The role of matrix metalloproteinase-3 functional 5A/6A promoter polymorphism in tumor cell progression and metastasis of breast cancer

Majid Motovali-Bashi*, Zohreh Hojati, Samaneh Hajihoseiny

Genetic Division, Department of Biology, Faculty of Sciences, Isfahan University, P.O. Box 1746-73441, Isfahan, I.R. Iran

Abstract

In the human genome, chromosome 11 contains a cluster of matrix metalloproteinase (MMP) genes. Single nucleotide polymorphisms in the promoter region of MMP genes are important for MMP expression. A common adenine deletion polymorphism (5A) at position -1171 of the MMP-3 gene promoter (5'AAACCAT-3' change to 5'AAAAACCACAT-3') facilitates transcriptional factor binding and MMP-3 promoter activity. A case-control study was performed including 120 breast cancer patients (60 patients with metastatic activity and 60 patients without metastatic activity); and 60 healthy controls. Whole blood samples were obtained from patients and healthy controls. Genomic DNA was extracted from samples and the MMP-3 5A/6A genotypes were determined using PCR-RFLP. MMP-3 genotype distributions between patients and controls were similar (OR= 0.89, 95%CI, 0.43-1.84, P= 0.047). It was observed that the 5A allele was more frequent among patients with metastatic activity than controls (OR= 2.9, 95%CI, 0.94-8.9, P= 0.074). Therefore, the 5A polymorphism in the MMP-3 promoter showed correlation with the metastasis group than patients without metastasis; both at the time of diagnosis. However our results do not show evidence for correlation between 5A/6A polymorphism and breast cancer susceptibility.

Keywords: Matrix metalloproteinase-3; Single nucleotide polymorphism; Breast cancer; Metastasis.

Matrix metalloproteinases (MMPs) are involved in all steps of tumor genesis, cancer invasion and metastasis (Sternlicht and Werb, 2001; Sternlicht et al., 2000; Declerck and Imren, 1994). Tumor invasion and metastasis formation always begin with blood lymphatic vessel inflammation. As these processes involve proteolytic digestion of the extracellular matrix, it is therefore suggested that MMPs play an important role in tumor progression (Somerville et al., 2003 and Shapiro, 1998). The basal expression of several MMPs such as MMP-1, MMP-3 and MMP-9 in cell culture have been detected at very low levels. However, their transcriptions are induced by a variety of extracellular stimuli including growth factors, cytokines, and chemical agents (Gotoh et al., 2004; Spurr et al., 1988). In the genome, chromosome 11 contains a cluster of MMP genes (Gatti et al., 1989; Pendas et al., 1996). The flanking regulatory regions of most inducible MMP genes exhibit common features, including the TATA box, activator protein-1 (AP-1) and regulatory elements in the proximal promoter region. In addition, MMP genes contain several potential AP-1 binding sites, and a transcriptionally important tumor suppressor P53 binding site (Posthumus et al., 2003), and also enhancing twenty six (ETS) family of oncprotein binding polyoma enhancing activator-3 (PEA-3) site and nuclear factor of KB (Fang et al., 2005). Figure 1 shows these conserved elements upstream of the MMP-3 gene.

Besides the signal transduction pathway, single nucleotide polymorphisms (SNPs) in the promoter regions of MMP genes affect level of transcription by
creating a regulatory box (Kang et al., 2005; Ke et al., 2004; Posthumus et al., 2003). Approximately 90% of DNA polymorphisms are SNPs due to single base substitutions or base insertion/deletions (Posthumus et al., 2003). Promoter polymorphisms have been identified in a number of MMP genes such as MMP-1 and MMP-3. Some of them have been shown to influence MMP expression and are therefore associated with susceptibility and/or severity of disease such as cancer (Shan et al., 2005; Blons et al., 2004; Zhang et al., 2004; Lei et al., 2003; Ghilardi et al., 2002). A single adenine insertion polymorphism at position-1171 of the MMP-3 promoter region modulates transcription and local expression of MMP-3. It has been demonstrated that the 6A allele expresses a roughly 2-fold lower amount of gene product as compared with the 5A allele (Kripple et al., 2004; Rhys, 2000). Additional studies have revealed binding of the putative transcriptional factors to this region. The difference in promoter activity is probably due to preferential binding of the transcriptional repressor to the 6A allele (Posthumus et al., 2003; Mercapide et al., 2003). Influence of this polymorphism has been assessed in breast cancer, head and neck cancer and atherosclerosis (Radisky et al., 2005; Blons et al., 2004; McGlinchey et al., 2004; Pollanen et al., 2002; Gnasso et al., 2000; Matt et al., 1999).

The presence of the 5A allele may be associated with breast cancer susceptibility and severity. Therefore, this study aimed to identify a subgroup of breast cancer patients who had more aggressive tumors or were prone to metastasis.

This case-control study included 120 female patients from Isfahan with confirmed diagnosis of breast cancer at different stages. Healthy women were included as controls and studied under the same conditions as the case studies. The median age of cases was 47 years old (age range, 27-68 years). The participants were also asked about any family history of cancer. Blood samples were collected from Omid hospital during 24 months, from 2004 to 2006. Cases were followed-up for 6-30 months. Women with breast cancer were subgrouped according to the tumor-node-metastasis (TNM) classification for breast cancer. Two subgroups of patients were considered: the M- subgroup without evidence of metastasis (n=60), and M+ subgroup with evidence of metastasis at the end of follow-up (n=60). Five ml of venous blood from each subject was drawn into a vacutainer tube containing EDTA and stored at 4°C for further studies and -80°C for long-term studies. Genomic DNA was extracted according to the salting out method (Miller et al., 1988) with some modifications. Besides using saturated NaCl, Chloroform was also used to increase DNA quality and quantity (Davis et al., 1994). Genetic polymorphism of the MMP-3 gene was detected using PCR followed by digestion with restriction enzymes. The promoter sequences of the MMP-3 gene were obtained from the Eukaryotic Promoter Database (EPD). According to available published primers, reverse and forward primers were selected and slightly modified. In the case of the 5A allele a substitution mutation from A to G in the second nucleotide close to the 3’ end of the reverse primer (showed “g” at the primer sequences; Figure 2) at the polymorphism region was made to create a Tth111I (PsyI) recognition site (Figure 2). PCR was carried out in a 25 µl volume containing 100 ng of DNA template, 2.5 µl of 10 X PCR buffer, 1.5 mM MgCl2, 1 U of Taq DNA polymerase, 200 nM dNTPs and 20 pM forward and reverses primers. The PCR cycling conditions were 5 min at 94°C followed by 30 sec at 94°C (denaturation), 30 sec at 65°C, 60 sec at 72°C (extension), repeated for 30 cycles followed by a final step at 72°C for 10 min. Detection of the allelotype was carried out by digestion of PCR products.
with the \textit{Tth111I} restriction enzyme (\textit{PsyI}). Digestion of PCR products was performed according to a standard protocol. Electrophoresis was performed using a 2-3\% (w/v) agarose gel stained with ethidium bromide. After digestion and electrophoresis, the 5A allele was expected to be represented by two bands of 97 and 32 bp and the 6A allele by a band of 129 bp. Statistical analyses were performed using the SPSS 12.0 software package. Hardy-Weinberg analysis was also carried out to compare the observed and expected genotype frequencies using the $\chi^2$ test. Odd Ratios (OR; approximate relative risk) were calculated as an index of the association of the \textit{MMP-3} promoter genotypes with breast cancer invasion and metastasis. For each OR, two-tailed probability values and 95\% confidence intervals (CIs) were calculated. P<0.05 was assumed to be statistically significant.

There was a statistical correlation between 5A allele in the M+ subgroup versus controls (P=0.074, OR=2.9, 95\%CI, 0.94-8.9). There was no statistical difference between the controls and M- subgroup with respect to the frequency of the 5A allele. (P=0.043, OR=0.77, 95\% CI, 0.23-2.5). Homozygotes for the 5A allele were more prevalent among the M+ patients than the controls (P=0.074). The difference between the M+ and M- subjects was not statistically significant (Table 2). As expected, the PCR products were 129 bp. Figure 3 shows the band after electrophoresis on the 2\% (w/v) agarose gel stained with ethidium bromide. The PCR products for the 5A and 6A alleles are of the same size for all samples, but these alleles may show up differently by RFLP. The 6A alleles do not have the \textit{Tth111I} recognition site; therefore, they show only one band of approximately 129 bp on the gel. But the 5A alleles have the \textit{Tth111I} recognition site, represented by one band of approximately 97 bp on the 3\% (w/v) agarose gel (the 32 bp band could not be detected by the agarose gel electrophoresis, Fig. 4). The \textit{MMP-3} genotyping was successfully determined in 120 patients and 60 controls. The distribution of \textit{MMP-3} alleles in controls and patients were consistent according to the Hardy-Weinberg principle ($\chi^2 = 4.4$, P =0.011). From the 120 participated patients in this study, 30 subjects were homozygous for the 6A allele, 28 subjects were homozygous for the 5A allele and 62 subjects were heterozygous. The 5A and 6A allele frequencies in the controls were 0.45 and 0.55, respectively (Table 1). In breast cancer patients, the \textit{MMP-3} allelic variation was significantly different compared

**Table 1. Distribution of \textit{MMP-3} promoter polymorphism.**

<table>
<thead>
<tr>
<th>\textit{MMP-3}</th>
<th>Patients (n=120)</th>
<th>Control (n=60)</th>
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<tbody>
<tr>
<td>5A/5A</td>
<td>28 (23.3)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td>5A/6A</td>
<td>62 (51.6)</td>
<td>38 (63.3)</td>
</tr>
<tr>
<td>6A/6A</td>
<td>30 (25.0)</td>
<td>14 (23.3)</td>
</tr>
<tr>
<td>5A frequency</td>
<td>(0.49)</td>
<td>(0.45)</td>
</tr>
<tr>
<td>6A frequency</td>
<td>(0.51)</td>
<td>(0.55)</td>
</tr>
</tbody>
</table>

P= 0.061, OR= 1.63, 95\%CI, 0.59-4.4. Parenthesis show \% of samples.
with that of the controls (P = 0.061, OR = 1.63, 95% CI, 0.59-4.4). Figure 5 shows distribution of various genotypes in patients and controls. No statistical correlation was found among the TNM stages at the time of breast cancer diagnosis (data not shown). According to our results the MMP-3 5A/6A polymorphism does not influence breast cancer susceptibility but there is a statistical correlation between tumor cell progression and metastatic activity among patients carrying the 5A allele.

Studies on the MMP-3 promoter polymorphism and its correlation with breast cancer have shown different results. Hughes and colleagues (2007) showed a significant and independent association of the C/T genotype for MMP-9 and the 1G/2G genotype of MMP-1 with the lymph node-positive disease of breast cancer. But he could not find any association between MMP-3 polymorphism and breast cancer. However, some studies have reported that the presence of the 5A allele is associated with susceptibility and severity of breast cancer. But in other cancers like colorectal cancer, the 6A/6A genotype in patients has been observed to occur more frequently among controls, however, the difference is not statistically significant (Zhang et al., 2004; Ghilardi et al., 2002). It is conceivable that the higher transcriptional activity associated with the 5A allele may enhance tumor invasiveness (Blons et al., 2004). Radisky and colleagues (2005) have reported that the 5A allele correlates with breast cancer metastatic activity. The results of this study do not show a statistically significant difference between the breast cancer patients without metastasis and the healthy controls. However, they show a close association between the 5A allele and metastasis of breast cancer. The 5A allele is substantially more frequent in the M+ subgroup than both the control group and M- subgroup (Table 2). Observed differences between reported risks are probably due to the differences in genetic background, environment and sample size of the studied population.

Acknowledgments

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Table 2. MMP-3 promoter polymorphism distribution in patients (M+/M-) and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>M+</th>
<th>M-</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td>5A/5A + 5A/6A</td>
<td>48</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>5A/5A</td>
<td>20</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>6A/6A</td>
<td>12</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>5A frequency</td>
<td>0.56</td>
<td>0.40</td>
<td>0.45</td>
</tr>
<tr>
<td>6A frequency</td>
<td>0.44</td>
<td>0.60</td>
<td>0.55</td>
</tr>
</tbody>
</table>

(Patients/ controls) 5A/5A: 6A/6A, OR = 1.63, 95%CI, 0.59-4.4, P = 0.061; (Patients/ controls) 5A/5A + 5A/6A, 6A/6A, OR = 0.89, 95%CI, 0.43-1.84, P=0.047; M+ controls, OR= 2.9, 95%CI, 0.84-8.9, P= 0.074; M+ M+, OR= 3.75, 95%CI, 1.25-11.24, P= 0.078; M+ : Patients with metastatic activity; M- : Patients without metastatic activity.
References


