A/G POLYMORPHISM OF THE MMP-7 GENE PROMOTER REGION IN COLORECTAL CANCER

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In gastrointestinal malignancies increased expression of matrilysin – MMP-7 – is often observed. Its high level positively correlates with clinical stage of malignancy and is a negative prognostic factor. This suggests a possible relationship between functional polymorphisms of the MMP-7 gene and susceptibility to development of colorectal cancer and an aggressive course of the disease.

The aim of the study was to assess the effects of A/G functional polymorphism at -181 site of the MMP-7 gene promoter region on development and progression of colorectal cancer.

Material and methods. In total, 184 patients treated surgically for colorectal cancer at the Department of General and Colorectal Surgery of the Medical University in Łódź in the years 2006-2009 and a control group of 205 cancer-free individuals with a negative family history for malignancy have been investigated. Polymorphic variants of the MMP-7 gene promoter region have been analysed using the RFLP-PCR method.

Results. A statistically significant difference in distribution of genotypes has been found between the investigated group and the control group, and the OR analysis confirmed a relationship between the A/G [1.67 (1.03-2.72); p= 0.038] and G/G [2.12 (1.34-3.38); p = 0.018] genotypes and an increased risk of colorectal cancer. The risk of lymph node involvement was more than twice higher for the G/G genotype (OR = 2.83 (1.18-6.79); P= 0.017). In addition, the analysis of genotype distribution in patients divided into groups according to the T parameter of the TNM classification revealed a relationship between the G/G genotype and advanced tumour infiltration. No relationship between the investigated A/G polymorphism and the presence of distant metastases has been found.

Conclusions. Obtained results indicate a possible relationship between -181 A/G polymorphism of the MMP-7 gene and malignant transformation of colorectal epithelial cells and progression of colorectal cancer. This suggests applicability of this polymorphism as a predisposing factor for the disease and a prognostic factor, which in the future may be useful in the management algorithm for colorectal cancer.

Key words: A/G polymorphism, MMP-7 gene, colorectal cancer

Matrix metalloproteinases are associated with development and progression of numerous malignancies, and their high level often positively correlates with poor prognosis for patients. In gastrointestinal malignancies, such as oesophageal, gastric, pancreatic, or colorectal cancer, increased expression of matrilysin (MMP-7) is often observed and its high level positively correlates with clinical stage of malignancy and is a negative prognostic factor (1, 2, 3). Like other MMPs, matrilysin is associated with invasiveness of tumours due to its role in proteolysis of extracellular matrix, as confirmed by positive immunohistochemical staining for MMP-7 at the so-called tumour cell invasive front and the ability to activate proMMP-2 and MMP-9 gelatinases (4). MMP-7 may contribute to development of colorectal adenomas by means of activation of growth factors, such as heparin-binding EGF-like growth factor (HB-EGF) activating the ErbB4 receptor or insulin-like growth factors (IGF) (5, 6). In addition, it has been demonstrated that MMP-7 increases (in a dose-dependent
manner) proliferation of vascular endothelial cells, suggesting a role of this metalloprotei
nase in the process of cancer-related angiogenesis (7). This has been confirmed by studies in
colorectal cancer, in which density of newly developed blood vessels correlated with immu
nohistochemical staining for MMP-7 expression (8).

In an animal model, inhibition of angiogenesis by matrilysin inhibitors in tumours de
veloped from implanted human colorectal cancer cells has been observed (9). In colorectal can-
cer, increased expression of MMP-7 positively correlated with the degree of tumour infiltr
ation and its clinical stage, and was a negative prognostic factor for patients. Using a more
sensitive RT-PCR method it has been demonstrated that the increase of MMP-7 expression
was proportional to the stage of colorectal cancer. This suggests a significant role of MMP-7 both in development and progression of colorectal cancer; therefore, functional poly
morphisms of the MMP-7 gene may be important for the course of this disease.

The objective of this study was to assess the effects of A/G functional polymorphism at -181
site of the MMP-7 gene promoter region on development and progression of colorectal cancer.

MATERIAL AND METHODS

In total, 184 patients treated surgically for colorectal cancer at the Department of Gen
eral and Colorectal Surgery of the Medical University in Łódź in the years 2006-2009 have been investigated.

Mean age of the patients was 58 years (47-77 years). In all investigated patients a diag
nosis of colorectal adenocarcinoma was confirmed by histopathological examination of post-operative specimens. Individuals with suspected familial colorectal cancer, including cases of FAP and Lynch syndrome (HNPCC), were excluded from the investigated group.

Clinical and pathological characteristics of the tumours is summarised in tab. 1. Laboratory
tests were performed on whole blood samples (5 ml) obtained from all patients using test
tubes containing anticoagulant (EDTA). The control group consisted of cancer-free indi
viduals with a negative family history for malignancy (n = 205). Mean age of these per
sons was 51 years (43-78 years). Blood samples

were obtained from these individuals. No sta
tistically significant differences in age or gen
der distribution were found between the investig
ated and control group. The study was ap
proved by the Bioethical Commission of the Medical University in Łódź.

DNA was isolated from frozen blood using a commercially available set of ion exchange columns – Genomic Mini AX BLOOD – according to the manufacturer’s recommendations. From 200 μl of frozen whole blood approximately 10 μg of DNA was obtained and sus
pended in 50 μl of TE buffer solution (pH 8).

Analysis of polymorphic variants of the MMP-7 gene promoter region was performed
using the RFLP-PCR method, in which restriction enzymes are used to detect changes in the
DNA sequence. The sequences of pairs of prim
ers encompassing polymorphic sites used in
the PCR reaction were as follows: EcoRI U:
5’-CTG AAT GAT ACC TAT GAG AGC AGT-3’
and EcoRI D: 5’- GCA GGA AGC ACA CAA
TGA ATT -3’. In the applied method of analy
sis of the A/G polymorphism of the MMP-7
gene, the EcoR I D primer was not fully comple
mentary to the gene sequence (underlined
nucleotides) in order to obtain restriction sites
detected by the EcoRI enzyme in the amplifica
tion product (10). Amplification was performed using 25 μl of reactive mixture of the following
composition: 50 ng of genomic DNA; 10 pmol
of each oligonucleotide; dATP, dCTP, dGTP,
and dTTP – 200 μmol each; 1.5 mM MgCl
2, 20 mM Tris-HCl (pH 8.4), 50 mM KCl and 1 U

Table 1. Clinical and pathological characteristics of patients with colorectal cancer

<table>
<thead>
<tr>
<th>Clinical and pathological characteristics</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNM classification</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
</tr>
<tr>
<td>T2</td>
<td>67</td>
</tr>
<tr>
<td>T3</td>
<td>111</td>
</tr>
<tr>
<td>T4</td>
<td>6</td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>102</td>
</tr>
<tr>
<td>N1</td>
<td>62</td>
</tr>
<tr>
<td>N2</td>
<td>20</td>
</tr>
<tr>
<td>Distant metastases</td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>147</td>
</tr>
<tr>
<td>M1</td>
<td>37</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>9</td>
</tr>
<tr>
<td>G2</td>
<td>156</td>
</tr>
<tr>
<td>G3</td>
<td>19</td>
</tr>
</tbody>
</table>
of Taq polymerase. The following thermal conditions were applied for PCR amplification: 95°C for 5 min followed by 35 cycles: 95°C for 30 s, 51°C for 30 s and 72°C for 30 s, followed by 72°C for 7 min. The amplification products (15 μl of the PCR reaction mixture) were enzyme-treated and underwent electrophoresis in 8% polyacrylamide gel at 10 V/cm for 1 h. Enzyme-treated amplification products were visualised using ethidium bromide (0.5 mg/ml) under ultraviolet light. In case of A/G polymorphism of the MMP-7 gene the EcoRI restriction enzyme detects a sequence containing the G allele and restriction cleavage of such a variant results in fragments 102 bp and 32 bp long. A sequence containing the A allele is not cleaved and a band 134 bp long is visible after electrophoresis. In case of A/G heterozygotes bands 134 bp and 102 bp long are visible (fig. 1).

The observed number of cases for each genotype in the investigated and control group was compared with the number expected according to the Hardy-Weinberg principle, using the χ² test. Significance of differences in the frequency of alleles and genotypes between the group was assessed using the χ² test. The risk of coexistence of specific genotypes with the disease and clinical and pathological parameters was evaluated with the odds ratio (OR), calculated using the wild-type genotype as the reference genotype.

### RESULTS

Distribution of the A/G polymorphism genotypes of the MMP-7 gene has been analysed in a group of patients with colorectal cancer and in individuals without evidence of malignancy, comprising a control group (tab. 2). Distributions in both groups were consistent with the Hardy-Weinberg principle (p > 0.05). A statistically significant difference in distribution of genotypes has been demonstrated between the investigated group and the control group. OR analysis revealed a relationship between the A/G and G/G genotypes and increased risk of development of colorectal cancer. In tab. 3 the results of analysis of genotype distribution in groups of patients with lymph node metastases and those in

### Table 2. Distribution of genotypes of the A/G polymorphism of the MMP-7 gene promoter region in patients with colorectal cancer (CRC) and in the control group. OR analysis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CRC patients (n = 184) number (proportion)</th>
<th>Control group (n = 205) number (proportion)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>39 (0,21)</td>
<td>66 (0,32)</td>
<td>Ref.</td>
</tr>
<tr>
<td>A/G</td>
<td>93 (0,51)</td>
<td>94 (0,46)</td>
<td>1,67 (1,03-2,72) p = 0,038</td>
</tr>
<tr>
<td>G/G</td>
<td>52 (0,28)</td>
<td>45 (0,22)</td>
<td>2,12 (1,34-3,38) p = 0,018</td>
</tr>
</tbody>
</table>

Consistence with the Hardy-Weinberg principle: MMP-7 CRC patients χ² = 0.046756 P > 0.05 and the control group: χ² = 1.098476 p > 0.05

### Table 3. Distribution of genotypes of the A/G polymorphism of the MMP-7 gene promoter region in patients with colorectal cancer depending on regional lymph node involvement. OR analysis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No involvement (n = 102) number (proportion)</th>
<th>Metastases (n = 82) number (proportion)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>27 (0,26)</td>
<td>12 (0,15)</td>
<td>Ref.</td>
</tr>
<tr>
<td>A/G</td>
<td>52 (0,51)</td>
<td>41 (0,50)</td>
<td>1,77 (0,80-3,92) p = 0,154</td>
</tr>
<tr>
<td>G/G</td>
<td>23 (0,23)</td>
<td>29 (0,35)</td>
<td>2,83 (1,18-6,79) p = 0,017</td>
</tr>
</tbody>
</table>
A/G polymorphism of the MMP-7 gene promoter region in colorectal cancer

whom no lymph node involvement was found. OR analysis demonstrated more than twice higher risk of lymph node involvement for the G/G genotype (OR = 2.83 (1.18-6.79); p = 0.017). In table 4 the results of analysis of genotype distribution in groups of patients with distant metastases and those in whom no such metastases were found are presented. OR analysis revealed no relationship between the investigated polymorphism and the presence of distant metastases. Distribution of genotypes in patients divided into groups according to the T parameter of the TNM classification was also analysed (tab. 5). The group of patients with T3 tumours and that of patients in whom T4 tumours were found were compared with the group of patients with T2 tumours. A relationship between the G/G genotype and T3 parameter was found.

**DISCUSSION**

A/G polymorphism at -181 site of the MMP-7 gene promoter region was demonstrated. In case of the G variant binding of an additional transcription factor is observed, resulting in a 2-3-fold increase in MMP-7 expression. A study in a small group of 58 patients with colorectal cancer revealed a relationship between the G allele and lymph node involvement and the presence of distant metastases (11). In case of gastric cancer, a relationship between the G allele and a higher risk of death due to this malignancy was found (12). In Caucasian population a relationship between the G/G genotype and the presence of lymph node metastases in breast cancer was observed, while in a group of patients of various ethnicity the G/G genotype increased the risk of death due to breast cancer (13). A study in a group of more than 1000 Chinese patients with breast cancer demonstrated that the G/G genotype was a negative prognostic factor associated with shorter survival time.

Our analysis of distribution of A/G polymorphism genotypes in a group of patients with colorectal cancer and a control group demonstrated a significantly higher prevalence of both A/G and G/G genotypes in the group of patients, indicating a relationship between this polymorphism and development of colorectal cancer. This is consistent with earlier studies, in which MMP-7 expression was observed not only in invasive colorectal cancer cells, but also at very early stages of development of adenomas, at which the cells show no invasive potential, suggesting that the role of MMP-7 in colorectal cancer is not limited to degradation of ECM components, but may affect (through activation of growth factors) the processes of proliferation and apoptosis and thus promote cancer development (14). Extracellular matrix contains precursors of heparin-binding EGF-like growth factors (HB-EGF), which, after cleavage with MMP-7, are capable of activating the EGFR, leading to the activation of the Akt signalling pathway and the initiation of cancer cell proliferation (15).}

### Table 4. Distribution of genotypes of the A/G polymorphism of the MMP-7 gene promoter region in patients with colorectal cancer depending on the presence of distant metastases. OR analysis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No distant metastases (n = 147) number (proportion)</th>
<th>Distant metastases (n = 37) number (proportion)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>32 (0.22)</td>
<td>7 (0.19)</td>
<td>Ref.</td>
</tr>
<tr>
<td>A/G</td>
<td>74 (0.50)</td>
<td>19 (0.51)</td>
<td>1.17 (0.44-2.48) p = 0.740</td>
</tr>
<tr>
<td>G/G</td>
<td>41 (0.28)</td>
<td>11 (0.30)</td>
<td>1.22 (0.42-3.52) p = 0.708</td>
</tr>
</tbody>
</table>

### Table 5. Distribution of genotypes of the A/G polymorphism of the MMP-7 gene promoter region in patients with colorectal cancer depending on the T parameter of the TNM classification

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T2 (n = 67) number (proportion)</th>
<th>T3 (n = 111) number (proportion)</th>
<th>T4 (n = 6) number (proportion)</th>
<th>OR (95% CI) T1/T2 vs T3</th>
<th>OR (95% CI) T1/T2 vs T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/G</td>
<td>20 (0.30)</td>
<td>18 (0.16)</td>
<td>1 (0.17)</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>G/G</td>
<td>36 (0.54)</td>
<td>54 (0.49)</td>
<td>3 (0.50)</td>
<td>1.66 (0.77; 3.57) p = 0.188</td>
<td>1.66 (0.16; 17.10) p = 0.562</td>
</tr>
<tr>
<td></td>
<td>11 (0.16)</td>
<td>39 (0.35)</td>
<td>2 (0.33)</td>
<td>3.93 (1.56; 9.92) p = 0.002</td>
<td>3.63 (0.29; 44.77) p = 0.321</td>
</tr>
</tbody>
</table>

Unauthenticated
of binding to the ErbB4 receptor, which activates proliferation and inhibits apoptosis (5). Other important factors strongly inhibiting apoptosis are insulin-like growth factors (IGF); their bioavailability is limited by IGF-binding proteins (IGFBP) blocking IGF functions. MMP-7 is capable of degradation of IGFBP and release of IGF, thus promoting tumour growth and facilitating survival of cancer cells by means of inhibition of apoptosis (6).

A relationship between the G/G genotype and lymph node involvement and tumour stage according to the TNM classification was also found. This may be associated with degradation of E-cadherin, an adhesive molecule forming intercellular junctions, by MMP-7. The loss of junctions formed by E-cadherins is necessary for tumour progression, as it makes it possible for cancer cells to release from the primary tumour and invade normal tissue. This is the reason for a relationship between the G allele associated with a high MMP-7 level and increased risk of local invasion. Despite the ability of MMP-7 to activate proMMP-2 and MMP-9 gelatinases, which make it possible for cancer cells to cross basal membranes of blood vessels, no relationship between the A/G polymorphism of the MMP-7 gene and the presence of distant metastases was demonstrated (4), which may be due to contribution of numerous other proteases to this process.

CONCLUSIONS

Obtained results indicate a role of MMP-7 in malignant transformation of colorectal epithelial cells and cancer progression, which may depend on the A/G polymorphism. This suggests applicability of the A/G polymorphism of the MMP-7 gene as a predisposing factor for the disease and a prognostic factor, which in the future may be useful in the management algorithm for colorectal cancer.

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