Prognostic Significance of MMP-1 and MMP-3 Functional Promoter Polymorphisms in Colorectal Cancer

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ABSTRACT

Purpose: Matrix metalloproteinase (MMP) belongs to a large group of proteases capable of breaking essentially all components of the extracellular matrix. They are implicated in all steps of tumorogenesis, cancer invasion, and metastasis. Among them, metalloproteinase type 1 (MMP-1) is implicated in tumor invasion and metastasis in different types of cancers including colorectal cancer in which its expression was correlated with poor prognosis. A polymorphism in the promoter region of the MMP-1 gene leads to a variation of its level of transcription.

Study Design: MMP-1-1607ins/delG and MMP-3-1612 ins/delA promoter polymorphisms were genotyped by multiplex PCR from 201 colorectal cancer patients. The median follow-up of patients was 30 months. The MMP genotypes were correlated to clinical outcome.

Results: Patients with the -1607insG/-1607insG MMP-1 genotype had significantly worse specific survival than the others in the whole series (P < 0.04), in stage I to III patients (P < 0.001), and in patients stage I and II (P < 0.01). In multivariate analysis, MMP-1-1607insG allele showed to be an independent poor prognostic factor after adjustment on stage, age, and the use of adjuvant chemotherapy. MMP-3 polymorphism was not associated with survival.

Conclusions: In the subgroups of nondistant metastatic patients (stages I and II, and stages I-III), an inverse relation between the number of MMP-1-1607insG allele and survival was observed suggesting a gene dosage effect. Our results are consistent with the importance of MMP-1 expression and cancer invasion, metastasis, and prognosis.

INTRODUCTION

Matrix metalloproteinase (MMP) belongs to a large group of proteases, which includes over 22 known human zinc-dependent proteolytic enzymes. These are capable of breaking essentially all components of the extracellular matrix (1, 2). MMPs take part in high tissue turnover and remodeling, both physiologic and pathologic conditions such as cancer. MMPs are also implicated in all steps of tumorogenesis, cancer invasion, and metastasis (3). Tumor invasion and metastasis formation always begin with blood and lymphatic vessel infiltration. As these processes involve proteolysis of the extracellular matrix, MMPs are suggested to play a major role in tumor progression.

Colorectal cancer is the most common malignancy of the gastrointestinal tract. It is the third cause of cancer overall and the second leading cause of cancer-related death in the Europe and the United States with an incidence of 300,000 new cases (4). It still carries a relatively poor prognosis despite recent improvements in early diagnosis, surgical techniques, and chemotherapy. About 40% of the patients who undergo operative resections will die within 5 years due to recurrent disease or metastases. MMPs are essential for tumor cells to penetrate the basement membrane and colonize distant sites. Their role was shown in colorectal cancer (5, 6). Among MMPs, MMP-1 is the most ubiquitously expressed interstitial collagenase. Overexpression of MMP-1 was implicated in tumor invasion and metastasis in different types of cancers including colorectal cancer (7) and its expression in colorectal cancer tumor cells was correlated with poor prognosis (8). Rutter et al. (9) reported in melanoma a functional single nucleotide polymorphism 1607del/insG in the MMP-1 promoter with a guanine (G) insertion. It creates an Ets binding site, 5-GGA-3, which increases the promoter activity. Thus, the MMP-1-1607insG allele has significantly higher transcriptional activity than the MMP-1-1607delG allele. An association between the presence of MMP-1-1607insG allele in the MMP-1 promoter and cancer susceptibility was described in ovarian (10) endometrial (11), and lung (12) cancers. Furthermore in melanoma (13), cervical cancer (14), and colorectal cancer (15), the presence of MMP1-1607insG allele was significantly associated with stage or invasiveness of the tumor.

MMP-3 is another important MMP interacting with tumor microenvironment. Its activities are complementary to MMP-1. It is located on the cluster gene 11q22, close to MMP-1 gene. A MMP-3-1612del/insA polymorphism in the promoter of the MMP-3 gene was described (16). In vitro analysis showed that the MMP-3-1612A allele is associated with low transcription levels (17). In a case-control study, the frequency of the MMP-3-1612A allele was higher in the breast cancer group than in controls and was more prevalent in the metastatic group than in the nonmetastatic one (18) suggesting a link between the presence of the MMP-3-1612A allele with breast cancer susceptibility and prognosis. In colorectal cancer case-control studies, discrepant results were observed concerning the association between MMP-3 polymorphism and cancer risk (19).

It is noteworthy that the haplotypes MMP-1-1607insG/MMP-3-1612insA and MMP-1-1607delG/MMP-3-1612delA are more frequently found than expected (20, 21) leading to a...
preferred association of the higher and the lower transcriptional activity alleles for MMP-1 and MMP-3 genes, respectively.

To evaluate the role of the single nucleotide promoter functional polymorphisms of MMP-1 and MMP-3 genes on patient survival in colorectal cancer, we genotyped them in 201 colorectal cancer patients. We found that MMP-1-1607insG allele was associated with a worse prognosis independently from tumor stage.

MATERIALS AND METHODS

Between April 1995 and May 2002, among the consecutive patients with colorectal cancer referred to the department of General and Oncological surgery of Georges Pompidou Hospital (tertiary care hospital), 357 newly diagnosed patients, without preoperative radiotherapy or previous cancer history were eligible. Among them, an informed consent was obtained from the 201 patients enrolled in this study. There were 96 males and 105 females, sex ratio was 1.1, and mean age 69 ± 11 years (range, 35-95 years). The median follow-up was 30 months (range, 3-87 months). Tumor locations were proximal in 63 cases (cephalad to the splenic flexure), distal colon in 98 cases, and rectal in 40 cases. According to the tumor-node-metastasis classification, 22, 63, 52, and 64 patients were, respectively, classified stage I, stage II, stage III, and stage IV. Chemotherapy regimens based on 5-fluorouracil were administrated in 48 patients in stages III and IV, depending on age and physical conditions. Seventy other patients received palliative chemotherapy for recurrence. Samples from tumor and nontumor colorectal tissues were harvested during surgery. Normal tissues were collected at least 5 cm distant from tumor. Samples were stored at −80°C until DNA extraction was done using the Qiamp tissue extraction kit (Qiagen, Courtaboeuf, France).

MMP-1 and MMP-3 Genotyping

Multiplex PCR was done on tumor tissues allowing coamplification of MMP-1 and MMP-3 fragments using the following primers: 5′6FAM-CCCTCTTGAACCTCA-CATGTTATG-3′ and 5′-ACTTTCCTCCTCTATTGAGTTC3′ for MMP-1 and 5′-TCTCATATCATGTTTGACAA-3′ and 5′6FAM-CGGCACCCTGGCTAAAACAC-3′ for MMP-3 as previously described (22). Briefly, 40 ng of genomic DNA were amplified by 0.5 units of HotStar Taq polymerase (Qiagen, Les Ulis, France), 200 nmol/L deoxynucleoside triphosphates, 2 mmol/L MgCl₂, and 300 nmol/L of each primer on a GeneAmp PCR system 9700 (Applied Biosystems, Courtaboeuf, France). Fragments were separated after dilution on an ABI 310 genetic analyzer (Applied Biosystems).

To rule out classification errors from our MMP-1 and MMP-3 genotyping methods, first we genotyped MMP-1 on a subset of 20 controls with another published method (12); We reproduce individually the same genotyping results. Second, we validated our MMP-3 genotyping method by characterizing the 23 CEPH polymorphisms that was observed in previous published studies (21). The number of MMP-1 heterozygous patients was less than expected. Thereby, we were not able to calculate the linkage disequilibrium between MMP-1 and MMP-3 polymorphisms that was observed in previous published studies (21, 22).

RESULTS

Among the 201 patients included in this series, DNA extraction failed in 13 patients, and genotyping could not be achieved in 7 and 10 patients for MMP-1 and MMP-3 polymorphism, respectively. Histologic and clinical data from the patients are given according to MMP-1 and MMP-3 genotype in Table 1. There were no significant differences in the frequency of MMP-1 and MMP-3 genotypes according to gender, age, tumor location, and stage (Table 1).

MMP-1 and MMP-3 Genotypes

MMP-1-1607delG and MMP-1-1607insG allele frequencies were 0.48 and 0.52, respectively, and for MMP-3-1612delA and MMP-3-1612insA allele frequencies were 0.44 and 0.56, respectively. Genotype distribution of MMP-3 was consistent with Hardy-Weinberg equilibrium, whereas genotype distribution of MMP-1 was not (P < 0.002). The number of MMP-1 heterozygous patients was less than expected. Thereby, we were not able to calculate the linkage disequilibrium between MMP-1 and MMP-3 polymorphisms that was observed in previous published studies (21, 22).

Survival Analysis

In a first attempt, we considered the whole series including patients with metastasis (n = 190). The median survival for homozygous MMP-1-1607delG patients was not reached, the median survivals were 76 months and 42 months.
for heterozygous and homozygous MMP-1-1607insG patients, respectively (Fig. 1A, P < 0.09 log-rank test). A significant difference for specific survival between MMP-1-1607delG homozygous patients, heterozygous patients and MMP-1-1607insG homozygous was observed (Fig. 1B, P < 0.05 log-rank test).

In a second analysis, we excluded stage IV patients. In these remaining patients (n = 132), significant differences were observed between MMP-1-1607delG homozygous patients, heterozygous patients and MMP-1-1607insG homozygous for overall survival (P = 0.007 log-rank test; Fig. 2A) as well as for specific survival (P < 0.001, log-rank test; Fig. 2B) and for disease-free survival (P = 0.005, log-rank test; Fig. 2C).

A multivariate analysis was done. We found that MMP-1 genotype was an independent prognostic factor of overall survival (Table 2) after adjustment on stage age and the use of adjuvant chemotherapy.

In a third analysis, we only included patients with stage I and II tumors (n = 81) in which the use adjuvant chemotherapy is matter of debate. Significant differences were observed among MMP-1-1607delG homozygous patients, MMP-1-1607delG heterozygous patients, and MMP-1-1607insG homozygous patients for overall survival (P = 0.007 log-rank test; Fig. 3A) as well as for specific survival (P < 0.01, log-rank test; Fig. 3B) and for disease-free survival (P < 0.03, log-rank test; Fig. 3C). The MMP-1 genotype was found to be an independent prognostic factor of survival after adjustment on age, stage, and the use of chemotherapy. Because no death occurred during the observations period time for MMP-1-1607delG homozygous patients taken as reference group, we were not able to calculate the odd ratio of the different genotypes.

Similar analyses were done for MMP-3 genotypes. No significant differences were observed in overall, specific, or disease-free survival according to the different MMP-3 genotypes.

**DISCUSSION**

We investigated the influence of MMP-1 and MMP-3 promoter polymorphisms known to modulate MMP-1 and MMP-3 expression level (23), as prognostic factors in a series of 201 colorectal cancer patients. The frequencies of the different MMP-1 and MMP-3 alleles were consistent with those observed by others in Caucasian populations (12, 19). However, the

![Fig. 1 A. overall survival for MMP-1 genotypes; P < 0.09, log-rank test. B. specific survival for MMP-1 genotypes; P < 0.05, log-rank test.](image-url)
distribution of MMP-1 genotypes in our series was not consistent with the Hardy-Weinberg equilibrium. This departure from the Hardy-Weinberg equilibrium either could be due to the association of the MMP-1 gene polymorphism with colorectal cancer (21) or could indicate the existence of stratification factors in our series of colorectal cancer patients. When we studied the MMP-1 genotypes distribution according to tumor stage, the distribution was consistent with the Hardy-Weinberg equilibrium.

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**Fig. 2**  
A. Overall survival of nonmetastatic patients for MMP-1 genotypes; \( P = 0.007 \), log-rank test.  
B. Specific survival of nonmetastatic patients for MMP-1 genotypes; \( P < 0.001 \), log-rank test.  
C. Disease-free survival of nonmetastatic patients for MMP-1 genotypes; \( P = 0.005 \), log-rank test.

**Fig. 3**  
A. Overall survival of stage I and stage II patients for MMP-1 genotypes; \( P < 0.02 \), log-rank test.  
B. Specific survival of stage I and stage II patients for MMP-1 genotypes; \( P < 0.01 \), log-rank test.  
C. Disease-free survival of stage I and stage II patients for MMP-1 genotypes; \( P < 0.03 \), log-rank test.
These results were confirmed in a multivariate analysis in which a significant inverse relation was observed between the number of MMP-1-1607delG allele and survival among colorectal cancer patients suggesting a gene dosage effect. This inverse relation was observed for overall, specific, and disease-free survivals. In 1996, Murray et al. (8) found that the MMP-1 protein expression in colorectal cancer cell was associated with a poor prognosis. This prognostic factor was independent of tumor-node-metastasis classification. Moreover, in 2001 Ghilardi et al. (15) found that the MMP-1 protein expression and cancer invasion, metastasis and prognosis described by others (13–15). Furthermore, it is interesting to underline that the genotype analysis was achieved in non-tumor tissues suggesting that this prognostic factor could be determine before tumor staging, independently of the tumor characteristics themselves.

One of the most important issues of colon cancer patient management is the determination of prognostic factors in early stages of the disease in order to determine which patients are at risk of recurrence or poor prognosis and then would benefit from a chemotherapy regimen. When we focused on stages I and II, patients bearing the MMP-1-1607delG allelic distribution did not allow us to infer MMP-1 and MMP-3 haplotype frequencies. Thus, we separately investigated the role of each genotype on patient survival.

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### Table 2: Survival analyses according to MMP-1 and MMP-3 genotypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Categories</th>
<th>n</th>
<th>Events</th>
<th>Time at risk</th>
<th>Crude hazard ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
<th>Adjusted hazard ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
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<td>MMP-1</td>
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<tr>
<td></td>
<td>-1607delG/-1607delG</td>
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<td>1691</td>
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<td></td>
<td></td>
<td></td>
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<td>-1607delG/-1607insG</td>
<td>49</td>
<td>13</td>
<td>1840</td>
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<td>3</td>
<td>1691</td>
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<td>1595</td>
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<td>1.69-20.38</td>
<td>0.005</td>
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<td>8.52</td>
<td>2.38-30.6</td>
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<td></td>
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<tr>
<td></td>
<td>-1607delG/-1607insG</td>
<td>49</td>
<td>6</td>
<td>1713</td>
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<td>1.25</td>
<td>0.37-4.22</td>
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<td>46</td>
<td>16</td>
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<td>1.26-9.48</td>
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<tr>
<td>MMP-3</td>
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<td>4</td>
<td>1071</td>
<td>1</td>
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<tr>
<td></td>
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<td>1993</td>
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<td>0.77-6.9</td>
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<td>44</td>
<td>12</td>
<td>1550</td>
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<td>0.68-6.7</td>
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<td>0.74-7.5</td>
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<td>Cancer-specific survival</td>
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<td>1071</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>-1612delA/-1612insA</td>
<td>54</td>
<td>10</td>
<td>1993</td>
<td>1.67</td>
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<td>0.44</td>
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<td>-1612insA/-1612insA</td>
<td>44</td>
<td>8</td>
<td>1550</td>
<td>2</td>
<td>0.52-7.6</td>
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<td>0.41-3.47</td>
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<td>1.07</td>
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NOTE. n, no. patients; events, no. events.
Adjusted hazard ratio on age, use of adjuvant chemotherapy, and stage.

MMP-1 genotype was found to be an independent prognostic factor of overall survival after adjustment on stage, age, and the use of adjuvant chemotherapy (Table 2).

Equilibrium in stages III and IV, whereas it was not in stages I and II, suggesting an association of one allele with prognosis. The MMP-1-1607insG and MMP-3-1612insA alleles and the MMP-1-1607delG and MMP-3-1612delA alleles where found in a linkage disequilibrium in several studies, the D’ coefficient varies from 0.50 to 0.92 according to the ethnic origin of the patients (21, 22). Our first intention was to investigate the MMP-1 and MMP-3 haplotypic effects on colorectal cancer patients. However, the absence of Hardy-Weinberg equilibrium for MMP-1 genotype distribution did not allow us to infer MMP-1 and MMP-3 haplotype frequencies. Thus, we separately investigated the role of each genotype on patient survival.

In 1996, Murray et al. (8) found that the MMP-1 protein expression in colorectal cancer cell was associated with a poor prognosis. This prognostic factor was independent of tumor-node-metastasis classification. Moreover, in 2001 Ghilardi et al. (15) found that the MMP-1-1607insG promoter allele may favor the tumor growth and the metastatic spread in colorectal cancer patients and then could be a factor of worse prognosis. In our study, considering the whole series, we found a significantly worse cancer-specific survival in the MMP-1-1607insG homozygous patients group as compared with both heterozygous and MMP-1-1607delG homozygous patients groups. When we excluded metastatic patients at the time of diagnosis, a significant inverse relation was observed between the number of MMP-1-1607insG allele and survival among colorectal cancer patients suggesting a gene dosage effect. This inverse relation was observed for overall, specific, and disease-free survivals. These results were confirmed in a multivariate analysis in which
REFERENCES


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