Polymorphisms in the MMP1 and MMP3 promoter and non-small cell lung carcinoma in North China

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Matrix metalloproteinases (MMPs) are proteolytic enzymes that regulate various cell behaviors in cancer biology, via their basic function of degradation of proteins. Genetic variations in several MMP promoters may influence transcription and expression of MMPs. The aim of this study is to assess the effects of the two single nucleotide polymorphisms (SNPs), the guanine insertion polymorphism in the MMP1 promoter and the adenosine insertion polymorphism in the MMP3 promoter, on risk of the development and lymphatic metastasis of non-small cell lung carcinoma (NSCLC). The MMP1 and MMP3 SNPs were genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis in 243 NSCLC patients and 350 control subjects in North China. The overall genotype and allelotype distribution of both the variants in cancer patients and controls was not significantly different (all P values are above 0.05). However, stratification analysis showed that smoking individuals with the MMP3 5A allele had a >1.5-fold increased risk to develop NSCLC, compared with those harboring the 6A homozygous [the age and gender adjusted odds ratio (OR) = 1.68, 95% confidence interval (CI) = 1.04–2.70]. In addition, the frequency of the MMP3 5A homozygote in NSCLC patients with lymphatic metastasis was significantly higher than that in lymph node negative ones (5.7 versus 0%, P = 0.04). Moreover, the MMP1 1G/5A haplotype significantly increased the risk of lymphatic metastasis (OR = 3.36, 95% CI = 1.42–7.94), compared with the 2G/6A haplotype. The present result suggested that the MMP3 promoter polymorphism may modify susceptibility to NSCLC, and the MMP1 1G/5A haplotype may precipitate the risk of lymphatic metastasis of this tumor.

Introduction

Matrix metalloproteinases (MMPs), a family of enzymes whose basic function is degrading the extracellular matrix (ECM) and basement membrane, have been involved in the regulation of various cell behaviors with relevance to cancer biology (1). At least 26 human MMPs have been identified currently, which are classified according to their substrate specificity and structural similarities (2). MMP1 belongs to the interstitial collagenase, a subfamily of MMPs that cleaves stromal collagens, which involve the ability of neoplastic cells to cross the basal membrane of both the epithelium and the vascular endothelium (2). MMP3 (stromelysin-1) is known to lyse basal membrane collagen and induce the synthesis of other MMPs such as MMP1 and MMP9 (2,3). Originally, MMPs were considered to play important roles in cancer invasion and metastasis; however, recent studies document that MMPs are involved in several steps of cancer development, including regulation of cell growth and apoptosis, angiogenesis and immune surveillance (1). In animals, tissue-specific over-expression of MMP1 may lead to hyperproliferative disease and an increase in cancer susceptibility (4), while the expression of MMP3 in the mammary gland may result in spontaneous breast cancer (5).

The MMP1 and MMP3 genes are neighbors on chromosome 11q. Both genes are expressed in various tumor cells and a wide variety of normal cells including stromal fibroblasts, macrophages, endothelial cells and epithelial cells (6). The level of MMP1 and MMP3 expression can be influenced by single nucleotide polymorphisms (SNPs) in the promoter region of their genes (7–10). The MMP1 SNP is located at position 1607 bp upstream of the transcriptional initiation site, where an insertion of a guanine base (G) creates the sequence of 5’-GGAT-3’, the core binding site for members of the EST family of transcription factors (7,8). The variation of the MMP3 SNP is located at position 1171 bp upstream of the transcriptional initiation site, containing either five or six adenosines that may affect the transcription activity of the promoter (9–11). The MMP1 and MMP3 SNPs have been correlated to the risk of several tumors such as renal cell carcinoma (12), colorectal cancer (13) and breast cancer (14). The polymorphisms have also been associated with the invasion of cutaneous malignant melanoma (6), ovarian cancer (15) and colorectal cancer (16).

The association between the MMP1 2G/2G genotype and increased susceptibility to lung cancer has been reported in Caucasians (17). However, the relationship between the MMP1 SNP and risk of the development of lung cancer in Chinese population has not been documented. In addition, the influence of the MMP3 SNP on the occurrence and progression of lung cancer has not been clarified so far. Based on the knowledge that the MMPs may play crucial roles in cancer formation, invasion and metastasis, we hypothesized that (i) the genotype leading high level of MMP1 expression (i.e. 2G/2G) may also increase susceptibility to lung cancer in Chinese population, (ii) the MMP3 SNP may modify risk of the development and metastasis of lung cancer and (iii) the MMP1 and MMP3 SNPs may work together to influence the development and progression of the tumor. Therefore, we conducted a case-control study in a population of North China with the aim to test the above hypotheses.

Abbreviations: CI, confidence interval; MMPs, matrix metalloproteinases; NSCLC, non-small cell lung carcinoma; SNP, single nucleotide polymorphisms; OR, odds ratio.
Materials and methods

Subject characteristics

This study included 243 incident cancer patients with non-small cell lung carcinoma (NSCLC) (126 with adenocarcinoma, 106 with squamous cell carcinoma, 11 with other histological types including five bronchioalveolar carcinoma, five mucocoeplid carcinoma and three pleomorphic tumors) and 350 healthy individuals without overt cancer. The cases were outpatients for bronchoscopic biopsy or inpatients for tumor resection in the Fourth Affiliated Hospital, Hebei Medical University between 2001 and 2003. This hospital is the only provincial tumor hospital of the Hebei province. The majority of lung cancer patients from Shijiazhuang city and its surrounding regions accept surgical treatment in this hospital. The patients who were diagnosed 3 months before the first examination and expected to have the possibility of tumor resection were randomly distributed into three sections of the Department of Thoracic Surgery. Since one important aim of our study is to analyze the role of the MMP polymorphisms in lymphatic metastasis of NSCLC, only patients from the first section were recruited in the study, to avoid bias induced by variation in operation approaches by different surgeon groups. Histological tumor typing was carried out on the basis of the biopsies or resected specimens in the Department of Pathology of the same hospital. To simultaneously assess the effect of the MMP polymorphisms on susceptibility and progression of different tumor types, we used the same panel of healthy controls as in our previous study on gastrooesophageal cancer. The detailed characteristics of the control individuals were described as before (18). Briefly, between 2001 and 2003, subjects who visited the same hospital for physical examination were invited to join the epidemiological study while having no history of cancerous or genetic diseases. Individuals volunteering to give their blood for the survey were recruited in the study. All of the cancer patients and control subjects were unrelated Han nationality and from Shijiazhuang city or its surrounding regions. The information on gender, age and smoking habit from cancer patients and healthy controls was obtained by two professional interviewers directly after sampling. For smoking habit, the former and present smoking status, the number of cigarettes per day, and the time of starting and quitting were inquired. Individuals who formerly or currently smoked 5 cigarettes/day for at least 2 years were defined as smokers. Information on lymphatic metastasis from 197 NSCLC patients who underwent surgical treatment was available from hospital recordings and pathological diagnosis. The study was approved by the Ethics Committee of Hebei Cancer Institute and informed consent was obtained from all recruited subjects.

DNA extraction

Five milliliters of venous blood from each subject was drawn in Vacutainer tubes containing EDTA and stored at 4°C. Genomic DNA was extracted within 1 week after sampling by using proteinase K (Merck, Darmstadt, Germany) digestion followed by a salting out procedure according to the method published by Miller et al. (19).

Genotyping of the MMP1 and MMP3 promoter polymorphism

The MMP1 and MMP3 genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay. The primers used for amplifying the MMP1 SNP were 5′-TACCTTTAAAACA-TAGTCTATGTTCA-3′ (forward) and 5′-TCTTGGATTGATTTGAGA-TAACGTAACAC3′ (reverse). A mutation from T to G at the second nucleotide close to the 3′ end of the reverse primer was made to create an AluI (AGCT) recognition site in the case of a 1G allele (17). The primers used for amplifying the MMP3 SNP were 5′-GGTCTCCCATTCCTTT-GATGGGGGGAAAG-3′ (forward) and 5′-CTTCTCGGAATTCTC-CACACTGCACAC-3′ (reverse). An A to G mutation at the second nucleotide close to the 3′ end of the forward primer was made to create a Tth111I (GACNNNGTC) recognition site in the case of a 5A allele. The PCR reactions were performed in a 25-μl volume containing 100 ng of DNA template, 2.5 μl of 10× PCR buffer, 2.0 mM MgCl2, 2.5 U of Taq-DNA polymerase (BioDev-Tech., Beijing, China), 0.2 mM dNTPs and 0.2 μM forward and reverse primer. The PCR cycling conditions were 5 min at 94°C followed by 35 cycles of 94°C for 0.5 min, 51.7°C for 1.5 min and 72°C for 1.5 min, and with a final step at 72°C for 5 min to allow for the complete extension of all PCR fragments. An 8-μl aliquot of PCR product was digested overnight at 37°C or for 4 h at 65°C in a 10-μl reaction containing 10 U of AluI (MMP1) or Tth111I (MMP3) enzyme (TakaRa Biotechnology, Dalian, China). After digestion, the products were subjected to electrophoresis on a 3% agarose gel stained with ethidium bromide. The MMP1 2G alleles were therefore represented by a DNA band with size of 269 bp, the 1G alleles were represented by two DNA bands with size of 241 and 28 bp, whereas the heterozygotes displayed a combination of both the alleles (269, 241 and 28 bp). For the MMP3 SNP, the 6A alleles were expected as a DNA band with size of 129 bp, the 5A alleles were expected as DNA bands of 97 and 32 bp, whereas the heterozygotes were expected as a combination of both the alleles (129, 97 and 32 bp).

For a negative control, distilled water instead of DNA in the reaction system was used for each panel of PCR. For 10% of samples, the PCR was repeated once for quality control.

Statistical analyses

Statistical analyses were performed using SPSS12.0 software package (SPSS Company, Chicago, IL). Hardy–Weinberg analysis was performed to compare the observed and expected genotype frequencies using χ² test. The distribution of the MMP1 and MMP3 SNP in the study groups was compared by means of two-sided contingency tables using χ² test or Fisher’s exact test. The MMP1 and MMP3 haplotype frequencies and linkage disequilibrium coefficient were estimated by using EH linkage software (1.2 version, Rockefeller University, NY). The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model and adjusted by age and gender accordingly. A probability level of 5% was considered significant for all statistic analyses.

Results

Subject characteristics

The demographic distribution of cancer patients and healthy controls was shown in Table I. The mean age of NSCLC cases was 57.2 ± 10.5 years (range 32–76) and that of controls was 51.7 ± 10.7 years (range 30–68). There was no statistical difference in age distribution between the two groups (P = 0.09). The gender distribution in NSCLC patients (70.4% men) was also comparable with that in healthy controls (65.4% men) (P = 0.21). Information on smoking status from 70 control subjects and nine cancer patients failed to be recorded. Among individuals with available information, the proportion of smokers in cancer patients was significantly higher than that in healthy controls (60.7 versus 42.9%, χ² = 16.2, P < 0.001). Therefore, smoking significantly increased risk of NSCLC development (the age and gender adjusted OR = 1.74, 95% CI = 1.16–2.60). Among 197 NSCLC patients with tumor resection, lymphatic metastasis was reported in 123 cases and the rest (74 cases) were diagnosed as lymph node negative.

Distribution of the MMP1 and MMP3 SNP in NSCLC patients and controls

Genotyping of the MMP1 and MMP3 SNP was successfully performed in all study subjects. The results from the re-genotyped samples completely matched the original ones. The distribution of neither the MMP1 nor the MMP3 SNP was correlated with gender and age both in NSCLC patients and healthy controls (data not shown). The distribution of the MMP3 genotypes in cancer patients and healthy controls did not significantly deviate from that expected by the Hardy–Weinberg equilibrium (χ² = 0.10 and 0.22, P = 0.95 and 0.90, respectively). The distribution of the MMP1 SNP in NSCLC patients was also consistent with the Hardy–Weinberg equilibrium (χ² = 1.79, P = 0.41). However, the distribution of the MMP1 genotypes in healthy controls was not in concordance with the Hardy–Weinberg equilibrium (χ² = 12.37, P = 0.002).

In healthy subjects, the frequencies of the MMP1 2G/2G, 1G/2G and 1G/1G genotypes were 55.4, 30.0 and 14.6%, of the MMP3 6A/6A, 5A/6A and 5A/5A genotypes were 67.7, 30.0 and 2.3%, respectively (Table II). Haplotype analysis showed that, the 2G/6A was the commonest haplotype in healthy controls (62.3%), followed by the 1G/6A (20.4%), 1G/5A (9.1%) and 2G/5A (8.2%) haplotypes (Table I). In addition, the MMP1 and MMP3 SNPs were imperfectly in linkage disequilibrium (D’ = 0.327, χ² = 37.15, P < 0.0001) in the study population,
i.e. the MMP1 2G allele tended to be linked to the MMP3 6A allele, although different haplotypes have been observed.

As shown in Tables I and II, the overall allelotype and genotype frequencies of the MMP1 SNP in NSCLC patients and healthy controls did not show significant difference (\(\chi^2 = 0.004\) and 3.44, \(P = 0.37\) and 0.18, respectively). When stratified by smoking status, there was still no significant difference in the MMP1 genotype distribution between cancer patients and healthy controls (all \(P\) values were above 0.05). Significant difference in the MMP3 genotype and allelotype frequencies in the overall NSCLC patients and healthy controls was also not observed (\(\chi^2 = 0.21\) and 0.08, \(P = 0.91\) and 0.78, respectively). However, the frequency of the MMP3 5A/6A + 5A/5A genotypes in smoking NSCLC patients was significantly higher than that in smoking controls (35.2 versus 23.3%, \(\chi^2 = 4.39, P = 0.04\)). By using 6A/6A, the genotype with low level of MMP3 expression as the reference, the 5A/5A + 5A/6A genotypes showed a significantly increased risk to the development of NSCLC (the age and gender adjusted \(OR = 1.68, 95\% CI = 1.04-2.70\)). Although the proportion of smokers in NSCLC patients was also significantly higher than that in healthy controls, the synergetic interaction between smoking and the MMP3 5A allele on the risk of NSCLC development was not observed (\(\chi^2 = 3.49, P = 0.06\)). In addition, the significant difference in the MMP1 and MMP3 SNP distribution between cancer patients and the overall controls was not found in the study, when stratified by pathological types (Table II).

Furthermore, the combined effects of the MMP1 and MMP3 SNP on the risk of developing NSCLC was analyzed by using the EH program. The overall distribution of the MMP haplotypes in NSCLC patients and healthy controls did not show significant difference, the similar trend was also observed when stratified by smoking status and pathological types (data not shown).

The MMP1 and MMP3 SNPs may modify the prone to lymphatic metastasis of NSCLC.

Since the prediction of local invasion and metastasis is crucial for making individualized treatment plans, we tried to identify whether the MMP1 and MMP3 polymorphisms play a role in the risk of lymphatic metastasis of NSCLC in the study population. Analyses were performed among cases with available

| Table II. Association analyses of the MMP1 and MMP3 SNP with risk of the development of NSCLC |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Groups          | \(1G/1G\) n (%) | \(1G/2G\) n (%) | \(2G/2G\) n (%) | \(6A/6A\) n (%) |
| Overall         | 51 (14.6)       | 105 (30.0)      | 194 (55.4)      | 237 (67.7)      |
| NSCLC           | 24 (9.9)        | 84 (34.6)       | 135 (55.5)      | 163 (67.1)      |
| Non-smokerc     | 25 (15.6)       | 46 (28.8)       | 89 (55.6)       | 96 (60.0)       |
| NSCLC           | 9 (9.8)         | 35 (38.0)       | 48 (52.2)       | 66 (71.7)       |
| Normal          | 18 (5.0)        | 38 (31.7)       | 64 (53.3)       | 92 (76.7)       |
| NSCLC           | 15 (0.6)        | 45 (31.7)       | 82 (57.7)       | 92 (64.8)       |
| AC              | 12 (9.5)        | 49 (38.9)       | 65 (51.6)       | 76 (60.3)       |
| SC              | 11 (10.4)       | 31 (29.2)       | 64 (60.4)       | 77 (72.6)       |

AC, adenocarcinoma; SC, squamous cell carcinoma.

\(^a\)The age and gender adjusted OR of the \(1G/2G + 2G/2G\) against the \(1G/1G\) genotype.

\(^b\)The age and gender adjusted OR of the \(5A/6A + 5A/5A\) against the \(6A/6A\) genotype.

\(^c\)Information on smoking status was available from a subset of subjects.

\(^d\)The frequency of the \(5A/6A + 5A/5A\) genotype in NSCLC patients was significantly higher than that in controls (\(\chi^2 = 4.39, P = 0.04\)).
clinical data at the time of diagnosis. As shown in Table III, the *MMP1* genotype frequencies in NSCLC patients with lymphatic metastasis were not significantly different from that in lymph node negative ones ($\chi^2 = 0.02, P = 0.99$). However, the *MMP3* 5A/5A genotype was significantly more frequent in patients with positive lymph nodes than those without lymphatic metastasis ($5.7$ versus $0\%$, Fisher’s exact test, $P = 0.04$), although the OR for the risk of lymphatic metastasis did not reach significance, compared with the 6A/6A genotype (the age and gender adjusted OR = 4.13, 95% CI = 0.88–19.41). More interestingly, when the combined effect of the *MMP1* and *MMP3* SNP was considered, a significant difference in the *MMP* haplotype distribution between NSCLC patients with and without lymphatic metastasis was observed ($\chi^2 = 12.41, df = 3, P = 0.006$). The frequency of the 1G/5A haplotype in patients with lymphatic metastasis was significantly higher than that in lymph node negative ones (13.3 versus 4.0%, $\chi^2 = 7.57, P = 0.006$). Thus, the *MMP1* 1G/5A haplotype significantly increased the risk of lymphatic metastasis, compared with 2G/6A, the most frequent haplotype in which the two alleles tend to be in linkage disequilibrium (OR = 3.36, 95% CI = 1.42–7.94).

### Discussion

Carcinogenesis is a multistage process, which involves various molecular events related to the fundamental alterations in cell physiology including self-support in growth signals, insensitivity to growth-inhibitory signals, escape from apoptosis, infenerative replication, sustained angiogenesis, and tissue invasion and metastasis (21). Natural variations in genes involved in the above molecular events may influence cancer development and progression. Some polymorphic genes such as vascular endothelial growth factor (22), transforming growth factor-β (23), cyclooxygenase-2 (24), cyclin D1 (25), plasminogen activator inhibitor-1 (26), xeroderma pigmentosum group A (27) and xeroderma pigmentosum group D (28), have been associated with susceptibility to chemically induced carcinomas including lung cancer.

MMPs, which can regulate tumor microenvironment, their expression and activity is increased in NSCLC tissues and may be inversely associated with metastasis and progression of this tumor (29–31). In Caucasians, the 2G allele of the *MMP1* promoter SNP has been associated with increased susceptibility to lung cancer, especially among smokers (17). However, the present study shows that, the *MMP1* SNP may not be independently associated with risk of the development and lymphatic metastasis of NSCLC in Chinese population. Although the sample size in this study may not have power to detect small effect, the very similar distribution of the *MMP1* genotypes between NSCLC patients and healthy controls indicates that the inconsistent result between the present study and the study in Caucasians is probably due to the different study ethnicities. The random recruitment of the healthy individuals, the reproducible genotyping method and the consistency with the Hardy–Weinberg equilibrium in several other polymorphic loci (18,32), suggests that the healthy individuals in the present study may reasonably be used in case-control investigations. The present study shows that the genotypes with the 5A allele of the *MMP3* promoter SNP may modify the risk of NSCLC development only in current or ex-smokers, suggesting that the *MMP3* 5A allele may interact with the metabolic changes induced by chemical carcinogens to influence susceptibility to NSCLC. However, this study did not observe a synergistic interaction between smoking and the *MMP3* 5A allele as observed by Yu et al. (33), who found an additive interaction between the *MMP2* promoter polymorphism and

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**Table III. Influence of the *MMP1* and *MMP3* SNP on lymphatic metastasis in NSCLC**

<table>
<thead>
<tr>
<th>Groups</th>
<th>LM negative n (%)</th>
<th>LM positive n (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMP1 genotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1G/1G</td>
<td>8 (10.8)</td>
<td>13 (10.6)</td>
<td>1.0 (ref.)</td>
</tr>
<tr>
<td>1G/2G</td>
<td>24 (32.4)</td>
<td>41 (33.3)</td>
<td>1.23 (0.41–3.69)</td>
</tr>
<tr>
<td>2G/2G</td>
<td>42 (56.8)</td>
<td>69 (56.1)</td>
<td>0.81 (0.28–2.27)</td>
</tr>
<tr>
<td><strong>MMP3 genotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A/6A</td>
<td>56 (77.0)</td>
<td>75 (69.1)</td>
<td>1.0 (ref.)</td>
</tr>
<tr>
<td>5A/6A</td>
<td>17 (23.0)</td>
<td>41 (25.2)</td>
<td>1.14 (0.55–2.38)</td>
</tr>
<tr>
<td>5A/5A</td>
<td>0</td>
<td>7 (5.7)</td>
<td>4.13 (0.88–19.41)</td>
</tr>
</tbody>
</table>

LM, lymphatic metastasis. The haplotype frequencies and deduced number of haplotypes were estimated by using the EH linkage software (1.2 version, Rockefeller University, NY).

*The ORs of *MMP* genotypes were adjusted by age and gender while that of haplotypes were not adjusted because the individual haplotype was unable to be deduced from subjects with double heterozygous genotypes.

The frequency was significantly higher in patients with lymphatic metastasis than lymph node negative ones (Fisher’s exact test, $P = 0.04$).

The overall distribution of the *MMP* haplotypes in NSCLC patients with and without lymphatic metastasis was significantly different ($\chi^2 = 12.41, df = 3, P = 0.006$).

The haplotype frequency in patients with positive LM was significantly higher than that in LM negative ones ($\chi^2 = 7.57, P = 0.006$).
smoking on the elevated risk of lung cancer. Therefore, the role of the MMP3 5A in smoking individuals needs to be verified by further studies with larger sample size. In addition, the dosage effect of smoking alone or in combination with the MMP3 SNP on susceptibility to NSCLC, which was not analyzed in this study because of the sample size, also needs to be further investigated.

Another interesting observation in this study is that the MMP3 5A/5A genotype is significantly more frequent in patients with lymphatic metastasis than in lymph node negative ones, although the OR for the risk of lymphatic metastasis does not reach significance, probably due to the relatively small sample size. In addition, the MMP 1G/5A haplotype has >3-fold increased risk to have lymphatic metastasis, compared with the 2G/6A haplotype, suggesting that NSCLC patients harboring the 1G/5A haplotype may need to be treated more actively in lymph node resection and need trimodality treatment after operation. Given that the combined effect of the MMP1 and MMP3 alleles on transcription and expression of MMPs has not been clarified so far, we used the 2G/6A as the reference because it is the most prevalent haplotype in the study population and the 2G and 6A alleles tend to be linked together, indicating that the 2G/6A may have biological advantage over other haplotypes. Since the result suggests that the MMP1 SNP may not independently modify the risk of metastasis, it might be speculated that the association between the 1G/5A haplotype and increased risk of lymphatic metastasis mainly due to the existence of the 5A allele. However, if this was the case, another haplotype with the 5A allele, 2G/5A, would also alter the potential of lymphatic metastasis, which was not observed in the study. Therefore, it is reasonable to postulate that the 1G/5A haplotype might be in linkage disequilibrium with the true causative allele, which is in the immediate chromosomal vicinity, to modify the risk of lymphatic metastasis in NSCLC. The attractive targets may be polymorphic alleles of other metalloproteinase genes, which are clustered in the 11q22-q23 and are found in the following order: MMP7-MMP20-MMP8-MMP10-MMP1-MMP3-MMP12-MMP13 (GeneBank accession no. NT_009151). Thus, studies on the association between the polymorphisms in the above MMP genes and development or progression of NSCLC may facilitate comprehension of the present observation. In addition, the joint effect of the MMP1 and MMP3 alleles on expression of MMP proteins and its subsequent influence on cell physiology need to be exploited by genotype and phenotype association studies, which was not investigated in the present study because of the unavailability of the tissue samples. Nevertheless, the selection bias may exist in the study because not all NSCLC patients in the hospital were recruited. A chance finding induced by the sample size may also not be excluded. Therefore, further studies with larger sample size and inclusion of all NSCLC patients in the hospital are needed to confirm our finding.

The modification of the MMP3 5A allele in the development and metastasis of NSCLC may be directly related to its 2-fold increased transcription activity (10,11). Elevated expression of MMP3 protein may also activate other MMPs such as MMP1 and MMP9, leading to local invasion and metastasis by facilitating degradation of ECM and promoting infiltration of tumor cells through the basal membrane (34). MMPs may also be involved in the early stage of tumor development by regulating cell growth, apoptosis and vessel formation, via cleaving diverse groups of substrate except for structure components of ECM, which include growth-factor-binding proteins, growth-factor precursors, receptor tyrosine kinases, cell adhesion molecules and other proteinases (1,4,5,35-37). Taken together, polymorphisms of the MMP genes may promote cancer development or progression via alteration of MMP protein expression, resulting in creation and maintenance of the microenvironment for tumor cell proliferation, migration and invasion.

In conclusion, our study suggests that the MMP3 5A allele may modify the susceptibility to NSCLC in Chinese smokers. In addition, the MMP1 and MMP3 1G/5A haplotype may be involved with increased risk of lymphatic metastasis of NSCLC.

Acknowledgements

We greatly acknowledge Mr Ming He and Mr Jifang Yao in the Fourth Affiliated Hospital of Hebei Medical University, China, for their assistance in recruiting study subjects.

References