Correlation between a Single Nucleotide Polymorphism in the Matrix Metalloproteinase-2 Promoter and Risk of Lung Cancer

Chunyuan Yu, Kaifeng Pan, Deyin Xing, Gang Liang, Wen Tan, Lian Zhang, and Dongxin Lin

Department of Etiology and Carcinogenesis, Cancer Institute, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021 [C. Y., D. X., G. L., W. T., D. L.]; and Department of Epidemiology, Peking University School of Oncology, Beijing Institute for Cancer Research, Beijing 100034 [K. P., L. Z.], China

Abstract

Matrix metalloproteinases (MMPs) play an important role in several steps of cancer development. A single nucleotide polymorphism (−1306C→T) in the MMP2 promoter sequence disrupts an Sp1 site and thus results in strikingly lower promoter activity. We examined the relationship between this polymorphism and risk for lung cancer in 781 cases and 852 age- and sex-matched controls in a Chinese population. We found that the allele frequency of MMP2-1306C was significantly higher among cases than among controls (0.91 versus 0.83). Subjects with the CC genotype had an overall 2-fold increased risk for developing lung cancer [adjusted odds ratio (OR) 2.18; 95% confidence interval (CI), 1.70–2.79] compared with those with the CT or TT genotype. The elevated risk was observed evenly among different subtypes of this cancer. Stratified analysis indicated an additive interaction between the CC genotype and smoking on the elevated risk. The ORs of lung cancer for the CC genotype, smoking, and both factors combined were 2.38 (95% CI 1.64–3.45), 4.26 (95% CI 2.57–6.84), and 7.64 (95% CI 4.74–12.33), respectively. Furthermore, when the data were stratified by the pack-years smoked, this joint effect was more evident and stronger in heavy smokers (OR 10.25, 95% CI 5.80–18.09) than in light smokers (OR 5.55, 95% CI 3.34–9.22). These results demonstrate a significant association between the MMP2 −1306C/T polymorphism and risk of developing lung cancer solely or in a manner of interaction with carcinogen exposure.

Introduction

The development of cancer is a complex, multistage process in which invading and colonizing of transformed cells to distant sites by degradation of extracellular matrix and basement membrane barriers is one of the key steps (1, 2). The matrix metalloproteinases (MMPs) constitute a family of >20 proteolytic enzymes that are capable of selectively degrading a wide spectrum of both extracellular matrix and nonmatrix proteins (3). Most MMPs are synthesized and secreted by cancer cells and by adjacent stromal cells as well. Therefore, it is generally believed that the MMPs, via breakdown of the physical barrier, play a pivotal role in tumor invasion and metastasis (4, 5). However, recent work has also suggested that, in addition to the historically considered features of promoting invasion and metastasis, MMPs also play an important role in several steps of cancer development (6–8).

MMP-2 (gelatinase A), among other MMPs, primarily hydrolyzes type IV collagen, the major structural component of basement membrane (4, 9). Numerous studies have shown that MMP-2 is expressed in various human cancer tissues and is implicated in tumor initiation, invasion, angiogenesis, and metastasis (7, 10–13). However, cancers in which a role for MMP-2 has been demonstrated are generally characterized by various individual susceptibility, implying the role of genetic factors. Recently, a functional single nucleotide polymorphism in MMP2 has been reported (14). The −1306C→T transition in the MMP2 promoter sequence disrupts an Sp1-type promoter site (CCACC box), and thus displays a strikingly lower promoter activity with the T allele in an in vitro assay system (14). It has been suggested that the −1306C/T polymorphism in the MMP2 gene may be informative in a test of association with cancer development in which a role for MMP-2 is implicated. However, this functional polymorphism has not been examined in any cancer studies to date. If this polymorphism actually causes variations in transcription and expression of MMP2 in vivo, it might affect individual susceptibility to carcinogenesis. On the basis of this hypothesis, we have examined the contribution of the −1306C/T polymorphism in the MMP2 gene to the risk of lung cancer in a large molecular epidemiological study in a Chinese population.

Materials and Methods

Study Subjects. This study included 781 patients with lung cancer and 852 healthy controls. All subjects were unrelated ethnic Chinese. Patients with lung cancer were consecutively recruited from January 1997 to November 2001, at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing, China). All cases diagnosed at the hospital during the study were recruited, yielding a 95% response rate; they were from Beijing City and surrounding regions. A portion of cases (351) were participants in a molecular epidemiological study of XPD and lung cancer described elsewhere (15). In the present study, we increased the sample size of lung cancer patients to 781. Population controls were accrued from a nutritional survey conducted in the same regions (15). Briefly, these controls were randomly selected from the nutritional survey database consisting of 2500 individuals. They had no history of cancer and were frequency matched to cases on sex and age (±5 years). At recruitment, informed consent was obtained from each subject, and each participant was then interviewed to solicit detailed information on demographic characteristics and lifetime history of tobacco use. This study was approved by the Institutional Review Board of the Chinese Academy of Medical Sciences Cancer Institute.

MMP-2 Genotyping. Genomic DNA was isolated from the peripheral blood of the controls or from surgically resected normal tissues adjacent to the tumor of lung cancer patients. PCR-based DHPLC analysis was used to determine the MMP2 genotypes. The primers used to amplify a 295-bp fragment of MMP2 promoter containing −1306 C/T site were: MMP-2F, 5′-CTG ACC CCC AGT CCT ATC TG C C; and MMP-2R, 5′-TGT TGG GAA CGC CTG ACT TCA G (14). PCR was accomplished with a 25-μL reaction mixture containing ~100 ng of DNA, 1.0 μM concentration of each primer, 0.2 mM dNTP, 2.0 mM MgCl2, 1.0 unit Taq DNA polymerase with 1 × reaction buffer (Promega, Madison, WI) and 2% DMSO. The reaction was conducted under the following conditions: an initial melting step of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 64°C, and 45 s at 72°C; and a final elongation of 7 min at 72°C. DHPLC analysis was performed on a Transgenomic WAVE System (Transgenomic Inc.) identical with that described previously (16). Briefly, each PCR

Received 8/8/02; accepted 10/2/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by Grants 39825122 and 39990370 from the National Natural Science Foundation and Grant G1998051304 from the State Key Basic Research Program.

2 To whom requests for reprints should be addressed, at Department of Etiology and Carcinogenesis, Cancer Institute, Chinese Academy of Medical Sciences, Beijing 100021 [C. Y., D. X., G. L., W. T., D. L.].

3 The abbreviations used are: MMPs, matrix metalloproteinases; DHPLC, denaturing high performance liquid chromatography; OR, odds ratio; CI, confidence interval; IGF, insulin-like growth factor.
product was applied to the DHPLC column, denatured for 1 min at 94°C, and then gradually reannealed by decreasing sample temperature from 94°C to 45°C over a period of 30 min to form homo- and/or heteroduplexes. PCR products were eluted with a linear acetonitrile gradient at a flow rate of 0.9 ml/min. The genotypes of MMP2 –1306 C/T revealed by DHPLC analysis were further confirmed by DNA sequencing. Three different allele PCR products were directly analyzed with an ABI PRISM 377 automatic sequencer using a dye terminator sequencing kit. Sequences were compared with a published MMP2 promoter sequence (17).

To ensure quality control, genotyping was performed with blinding to case/control status; a 15% masked, random sample of cases and controls was tested twice by different persons; and the results were concordant for all masked duplicate sets.

Statistical Analysis. The association between the MMP2 polymorphism and risk of lung cancer was estimated by ORs and their 95% CIs, which were calculated by unconditional logistic regression models. Smokers were considered current smokers if they smoked up to 1 year before the date of diagnosis, or up to the date of the interview for controls. Information was collected on the amount of cigarettes smoked per day, the age at which the subjects started smoking, and the age at which ex-smokers stopped smoking. Light or heavy smokers were categorized by the approximate 50th percentile pack-year value among controls, i.e., <27 or ≥27 pack-years [(cigarettes per day / 20) × (years smoked)]. The ORs were adjusted for age, sex, and smoking status or pack-years smoked. We tested the null hypotheses of additivity and multiplicativity and evaluated the departures from additive and multiplicative interaction models (18). A more than additive interaction was indicated when OR$_{11}$ > OR$_{01}$ + OR$_{00}$ = 1, where OR$_{11}$ = OR when both factors are present, OR$_{01}$ = OR when only factor 1 is present, and OR$_{00}$ = OR when only factor 2 is present. A less than multiplicative interaction was suggested when OR$_{11}$ > OR$_{01}$ × OR$_{00}$. The departures from these additive and multiplicative models were assessed by including main effect variables and their product terms in the logistic regression model. All analysis was conducted with Statistical Analysis System software (version 6.12; SAS Institute, Cary, NC).

Results

The distributions of age, sex, and smoking status among the study subjects are summarized in Table 1. No statistical differences were observed between cases and controls in the distributions of age and sex, suggesting that the frequency matching was adequate. However, observed between cases and controls in the distributions of age and smoking status (Table 1). No statistical differences were found among histological types of lung cancer (95% CI, 1.64–3.45). However, among smokers, subjects carrying the CC genotype had an OR of 7.64 (95% CI, 4.74–12.33) that was significantly different from that for those carrying CT or TT genotype (OR 4.26; 95% CI 2.57–8.44; P = 0.0001, test for homogeneity). Because the OR for the presence of both smoking and CC genotype were then compared among different subtypes of lung cancer, i.e., squamous cell carcinoma, adenocarcinoma, and other histological types of lung cancer. No significant difference in terms of risk related to the CC genotype was found among histological types of lung cancer (P = 0.33, test for homogeneity) and the elevated risk was consistently observed in all subtypes of the cancer (Table 2).

The risk of lung cancer related to MMP2 polymorphism was further examined with stratification by smoking status and pack-years smoked (Table 3). It was found that among nonsmokers, the adjusted OR of lung cancer for subjects carrying the CC genotype was 2.38 (95% CI, 1.64–3.45). However, among smokers, subjects carrying the CC genotype had an OR of 7.64 (95% CI, 4.74–12.33) that was significantly different from that for those carrying CT or TT genotype (OR 4.26; 95% CI 2.57–8.44; P = 0.0001, test for homogeneity). Because the OR for the presence of both smoking and CC genotype was greater than the sum of OR for smoking and OR for the genotype, these data suggested an additive interaction between smoking and the MMP2 CC genotype. When the ORs related to the MMP2 polymorphism were further evaluated within strata of 0, 1–26, and ≥27 pack-years smoked, a significant interaction between the susceptible genotype and pack-years smoked existed in a clear dose-response manner; the ORs were 1.00, 2.23, and 5.32, respectively, for the TT or CT genotype but 2.38, 5.55, and 10.25, respectively, for the CC genotype (trend test, P < 0.0001).

Discussion

In the present study, we have examined the relationship between the –1306C→T polymorphism in the promoter of MMP2, a gene that plays a role in several steps of cancer development, and risk of lung cancer in a Chinese population. We found that MMP2 –1306CC genotype was overrepresented among patients with lung cancer compared with cancer-free controls. Subjects carrying the CC genotype had a ≥2-fold increased risk for this disease. In addition, we observed a possible additive interaction between this genetic polymorphism and tobacco smoking on risk of lung cancer, with the OR being 10.25 among...
heavily smokers who had the CC genotype. To our best knowledge, this is the first study to investigate the impact of the MMP2 polymorphism on susceptibility to cancer and provides a substantial evidence, for the first time, that MMP2 may play an important role in the development of lung cancer.

Although the design of hospital-based case-control study has potential drawbacks such as selection bias, the results in this study, which had large sample size and included >90% of the eligible cases, solid and reproducible genotyping procedures, and significantly increased ORs with very small P values, are unlikely to be attributable to selection bias. The fact that genotype frequencies among the control population fit the Hardy-Weinberg law further supports the randomness of our control selection. Moreover, the observed effect of MMP2 polymorphism was not fluctuated by other potential predictive factors of lung cancer such as age, sex, and smoking. Hence, our results are unlikely to be biased by subject selection or unknown confounding factors.

These molecular epidemiological results are consistent with the previous findings showing that the C→T transition at -1306, which disrupts an Sp1-type promoter site (CCACC box) and results in a strikingly lower promoter activity with the T allele of the MMP2 gene (14). The Sp1 site, among other promoter elements such as AP-2, has been shown to be necessary for regulating constitutive expression of MMP-2 (19). Therefore, the presence of the Sp1 promoter site in the MMP2-1306C allele may enhance transcription, which has in fact been demonstrated in vitro in transient transfection experiments (14), so MMP-2 protein expression would be higher in individuals who carry the CC genotype than those who carry the TT or CT genotype. Because MMP-2 and other forms of MMPs may contribute in multiple ways to all stages of carcinogenesis (8), the increased level of this enzyme over a lifetime may render the hosts and their target tissues at increased susceptibility to cancer development. This postulation is strongly supported by experimental cancer models. It has been shown that when induced by carcinogenic stimulus, wild-type mice developed more cancers than mice that lack the Mmp2, -7, -9, or -11 gene (20–22), and the development of squamous cell carcinomas in mice that lack Mmp9, another form of gelatinase, could be restored by transplanting Mmp9-expressing bone marrow cells (21). In another experiment, cancer cells injected via vein were found to be more capable of colonizing the lungs of wild-type mice than the lungs of

Table 2  MMP2 genotypes in cases and controls and their association with risk of lung cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Overall cases</th>
<th>Cases with SCC a</th>
<th>Cases with AC</th>
<th>Cases with other b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>CC</td>
<td>585 68.7</td>
<td>644 82.5</td>
<td>272 84.5</td>
<td>176 82.6</td>
<td>196 79.7</td>
</tr>
<tr>
<td>CT</td>
<td>248 29.1</td>
<td>127 16.3</td>
<td>48 14.9</td>
<td>34 16.0</td>
<td>45 18.3</td>
</tr>
<tr>
<td>TT</td>
<td>19 2.2</td>
<td>10 1.2</td>
<td>2 0.6</td>
<td>3 1.4</td>
<td>5 2.0</td>
</tr>
<tr>
<td>C allele frequency</td>
<td>0.83</td>
<td>0.91</td>
<td>0.92</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>Crude OR (95% CI)</td>
<td>2.15 (1.70–2.71)</td>
<td>2.48 (1.78–3.47)</td>
<td>2.17 (1.48–3.18)</td>
<td>1.79 (1.27–2.52)</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)</td>
<td>2.18 (1.70–2.79)</td>
<td>2.54 (1.75–3.68)</td>
<td>2.14 (1.45–3.15)</td>
<td>1.73 (1.22–2.45)</td>
<td></td>
</tr>
</tbody>
</table>

a SCC, squamous cell carcinoma; AC, adenocarcinoma.
b Includes undifferentiated cancer (n = 94), bronchioalveolar carcinoma (n = 87), and mixed cell carcinoma (n = 65).

Table 3  Risk of lung cancer related to MMP2 genotypes by smoking

<table>
<thead>
<tr>
<th>MMP2 genotype</th>
<th>Smoking status</th>
<th>CT + TT a</th>
<th>OR (95% CI) b</th>
<th>CC a</th>
<th>OR (95% CI) b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonsmokers</td>
<td>47/157</td>
<td>1.00</td>
<td>224/309</td>
<td>2.38 (1.64–3.45)</td>
</tr>
<tr>
<td></td>
<td>Smokers</td>
<td>90/110</td>
<td>4.26 (2.57–8.44)</td>
<td>420/276</td>
<td>7.64 (4.74–12.33)</td>
</tr>
<tr>
<td></td>
<td>&lt;27 pack-years</td>
<td>22/55</td>
<td>2.23 (1.06–4.66)</td>
<td>155/141</td>
<td>5.55 (3.34–9.22)</td>
</tr>
<tr>
<td></td>
<td>≥27 pack-years</td>
<td>6/57</td>
<td>5.32 (3.16–11.11)</td>
<td>265/135</td>
<td>10.25 (5.80–18.09)</td>
</tr>
</tbody>
</table>

a Numbers of cases/numbers of controls.
b ORs and 95% CIs were calculated by logistic regression, with the CT or TT genotype as the reference group and adjusted for age and sex.

Table 2: MMP2 genotypes in cases and controls and their association with risk of lung cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Overall cases</th>
<th>Cases with SCC a</th>
<th>Cases with AC</th>
<th>Cases with other b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>CC</td>
<td>585 68.7</td>
<td>644 82.5</td>
<td>272 84.5</td>
<td>176 82.6</td>
<td>196 79.7</td>
</tr>
<tr>
<td>CT</td>
<td>248 29.1</td>
<td>127 16.3</td>
<td>48 14.9</td>
<td>34 16.0</td>
<td>45 18.3</td>
</tr>
<tr>
<td>TT</td>
<td>19 2.2</td>
<td>10 1.2</td>
<td>2 0.6</td>
<td>3 1.4</td>
<td>5 2.0</td>
</tr>
<tr>
<td>C allele frequency</td>
<td>0.83</td>
<td>0.91</td>
<td>0.92</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>Crude OR (95% CI)</td>
<td>2.15 (1.70–2.71)</td>
<td>2.48 (1.78–3.47)</td>
<td>2.17 (1.48–3.18)</td>
<td>1.79 (1.27–2.52)</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)</td>
<td>2.18 (1.70–2.79)</td>
<td>2.54 (1.75–3.68)</td>
<td>2.14 (1.45–3.15)</td>
<td>1.73 (1.22–2.45)</td>
<td></td>
</tr>
</tbody>
</table>

a SCC, squamous cell carcinoma; AC, adenocarcinoma.
b Includes undifferentiated cancer (n = 94), bronchioalveolar carcinoma (n = 87), and mixed cell carcinoma (n = 65).

Table 3: Risk of lung cancer related to MMP2 genotypes by smoking

<table>
<thead>
<tr>
<th>MMP2 genotype</th>
<th>Smoking status</th>
<th>CT + TT a</th>
<th>OR (95% CI) b</th>
<th>CC a</th>
<th>OR (95% CI) b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonsmokers</td>
<td>47/157</td>
<td>1.00</td>
<td>224/309</td>
<td>2.38 (1.64–3.45)</td>
</tr>
<tr>
<td></td>
<td>Smokers</td>
<td>90/110</td>
<td>4.26 (2.57–8.44)</td>
<td>420/276</td>
<td>7.64 (4.74–12.33)</td>
</tr>
<tr>
<td></td>
<td>&lt;27 pack-years</td>
<td>22/55</td>
<td>2.23 (1.06–4.66)</td>
<td>155/141</td>
<td>5.55 (3.34–9.22)</td>
</tr>
<tr>
<td></td>
<td>≥27 pack-years</td>
<td>6/57</td>
<td>5.32 (3.16–11.11)</td>
<td>265/135</td>
<td>10.25 (5.80–18.09)</td>
</tr>
</tbody>
</table>

a Numbers of cases/numbers of controls.
b ORs and 95% CIs were calculated by logistic regression, with the CT or TT genotype as the reference group and adjusted for age and sex.

Fig. 1. Representative DHPLC profiles for different allelic PCR products containing the MMP2-1306C/T polymorphism site and DNA sequencing analysis. At the first DHPLC, the CT genotype was discriminated from the homozygous genotypes (a). To determine the CC or TT genotype, the second DHPLC was run for the homozygous DNA mixed with a DNA sample known as CC genotype, by which the CC and TT genotypes were readily discerned (b). The PCR products with different DHPLC profiles were sequenced to confirm the mutation (c). The conditions for DHPLC and DNA sequencing analysis are described in “Materials and Methods.”
Mmp2-deficient mice (12). It is also documented that overexpression of MMPs in transgenic mice results in elevated cancer susceptibility (23, 24).

The involvement of MMPs in carcinogenesis is biologically plausible because they can alter the cellular microenvironment and consequently affect the process of neoplastic transformation and cancer development. It has been shown that by cleaving IGF-binding proteins, MMPs can release IGFs (25), IGFs such as IGF-1 are well known to have a strong effect on stimulating cell proliferation and inhibiting apoptosis. High levels of circulating IGF-1 and low levels of IGF-binding protein 3 are associated with increased risk of several common cancers, including lung cancer (26). MMPs may also release the cell membrane-bound precursor of transforming growth factor α (27), another important growth factor involved in neoplastic transformation and cancer development. Moreover, MMPs are also involved in cleavage of a number of molecules on the cell surface, which may alter cell cycle checkpoint controls and conceivably promote genomic instability by affecting cell adhesion (28), may disrupt cell signaling, and may foster cancer cells to escape immunosurveillance (8). Taken together, these data provide very plausible molecular mechanisms through which the genetic polymorphism resulting in high expression of MMP-2 over a lifetime could increase cancer risk.

In our study, we found that the MMP2-1306CC genotype was significantly associated with lung cancer risk in nonsmokers; however, the risk rose markedly in smokers, particularly heavy smokers, suggesting an additive interaction between the MMP2 polymorphism and smoking. Several possibilities exist to explain these findings. Because MMPs expression can be induced by smoking (29, 30), one hypothesis is that, in addition to higher constitutive expression because of gain of an Sp1 promoter site, the inducibility by smoking of the C allele of MMP2 may also be higher than that of the T allele, which loses an Sp1 site. Given these conditions, it would be expected that subjects who smoked and carried the CC genotype were more susceptible to developing lung cancer. Alternatively, a higher risk of lung cancer among heavy smokers with the C allele polymorphism to lung cancer among heavy smokers with the CC genotype may attribute to the occurrence of larger numbers of transformed cells caused by smoking in the target tissue, which, in turn, increases the possibility that one of these cells will become malignant under the condition of higher expression of MMP2. Another interesting finding in our present study was that the increased risk related to the MMP2 polymorphism was evenly observed in different subtypes of lung cancers, i.e., squamous cell carcinoma, adenocarcinoma, and other histological types of lung cancer. This result suggests that the MMP2 polymorphism might be a general, but not a specific, risk factor for common cancers, further supporting the likelihood that MMPs profoundly influence early tumor initiation and development.

In summary, this study demonstrated a significant association between the MMP2 −1306CT polymorphism and the risk of developing lung cancer solely or in a manner of interaction with tobacco smoking in a Chinese population. Because this is the first report demonstrating the contribution of the MMP2 polymorphism to lung cancer risk and because MMP2 is expressed in many types of cancer and normal stromal cells, additional studies on lung cancer and other types of common cancers would be warranted. Moreover, the possible role of the MMP2 −1306CT polymorphism in cancer invasiveness and metastasis should also be addressed.

References

Correlation between a Single Nucleotide Polymorphism in the Matrix Metalloproteinase-2 Promoter and Risk of Lung Cancer

Chunyuan Yu, Kaifeng Pan, Deyin Xing, et al.


**Updated version**
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/22/6430

**Cited articles**
This article cites 29 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/22/6430.full.html#ref-list-1

**Citing articles**
This article has been cited by 21 HighWire-hosted articles. Access the articles at:
/content/62/22/6430.full.html#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.