Matrix Metalloproteinase-1 Promoter Polymorphism 1G/2G Is Correlated with Colorectal Cancer Invasiveness

Giorgio Ghilardi,[1] Maria Luisa Biondi, Jacopo Mangoni, Simona Leviti, Marco DeMonti, Emma Guagnellini, and Roberto Scorza

Dipartimento di Medicina, Chirurgia e Odontoiatria, Clinica Chirurgica Generale, Università degli Studi di Milano-Polo S. Paolo [G. G., J. M., M. D., R. S.], and Laboratorio di Chimica Clinica e Microbiologia, Ospedale S. Paolo-Polo Universitario [M. L. B., S. L., E. G.], I-20142 Milano, Italy

ABSTRACT

***Purpose*: Matrix metalloproteinase-1 (MMP-1) is likely to be involved in invasion and metastasis of several tumors by degrading the extracellular matrix. A single guanine insertion polymorphism (2G) in the MMP-1 promoter region creates an Ets binding site causing the elevation of transcriptional level and local expression of MMP-1. The aim of this study was to evaluate the impact of this 2G insertion type polymorphism on invasion and metastasis of colorectal cancer (CRC).**

***Experimental Design:* We genotyped for this 1G/2G polymorphism 60 patients, who were operated on for CRC and followed for 6–30 months (median: 21). A control population of 164 age- and sex-matched tumor-free subjects was also genotyped for the same polymorphism.**

***Results:* The proportion of 2G homozygotes was higher in the CRC group than in the controls ($P = 0.014$; odds ratio, 2.21; 95% confidence interval, 1.17–4.16). The CRC group was divided in a group without metastasis (M−) and a group that had developed metastasis (M+). At the time of diagnosis, 2G homozygotes were more represented in the M+ group than in M− ($P = 0.0082$; odds ratio, 4.73; 95% confidence interval, 1.46–15.26). The difference between M− patients and controls did not achieve statistical significance ($P = 0.52$).**

***Conclusions:* Our results suggest that the presence of 2G polymorphism at the MMP-1 promoter region may favor the growth and the metastatic process in CRC patients and could be looked at as a risk factor for a worse prognosis.**

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[1] To whom requests for reprints should be addressed, at Dipartimento di Medicina, Chirurgia e Odontoiatria, Clinica Chirurgica Generale, Università degli Studi di Milano-Polo S. Paolo, Via A. Di Rudinì, 8, I-20142 Milano, Italy. Fax: 39-02-8137613; E-mail: giorgio.ghilardi@unimi.it.

INTRODUCTION

CRC[2] is the second most common cancer in males and the third most common in females in Italy.

An orderly sequence of nonrandom events leads to the development of CRC through the transformation of epithelial tissue into premalignant adenomatous polyp and eventually into invasive carcinoma (1).

The outcome depends on the degree of bowel wall invasion and on metastatic spread to regional lymph nodes or distant organs.

Disease staging takes into account the histopathological evaluation of the surgical specimen that includes locoregional and interaortocaval nodes.

Basal membrane and extracellular matrix represent two physical barriers to malignant invasion: their degradation by MMPs plays a key role in tumor progression and metastatic spread (2).

Among proteolytic enzymes, the MMP family has a substrate-specific degradation activity; specifically, MMP-3 (stromelysin-1) acts on type IV collagen that forms the basal membrane, and MMP-1 (interstitial collagenase) acts on fibrillar collagen and gelatin; MMP-3 has an additional activity activating pro-MMP-1 to MMP-1.

A prognostic value of MMP expression in tumor tissue has been shown (3); the relevance of MMP-1 in both CRC and esophageal cancer has been reported by Murray *et al.* (4, 5), and the relevance of MMP-3 in both CRC and breast cancer has also been observed (6, 7). The metastatic process is associated with local overexpression of MMPs (2).

Heppner *et al.* (8) have shown in breast cancer that the expression of many metalloproteinases in tumor tissue may represent a tumor-induced host response.

Rutter *et al.* (9) have reported recently that the insertion of a G nucleotide at −1607 bp in the nucleotide sequence of the MMP-1 gene promoter generates a new 5′-GGA-3′ sequence that corresponds to a core recognition sequence of the binding site for members of the Ets family of transcription factors (10, 11). The 2G homozygous polymorphism of the promoters results in an increased transcription activity in melanoma cell lines and in normal fibroblasts compared with 1G homozygotes and controls (9).

Another two reports have demonstrated the correlation between the 2G allele and ovarian carcinoma and endometrial carcinoma, respectively, both in their homozygous and heterozygous forms (12, 13).

Similarly, the insertion/deletion mechanism of an A nucleotide at −1171 bp in the MMP-3 gene promoter sequence

[2] The abbreviations used are: CRC, colorectal carcinoma; MMP, matrix metalloproteinase; OR, odds ratio; CI, confidence interval; G, guanine; A, adenosine.

results in a polymorphism (5A/6A) in which the transcriptional activity of the 5A homozygous is approximately double that of the 6A homozygous (14). In addition to its proteolytic activity, MMP-3 releases several cell surface molecules such as E-cadherin, a known contributor to cancer development (15).

The aim of this study was to investigate possible correlations between MMP-1 and MMP-3 promoter polymorphisms and CRC clinical phenotypes and, specifically, if genetic analysis is capable of identifying a subgroup of CRC patients with a disease which is more aggressive or prone to metastasize.

MATERIALS AND METHODS

MMP-1 and MMP-3 gene promoter sequences were obtained form peripheral blood samples of 60 patients with CRC of different stages who underwent surgery and of 164 sex- and age-matched healthy subjects (control group). CRC patients were 40 males and 20 females; the median age was 68 years (range: 32–88).

CRC patients were grouped according to Duke's classification modified by Astler and Coller, on the basis of the postoperative histopathological evaluation. The group assignment was than reevaluated at the end of the follow-up period that ranged from 6 to 30 months (median: 21 months); the 60 patients with CRC were assigned to two subgroups according to the presence (M+) or absence of detectable metastasis (M−) at the time of diagnosis and at the end of the follow-up.

The INSTA GENE (Bio-Rad) commercial kit was used for DNA extraction from whole blood collected using K_3EDTA as anticoagulant.

The PCR reaction for MMP-1 and MMP-3 was carried out in a total volume of 25 μl with 5 ml of extracted genomic DNA; 100 μM dATP, dGTP, dTTP, and dCTP; 1.5 mM $MgCl_2$; and 1 unit of Taq polymerase; the two primers, forward and reverse, were each at a concentration of 80 nM. The primers were designed with the Primer Express software. The MMP-1 primer sequence is: forward primer 5′-CCCTCTTGAACTCACATGTTATG-3′; reverse primer 5′-ACTTTCCTCCCCTTATGGATTCC-3′. For MMP-3: forward primer 5′-TCCTCATATCAATGTGGCCAAA-3′; reverse primer 5′-CGGCACCTGGCCTAAAGAC-3′.

The PCR reaction starts with 5-min incubation at 94°C to activate the enzyme, followed by 35 cycles of 20 s at 94°C, 20 s at 55°C, and 30 s at 72°C.

The amplification was verified on an agarose gel (2%) followed directly by sequencing with an automatic sequencer in fluorescent DNA capillary electrophoresis (ABI Prism 310; Applied Biosystems).

Statistical analysis was performed by χ^2 test with the Sigmastat for Windows Version 2.03 software (SPSS, Inc.). Yates correction for continuity was used, and OR was calculated when required.

RESULTS

The polymorphism distribution in the controls was as expected, according to the Hardy-Weinberger principle.

In CRC patients, the MMP-1 allelic variation was significantly different compared with controls ($P = 0.018$; OR, 2.21; 95% CI, 1.17–4.16), with the distribution curve shifted to the right (greater frequency of 2G homozygosity).

Table 1[a] MMP-1 and MMP-3 promoter polymorphisms in patients and controls

	Controls	CRC	OR (95% CI)	*P*
1G/1G + 1G/2G	128	37	2.21 (1.17–4.16)	0.0137
2G/2G	36	23		
5A/5/A	42	9	1.95 (0.89–4.23)	0.0936
5A/6A + 6A/6A	122	51		

[a] ORs (equivalent to relative risk) were calculated as the measure of the association of the MMP-1 and MMP-3 genotypes with CRC.

Table 2[a] MMP-1 promoter polymorphism in M+ and M− CRC subgroups

		M+	M−	OR (95% CI)	*P*
T_0[b]	1G/1G +2G/2G (37)	6	31	4.73 (1.46–15.26)	0.0082
	2G/2G (23)	11	12		
$T_{f\text{-}u}$	1G/1G +1G/2G (37)	12	25	3.90 (1.32–11.54)	0.0131
	2G/2G (23)	15	8		

[a] ORs (equivalent to relative risk) were calculated as the measure of the association of the MMP-1 genotypes with the presence (M+) or the absence (M−) of metastases.

[b] T_0, time of diagnosis; $T_{f\text{-}u}$, end of follow-up.

In CRC patients, no difference in MMP-3 allelic variant distribution compared with controls was noted.

The relevant figures are summarized in Table 1.

Subgroups have been compared with controls and among allelic variants.

MMP-3 promoter allelic variant distribution has shown no statistically significant differences between subgroup M+ (presence of metastases) *versus* controls, nor between subgroups M+ *versus* M− (absence of metastases) or M− *versus* controls.

As to MMP-1 promoter polymorphism, a strong correlation between 2G homozygosity and the M+ subgroup (presence of metastases) has been observed, both at the time of diagnosis (OR, 4.73; $P = 0.0082$) and at the end of the follow-up period considered (OR, 3.90; $P = 0.0131$; Table 2).

The frequency of the 2G allele showed no statistical difference between controls and metastasis-free (M−) patients ($P = 0.52$).

During follow-up, six deaths were recorded which were directly related to malignancy; 3 patients were homozygous for 2G, and 3 were heterozygous (1G/2G) for MMP-1 promoter.

DISCUSSION

A correlation between the transcription-enhancing insertion of a single G nucleotide in the MMP-1 gene promoter region and MMP-1 overexpression has been shown previously in ovarian cancer tumor tissue (12). Similarly, an association between local MMP-1 overexpression and unfavorable prognosis has been reported for both CRC and esophageal cancer (4, 5).

The elevated frequency of 2G homozygosity in melanoma and breast cancer cell lines has been considered responsible of a more aggressive behavior by virtue of higher MMP-1 levels present in tumor tissues (9, 12).

The attempt to correlate MMP-1 gene promoter insertion/deletion polymorphisms with CRC clinical stage and prognosis

shifts the focus from tumor-related factors to genetically predetermined host response mechanisms that relate to cancer invasion susceptibility.

Our results point out a significant difference in MMP-1 allelic variants distribution with a 2.21 exceeding risk of CRC for 2G homozygous patients.

The subsequent analysis of initial staging and clinical outcome seems to suggest differences in tumor biological behavior which are associated with host polymorphism. The frequency of metastases at the time of diagnosis is 4.7-fold higher in 2G homozygous patients. At the end of the follow-up, the percentage of metastases in 2G homozygous patients was twice (2G/2G: 65%; 1G/1G + 1G/2G: 32%).

The increased aggressiveness of the tumor associated with the host 2G allele is paralleled by the observation that the frequency of the 2G allele is substantially higher in the M+ subgroup relative to both the control group and the M− subgroup. The lack of a statistically significant difference in the frequency of the 2G allele between controls and metastasis-free CRC patients seems to support the relevance of host-dependent factors in the limitation of CRC invasiveness.

MMP-3 is known to lyse basal membrane collagen and to induce the synthesis of other MMPs, among which MMP-1. It may play a role both in local invasiveness and metastatic spread; the latter in fact 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 (16) is consistent with this hypothesis. The insertion of an A in the MMP-3 gene promoter sequence halves its transcription activity. It is conceivable the higher transcription activity associated with the 5A allele may enhance tumor invasiveness.

Our study demonstrates the lack of correlation between CRC, its clinical stage, the presence of metastases, and the 5A/6A polymorphism of the MMP-3 promoter, questioning the role of host 5A allele as a relevant factor inducing the well-known local MMP-3 overexpression. This feature seems to differentiate CRC patients from other patients with breast, lung, and ovarian cancer (15, 17).

In conclusion, our study seems to suggest that the presence of the 2G allele in the MMP-1 gene promoter sequence may be a facilitating factor for cancer growth and metastasis in CRC patients. This activity might be mediated by host MMP-1 overexpression induced by the tumor, which seems to decrease the ability of CRC-affected individuals to limit, both locally and systemically, tumor cell invasiveness.

We may hypothesize that correcting for other potentially relevant variables (social status, quality of health care services, and environmental and nutritional variables), the presence of the 2G allele is associated with increased tumor growth and invasiveness, and thus, the higher frequency of the 2G allele in advanced-stage CRC patients compared with more localized cases may not be explained by a delay in diagnosis but, rather, may represent a true additional risk factor.

REFERENCES

1. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–767, 1990.

2. Forget, M. A., Desrosiers, R. R., and Beliveau, R. Physiological roles of matrix metalloproteinases: implications for tumor growth and metastasis. Can. J. Physiol. Pharmacol., *77:* 465–480, 1999.

3. McDonnel, S., and Matrisian, L. M. Stromelysin in tumor progression and invasion. Cancer Metastasis Rev., *9:* 305–319, 1991.

4. Murray, G. I., Duncan, M. E., O'Neal, P., Melvin, W. T., and Fothergill, J. E. Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. Nat. Med., *2:* 461–462, 1996.

5. Murray, G. I., Duncan, M. E., O'Neal, P., McKay, J. A., Melvin, W. T., and Fothergill, J. E. Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. J. Pathol., *185:* 256–261, 1998.

6. Remacle, A. G., Noel, A., Duggan, C., McDermott, E., O'Higgins, N., Foidart, J. M., and Duffy, N. J. Assay of matrix metalloproteinase type 1, 2, 3 and 9 in breast cancer. Br. J. Cancer, *77:* 926–931, 1998.

7. Gallegos, N. C., Smales, C., Savage, F. J., Hembry, R. M., and Boulos, P. B. The distribution of matrix metalloproteinase and tissue inhibitor of metalloproteinases in colorectal cancer. Surg. Oncol., *4:* 111–119, 1995.

8. Heppner, K. J., Matrisian, L. M., and Jensen, R. A. Expression of most matrix metalloproteinase family members in breast cancer represents a tumor induced host response. Am. J. Pathol., *149:* 273–282, 1996.

9. Rutter, J. L., Mitchell, T. I., Butticé, G., Meyers, J., Gusella, J. F., Ozelius, L. J., and Brinckerhoff, C. E. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res., *58:* 5321–5325, 1998.

10. Wang, D. G., Fan, J. B., Siao, C. J., Berno, A., Young, P., Sapolsky, R., Ghandour, G., Perkins, N., Winchester, E., Spencer, J., Kruglyak, L., Stein, L., Hsie, L., Topaloglou, T., Hubbel, E., Robinson, E., Mittmann, M., Morris, M. S., Shen, N., Kilburn, D., Nusbaum, C., Rozen, S., Hudson, T. J., and Lander, E. S., *et al.* Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. Science (Wash. DC), *280:* 1077–1082, 1998.

11. Graves, B. J. Inner workings of a transcription factor partnership. Science (Wash. DC), *279:* 1000–1001, 1998.

12. Kanamori, Y., Matsushima, M., Minaguchi, T., Kobayashi, K., Sagae, S., Kudo, R., Terakawa, N., and Nakamura, Y. Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Res., *59:* 4225–4227, 1999.

13. Nishioka, Y., Kobayashi, K., Sagae, S., Ishioka, S., Nishikawa, A., Matsushima, M., Kanamori, Y., Minaguchi, T., Nakamura, Y., Tokino, T., and Kudo, R. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter in endometrial carcinomas. Jpn. J. Cancer Res., *91:* 612–615, 2000.

14. Ye, S., Eriksson, P., Hamsten, A., Kurkinen, M., Humphries, S. E., and Henney, A. M. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J. Biol. Chem., *271:* 13055–13060, 1996.

15. Sternlicht, M. D., Bissel, M. J., and Werb, Z. The matrix metalloproteinase stromelysin-1 acts as natural mammary tumor promoter. Oncogene, *19:* 1102–1113, 2000.

16. Chambers, A. F., and Matrisian, L. M. Changing views of the role of matrix metalloproteinases in metastasis. J. Natl. Cancer Inst. (Bethesda), *89:* 1260–1270, 1997.

17. Biondi, M. L., Turri, O., Leviti, S., Seminati, R., Cecchini, F., Bernini, M., Ghilardi, G., and Guagnellini, E. MMP-1 and MMP-3 polymorphism in promoter regions and cancer. Letter. Clin. Chem., *46:* 2023–2024, 2000.

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