Gene Polymorphism of Matrix Metalloproteinase-12 and -13 and Association with Colorectal Cancer in Swedish Patients

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Abstract. *Background: It has been widely reported that matrix metalloproteinases (MMPs) have fundamental roles in pathological processes in cancer through degradation of basal membranes and extracellular matrix. For MMP12 and MMP13, a functional single nucleotide polymorphism (SNP) has been detected -82A→G (rs2276109) and -77A→G (rs2252070), respectively. These SNPs are suggested to have an influence on different diseases. The present study evaluated the association between these SNPs in patients with colorectal cancer (CRC) patients and healthy controls. Patients and Methods: Using the TaqMan system, these SNPs were screened in 385 patients with CRC and 619 controls. Results: No significant difference in genotype distribution or in allelic frequencies was found between the two groups. However, we showed that the AA MMP-12 genotype is connected with a higher risk of disseminated CRC (Odds Ratio=1.77; 95% Confidence Interval=1.11-2.81, p=0.018). Conclusion: The results of this study suggest that the -82A→G (rs2276109) polymorphism of the MMP12 gene reflects clinical outcome of patients with CRC.*

Worldwide, colorectal cancer (CRC) is the third and the fourth most common cancer in women and men respectively (1). CRC progression involves the accumulation of multiple mutations in oncogenes, tumour-suppressor genes and DNA repair genes but epigenetic changes also affect CRC development. Moreover, genetic variation such as single nucleotide polymorphisms (SNPs), are thought to play an important role in individual variation in CRC susceptibility (2, 3). The prognosis of CRC depends on the extent of local, and metastatic tumour spread and the degradation of the extracellular matrix surrounding cancerous tissue is a pivotal step in tumour invasion (4). Matrix metalloproteinases (MMPs) have fundamental roles in pathological processes in cancer and gut inflammation through degradation of basal membranes and extracellular matrix (5, 6). Expression of MMPs has been described in a wide range of cancer types including CRC and associated with tumour invasion and poor prognosis (5, 7-13). Knowledge of the elevation of MMP expression in cancer tissue has led to an area of development of MMP inhibitors for the treatment of various malignancies (14). Both MMP12 (human macrophage metalloelastase) and MMP13 (human collagenase-3) are expressed in CRC (12, 13, 15), and MMP13 expression has been demonstrated to correlate with poor survival and the presence of liver metastases in CRC (12, 16).

In recent years, several SNPs in the promoter region of the *MMP* genes have been described (9, 14, 17, 18). For *MMP12* and *MMP13*, SNPs *-82A→G* (rs2276109) and *-77A→G* (rs2252070), respectively, have been detected (9, 19, 20). Functional analysis of these SNPs has led to the proposed of their modulatory role on transcriptional activity, leading to alterations in the gene expression (19, 20). It has been suggested that these SNPs are associated with the development of epithelial ovarian carcinoma (21), and gastric cardia adenocarcinoma and esophageal squamous cell carcinoma (22). Moreover, data show that these SNPs are implicated in endometriosis progression (17).

Based on the suggested role of the functional gene polymorphisms *MMP12 -82A→G* (rs2276109) and *MMP13 -77A→G* (rs2252070) in the pathogenesis of certain diseases, we analyzed these SNPs to assess their value as risk factor and as predictors of disease outcome in Swedish patients with CRC.

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Patients and Methods

Patients and controls. This study was carried out on blood samples from 385 consecutive patients from southeastern Sweden who underwent surgical resection for primary colorectal adenocarcinomas at the Department of Surgery, Ryhov County Hospital, Jönköping, Sweden. Clinicopathological characteristics of the patients were confirmed by surgical and pathological records. The investigation was approved by the local Ethical Committee (Dnr. 98113) and informed consent was obtained from the participants.

The patient group included 209 males and 176 females with a mean age of 70 years (range=25-93 years). The tumours were localized in the colon in 216 and rectum in 169, and were classified by Dukes' classification system: Stage A, n=65; stage B, n=144; stage C, n=118; and stage D, n=58. Blood donors (n=619) with no known CRC history and from the same geographical region as the patients with CRC were selected as controls. The control group consisted of 337 males and 282 females, with a mean age of 61 years (range=50-83 years). Blood samples were centrifugated to separate plasma and blood cells and then stored at –78˚C.

DNA extraction and genotype determination. Genomic DNA was isolated from blood samples using the QiaAmp DNA Blood Kit (Qiagen, Valencia, CA, USA). DNA samples were genotyped using a 5'-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). The Taqman SNP Genotyping Assay was used for analysis of the MMP12 (rs2276109) and MMP13 (rs2252070) (Applied Biosystems). DNA samples (10 ng) were amplified in a 12 μl total volume containing TaqMan Universal PCR Master Mix (Applied Biosystems) including 1x TaqMan SNP Genotyping Assay. Amplification was performed using by an initial cycle of 50˚C for 2 min, 95˚C for 10 min followed by 40 cycles at 95˚C for 15 s and then 60˚C for 1 min. A post-PCR end-point reading was performed on each plate using the 7500 Fast Real-Time PCR System (Applied Biosystems). The manual calling option in the allelic discrimination application ABI PRISM 7500 SDS software version 1.3.1 (Applied Biosystems) was then used to assign genotypes.

Statistical analysis. Differences in the frequencies of the *MMP12* and *MMP13* gene polymorphism between patients and the control group and between clinical characteristics within the CRC subgroup were analyzed using the Chi-squared test, and the Hardy–Weinberg equilibrium was tested for the genotypes. Statistical analysis was performed using SPSS Statistics Software (version 19, 2012; Chicago, IL, USA). Results were considered significant at $p<0.05$.

Results

Genotypic frequencies of the *MMP12* and *MMP13* gene polymorphisms in patients and control group indicated no difference in genotypic distribution (Table I) or allelic frequencies (data not shown). Moreover, the genotypes in patients and the control group were not associated with clinical characteristics, such as age and gender (data not shown). When subdividing the patients into groups of colon and rectal cancer or localized Dukes' A and B and disseminated Dukes' C and D disease, we were unable to detect any significant difference between disease of the rectum and colon regarding genotypes (Table II). However, a significant ($p=0.015$) difference in genotypic distributions for MMP12 in patients regarding disease stage was found (Table II). We found the rate of genotype AA to be 68.9% (144/209) and that of AG+GG to be 31.1% (65/209) in cases with localized disease. In patients with disseminated disease, the rate of AA was 79.5% (140/176) and the rate of AG+GG was 20.5% (36/176). The dominance of the AA genotype in patients with disseminated disease was significant ($p=0.018$) with an (OR)=1.77 [95% (CI)=1.11-2.81].

We found eight combined genotypes out of the nine theoretically possible and there was no significant difference in combined genotypic distribution between patients and controls (Table III). Neither the patient nor the control group showed significant deviation in genotypic frequencies from the Hardy–Weinberg.

Table I. *Genotypic frequencies of matrix metalloproteinase 12 (MMP12) and 13 (MMP13) in patients with colorectal cancer and healthy controls*.

Genotype	CRC patients (n=385)	Controls (n=619)
MMP12 (rs2276109)		
A/A	284 (73.8)	437 (70.6)
G/A	91 (23.6)	169 (27.3)
G/G	10 (2.6)	13 (2.1)
G/A+G/G	101 (26.2)	182 (29.4)
MMP13 (rs2252070)		
A/A	185 (48,0)	276 (44.6)
G/A	158 (41.0)	276 (44.6)
G/G	42 (11.0)	67 (10.8)
G/A+G/G	200 (51.9)	343 (55.4)

CRC patients *vs*. healthy controls: *MMP12* genotype overall $p=0.418$, A/A *vs*. G/A+G/G $p=0.278$. *MMP13* genotype overall $p=0.432$, A/A *vs*. G/A+G/G $p=0.284$.

Discussion

Collectively, MMPs have fundamental roles in pathological processes through degradation of basal membranes and extracellular matrix. Some MMPs contribute to tumour invasion and metastasis (5, 6). MMP12 and MMP13 have been found to have high expression in CRC (12, 13, 15). For MMP12 and MMP13, functional SNPs have been detected, *-82A→G* (rs2276109) and *-77A→G* (rs2252070), respectively (19, 20). These SNPs have been studied in relation to epithelial ovarian carcinoma (21), gastric cardia adenocarcinoma and esophageal squamous cell carcinoma

Table II. *Numbers of individuals with each genotype for matrix metalloproteinase-12 (MMP12) and -13 (MMP13) regarding tumour location and disease stage in patients with colorectal cancer.*

Genotype	Colon (n=216)	Rectum (n=169)	Dukes' A+B (n=209)	Dukes' C+D (n=176)
MMP12 (rs2276109)				
A/A	156 (72.2)	128 (75.7)	144 (68.9)	140 (79.5)
G/A	57 (26.4)	34 (20.1)	58 (27.8)	33 (18.8)
G/G	3 (1.4)	7 (4.2)	7 (3.3)	3 (1.7)
G/A+G/G	60 (27.8)	41 (24.3)	65 (31.1)	36 (20.5)
MMP13 (rs2252070)				
A/A	104 (48.2)	81(47.9)	101(48.3)	84 (47.7)
G/A	91 (42.1)	67 (39.7)	85 (40.7)	73 (41.5)
G/G	21 (9.7)	21 (12.4)	23 (11.0)	19 (10.8)
G/A+G/G	112 (51.9)	88 (52.1)	108 (51.7)	92 (52.3)

Colon *vs*. rectum: MMP12 genotype overall p=0.881, A/A *vs*. G/A+G/G p=0.606. MMP13 genotype overall p=0.665, A/A *vs*. G/A+G/G p=0.964. Dukes' A+B *vs*. Dukes' C+D: MMP12 genotype overall p=0.015, A/A *vs*. G/A+G/G p=0.018. MMP13 genotype overall p=0.682, A/A *vs*. G/A+G/G p=0.459.

Table III. *Distribution of combined genotypes for MMP12 (rs2276109) and MMP13 (rs2252070) gene polymorphisms in patients with colorectal cancer and healthy controls.*

Combined genotype	MMP12	MMP13	CRC (n=385)	Controls (n=619)
1	A/A	A/A	46.7 (180)	42.5 (263)
2	A/A	G/A	24.2 (93)	25.2 (156)
3	G/A	G/A	16.3 (63)	18.7 (116)
4	G/A	G/G	6.0 (23)	6.5 (40)
5	A/A	G/G	2.9 (11)	2.9 (18)
6	G/G	G/G	2.1 (8)	1.5 (9)
7	G/A	A/A	1.3 (5)	2.1 (13)
8	G/G	G/A	0.5 (2)	0.6 (4)

CRC patients *vs*. controls: not significant.

(22), and breast cancer (9). To the best of our knowledge, such studies are very limited regarding CRC. In the current study, we attempted to determine whether an association exists between the functional polymorphisms MMP12 *-82A→G* or MMP13 *-77A→G* and CRC in Swedish patients. We observed no differences between CRC and controls in the genotypic and allelic frequencies. On the other hand, we found a significant difference in genotypic distributions for MMP12 in patients with CRC between those with localized and those with disseminated disease. Moreover, we found the AA genotype at a rate of 68.9% in localized disease in comparison with 79.5% in the patients with disseminated disease. The dominance of the AA genotype in those with disseminated disease was significant, with a OR=1.77.

It has been shown that *MMP12 -82A→G* gene polymorphism is located in the activator protein-1 (AP1) transcription factor-binding site and that greater binding affinity of AP1 to the A allele is associated with higher MMP12 promoter activity (19). The level of AP1 is increased in CRC (23) and may affect MMP12 expression. There are multiple distinct genetic pathways to CRC development. Later stages of cancer differ from early-stage cancer due to different activated pathways (2, 3). In CRC, it is reported that AP1 activity is stimulated through activation of upstream signaling pathways, such as mitogen-activated protein kinases (23). By this pathway, AP1 is involved in colorectal carcinogenesis and may have impact on the control of MMP12 expression.

Our study demonstrated that the carriers of the AA MMP12 genotype were at a higher risk of developing disseminated CRC. This could support the hypothesis that higher transcriptional activity of the *MMP12* gene leads to potentially higher MMP12 levels, with subsequent enhanced cleavage of structural components of the extracellular matrix, and thereby contributes to an elevated risk for dissemination of CRC.

In conclusion, our study indicated that the functional polymorphism *-82A→G* of the *MMP12* gene may play an important role in CRC progression to disseminated disease. Further studies on a larger population of Swedish patients with CRC and on a different ethnic population will be carried out to further define the importance of *MMP12* gene polymorphism as a marker for disseminated disease and survival rate.

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