Y, et al. Functional polymorphisms of matrix metalloproteinase-9 are associated with risk of occurrence and metastasis of lung cancer. *Clin Cancer Res.* 2005; 11(15): 5433–5439.
36. Sellis D, Callahan BJ, Petrov DA, Messer PW. Heterozygote advantage as a natural consequence of adaptation in diploids. *Proc Natl Acad Sci U S A.* 2011; 108(51): 20666–20671.
37. Belvisi MG, Bottomley KM. The role of matrix metalloproteinases (MMPs) in the pathophysiology of chronic obstructive pulmonary disease (COPD): a therapeutic role for inhibitors of MMPs? *Inflamm Res.* 2003; 52(3): 95–100.
38. Vandembroucke RE, Dejonckheere E, Libert C. A therapeutic role for matrix metalloproteinase inhibitors in lung diseases? *Eur Respir J.* 2011; 38(5): 1200–1214.
39. Bigelow RL, Williams BJ, Carroll JL, Daves LK, Cardelli JA. TIMP-1 overexpression promotes tumorigenesis of MDA-MB-231 breast cancer cells and alters expression of a subset of cancer promoting genes in vivo distinct from those observed in vitro. *Breast Cancer Res Treat.* 2009; 117(1): 31–44.
40. Ridnour LA, Barasch KM, Windhausen AN, Dorsey TH, Lizardo MM, Yfantis HG, et al. Nitric oxide synthase and breast cancer: role of TIMP-1 in NO-mediated Akt activation. *PLoS One.* 2012; 7(9): 44081.
41. Zhu Y, Spitz MR, Lei L, Mills GB, Wu X. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. *Cancer Res.* 2001; 61(21): 7825–7829.
42. Gnasso A, Motti C, Irace C, Carallo C, Liberatoscioli L, Bernardini S, et al. Genetic variation in human stromelysin gene promoter and common carotid geometry in healthy male subjects. *Arterioscler Thromb Vasc Biol.* 2000; 20(6): 1600–1605.
43. Peres RC, Line SR. Analysis of MMP-9 and TIMP-2 gene promoter polymorphisms in individuals with hypodontia. *Braz Dent J.* 2005; 16(3): 231–236.
44. Joos L, He JQ, Shepherdson MB, Connett JE, Anthonisen NR, Pare PD, et al. The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet.* 2002; 11(5): 569–576.

