A Single Nucleotide Polymorphism in the Matrix Metalloproteinase-3 Promoter Enhances Breast Cancer Susceptibility

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ABSTRACT

Purpose: Matrix metalloproteinases (MMPs) are likely to be involved in invasion and metastasis of several tumors by degrading the extracellular matrix. A single adenine insertion/deletion polymorphism (5A/6A) in the MMP-3 promoter region causes the elevation of transcriptional level and local expression of MMP-3. The aim of this pilot study was to evaluate the impact of this 5A/6A polymorphism on susceptibility and metastasis in breast cancer.

Experimental Design: Genotyping for 5A/6A polymorphism was performed in 86 Italian women operated on for breast cancer and followed for 6–30 months (median follow-up, 21 months). A control population of 110 Italian age-matched tumor-free women was also genotyped for the same polymorphism. The 1G/2G gene promoter polymorphism for MMP-1 was additionally tested.

Results: The frequency of 5A allele was higher in the breast cancer group than in controls (P = 0.035; odds ratio, 1.53; 95% confidence interval, 1.02–2.29). The breast cancer group was divided into a group without metastasis (M–) and a group that had developed metastasis (M+). At the time of diagnosis, the 5A allele was more prevalent in the M+ group than in controls (P = 0.010; odds ratio, 1.96; 95% confidence interval, 1.16–3.30). The difference between M– patients and controls did not reach statistical significance (P = 0.37). This study was not able to demonstrate any statistical differences with respect to 1G/2G polymorphism between controls and cases and between M+ and M– subgroups.

Conclusions: Although this should be considered only as a pilot study, our results suggest that the presence of 5A polymorphism at the MMP-3 promoter region may represent an unfavorable prognostic feature in breast cancer patients associated with more invasive disease.

INTRODUCTION

Degradation of ECM2 is essential in many physiological processes, e.g., during development, growth, and repair of tissue. Tumor invasion, metastasis, and angiogenesis require controlled degradation of ECM. Increased expression of MMPs is associated with invasion and metastasis of different malignancies (1, 2). The proposed role of MMPs in tumor invasion is based mainly on the observation of high-level expression of distinct MMPs in invasive malignant tumors (3, 4); however, evidence for the activity of distinct MMPs in tumor tissues in vivo is limited. The expression of MMPs in tumor is regulated in a paracrine manner by growth factors and cytokines secreted by tumor-infiltrating inflammatory cells as well as by tumor or stromal cells; recent studies have suggested continuous cross-talk between tumor cells, stromal cells, and inflammatory cells during the invasion process (1, 2, 5, 6). Expression of most MMPs is normally low in tissues and is induced when remodeling of the ECM is required. MMP gene expression is regulated primarily at the transcriptional level, but there is also evidence of modulation of mRNA stability in response to growth factor and cytokines (2, 7). The promoter region of inducible MMP genes (i.e., MMP-1 and MMP-3) shows remarkable conservation of regulatory elements, and their expression of MMP-1 and MMP-3 is induced by growth factors, cytokines, and other environmental factors such as contact with the ECM (8–10).

Recently, a naturally occurring sequence variation in the human MMP-1 gene promoter was reported (11). This genetic variation arises from insertion or deletion of a guanine nucleotide (G) at position –1607 relative to the transcriptional start site; consequently, one allele (insertion) has two Gs (2G), whereas the other allele (deletion) has only one G at this position (1G). The insertion creates the core sequence (5′-GGA-3′) of a binding site for the Ets transcription factors, and it was demonstrated in vitro that the 2G allele had a higher transcriptional activity (11). Several studies have demonstrated the correlation between the 2G allele and a number of malignant tumors with different histogenetic origin (12–16). To our knowledge, this is the first report addressing this issue in breast cancer.

In mice, Sternlicht et al. (17) demonstrated that expression of active stromelysin-1 (MMP-3) in normal mammary gland epithelium is able to induce development of invasive mesenchymal-like tumors. Recently, a common variant in the promoter of the MMP-3 gene has been described (18). In vitro assays of promoter activity showed that the 5A allele had 2-fold higher promoter activity than the 6A allele (19). Our preliminary re-

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2 The abbreviations used are: ECM, extracellular matrix; MMP, matrix metalloproteinase; OR, odds ratio; CI, confidence interval.
Table 1  Clinical and histochemical characteristics of patients

<table>
<thead>
<tr>
<th>Staging</th>
<th>Patients (n = 86)</th>
<th>Controls (n = 110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>IIa</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>IIb</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>IIIa</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IIIb</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>G2</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>G3</td>
<td>38</td>
<td>45</td>
</tr>
<tr>
<td>Estrogen receptor positive</td>
<td>66</td>
<td>77</td>
</tr>
<tr>
<td>Progesterone receptor positive</td>
<td>69</td>
<td>80</td>
</tr>
<tr>
<td>Ki67 &gt; 20%</td>
<td>49</td>
<td>57</td>
</tr>
</tbody>
</table>

Results (20) show a correlation between this polymorphism and breast cancer.

This is a pilot study aimed at investigating possible correlations between MMP-1 and MMP-3 promoter polymorphisms and breast cancer clinical phenotypes and, specifically, the ability of genetic analysis to identify a subgroup of breast cancer patients with a disease that is clinically more aggressive or prone to metastasize.

MATERIALS AND METHODS

MMP-1 and MMP-3 gene promoter sequences were obtained from peripheral blood samples of 86 patients with breast cancer of different stages who underwent surgery and of 110 tumor-free women (control group). This study was conducted among Italian women (all Caucasian). Controls were healthy volunteers. Median age of cases was 62 years (age range, 24–91 years), and controls were age-matched. Cases were collected from a consecutive incident series of 107 women operated on during 24 months of surgical activity at our department: 21 patients either refused consent or were lost at follow-up and were not included in this study. Cases were prospectively followed-up for 6–30 months (median follow-up, 21 months), and adjuvant protocols were not modified.

Women with breast cancer were grouped according to the tumor-node-metastasis (TNM) classification for breast cancer.

The list of clinical and histochemical characteristics of breast cancer women is reported in Table 1. Two further subgroups of patients were considered: (a) the M− subgroup without evidence of metastasis (n = 46); and (b) the M+ subgroup with evidence of metastasis at the end of the follow-up (n = 40).

Genetic polymorphisms were detected with PCR followed by direct sequencing, as reported previously (14).

Differences between groups were examined by χ² test or unpaired Student’s t test when appropriate. ORs (approximate relative risk) were calculated as an index of the association of the metalloproteinase genotypes with each phenotype. For each OR, two-tailed probability values and 95% CIs were calculated. P < 0.05 was assumed to be statistically significant.

All statistical analyses were two-sided and were performed with Stata Statistical Software (Stata Corp., College Station, TX).

RESULTS

The distributions of the polymorphisms in controls and patients were consistent with the Hardy-Weinberg principle.

In breast cancer patients, the MMP-3 allelic variation was significantly different compared with controls (P = 0.035; OR, 1.53; 95% CI, 1.02–2.29), with a distribution curve shifted to a greater frequency of 5A homozygosity. In breast cancer patients, no difference in MMP-1 allelic variant distribution was noted compared with controls.

The relevant figures are summarized in Tables 2 and 3.

Allelic variants have been compared among subgroups as well as with controls.

No statistical correlations were found among the TNM stage at the time of breast cancer diagnosis and the polymorphisms of the gene promoters of MMP-1 and MMP-3.

With regard to MMP-3 promoter polymorphism, a strong correlation between 5A allele and the M+ subgroup (presence of metastases) versus controls was observed (P = 0.010; OR, 1.96; 95% CI, 1.16–3.30). The frequency of the 5A allele showed no statistical difference between controls and M− (metastasis-free) patients (P = 0.37).

Homzygotes for 5A allele were more prevalent among M+ patients than controls (P = 0.027); comparing M+ and M− subjects, this difference did not reach statistical significance (P = 0.064).

MMP-1 promoter allelic variant distribution showed no statistically significant differences between subgroup M+ (pres-
Table 4  MMP-3 gene promoter polymorphism distribution in patients, according to the presence or absence of metastasis, and controls

<table>
<thead>
<tr>
<th></th>
<th>M+</th>
<th>M−</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>5A/6A + 6A/6A</td>
<td>25</td>
<td>37</td>
<td>88</td>
</tr>
<tr>
<td>5A/5A</td>
<td>15</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>5A frequency</td>
<td>0.61</td>
<td>0.50</td>
<td>0.44</td>
</tr>
</tbody>
</table>

DISCUSSION

Our data support that the 5A allele is correlated to breast cancer susceptibility and demonstrate that 5A homozygosity is a worst prognostic factor. No correlation was observed between 1G/2G polymorphism and breast cancer.

MMP-3 is known to lyse basal membrane collagen and to induce the synthesis of other MMPs, including MMP-1. It may play a role in both local invasiveness and metastatic spread, the latter of which involves the ability of neoplastic cells to cross the basal membrane of both the epithelium and the vascular endothelium; MMP-3 overexpression by some tumor types (21) is consistent with this hypothesis. Apoptosis (programmed cell death) is suppressed in the presence of intact ECM basement membrane (22). Increased MMP-3 expression leads to apoptosis in mammary tissue. MMPs may therefore be involved in apoptosis by their ability to degrade the ECM. The insertion of an adenosine in the MMP-3 gene promoter sequence halves its transcriptional activity (19). It is conceivable that the higher transcriptional activity associated with the 5A allele may enhance tumor invasiveness. Our results not only confirm that the frequency of 5A allele was higher in women with breast cancer (OR, 1.53; \( P = 0.035 \)) when compared with tumor-free controls but, for the first time, demonstrated that 5A/5A homozygotes have a 2.4-fold higher risk of metastatic disease. The increased aggressiveness of the tumor associated with the host 5A/5A genotype is paralleled by the observation that the 5A/5A genotype is substantially more frequent in the M+ subgroup relative to both the control group (\( P = 0.027 \)) and, at a borderline level, the M− subgroup (\( P = 0.064 \); Table 4). The lack of statistically significant difference in the frequency of the 5A allele between controls and metastasis-free breast cancer patients (\( P = 0.37 \)) seems to support the relevance of host-dependent factors in the limitation of breast cancer invasiveness.

Recently, we demonstrated a correlation between 1G/2G polymorphism of the MMP-1 gene promoter and colorectal carcinoma invasiveness: this study also failed to demonstrate correlations of MMP-3 gene promoter polymorphism and colorectal carcinoma (14). It seems noteworthy that breast cancer invasiveness and the examined polymorphisms show an inverted relation. If confirmed by additional studies, these results may provide the basis for further research into the relationships between solid tumors and host, focusing attention on minor genetic variants of the host that determine the intensity of host antitumor response. Prevention programs for specific tumors, such as breast cancer or colorectal carcinoma, could then be addressed to population subsets considering minor genetic variations as new risk factors.

In conclusion, our study seems to suggest that the presence of the 5A allele in the MMP-3 gene promoter sequence may be a facilitating factor for cancer growth and metastasis in breast cancer patients. This activity might be mediated by genetically determined host MMP-3 overexpression induced by the tumor, which seems to decrease the ability of individuals with breast cancer to limit tumor cell invasiveness both locally and systemically.

The reported data need to be validated in larger studies and eventually compared with studies conducted in populations other than Caucasian.

REFERENCES


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