Matrix metalloproteinase-7 expression in gastric carcinoma

M Honda, M Mori, H Ueo, K Sugimachi, T Akiyoshi

Abstract

Background/Aims—Matrix metalloproteinase-7 (MMP-7) belongs to the same family as matrix degrading metalloproteinase (MMPs) that may play an important part in cancer cell invasion and metastasis. This study reports on the MMP-7 mRNA expression level both in human gastric carcinomas and the normal gastric mucosa.

Methods—From fresh specimens of 47 surgical pairs of primary gastric carcinomas and corresponding normal tissue specimens, cDNA was obtained by reverse transcription (RT) and thereafter MMP-7 mRNAs were detected by means of a polymerase chain reaction. The tumour/normal (T/N) ratio of MMP-7 expression was calculated after correcting for glyceraldehyde-3-phosphate dehydrogenase as an internal control.

Results—The expression corrected levels of MMP-7 mRNA of the tumour was greater than that of the normal mucosa in 41 of 47 cases (87%). The 13 cases whose T/N ratio was more than 2.1 showed a deeper invasion of the gastric wall, and more frequent lymphatic or vascular permeations than the 34 cases whose T/N ratio was less than 2.0. An immunohistochemical study showed that MMP-7 was predominantly expressed in the cancer cells, weakly expressed in normal epithelial cells, and not expressed in the surrounding stromal cells.

Conclusions—These findings suggest that the overexpression of MMP-7 may thus play an important part in tumour invasion in gastric carcinomas while, in addition, MMP-7 may also prove to be a useful marker for determining the biological aggressiveness of gastric carcinoma.

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Keywords: gastric carcinoma, matrix metalloproteinase-7.

There are many steps involved in the invasion or metastasis of cancer cells to either surrounding or distant organs.¹ An initial step of the process may be the degradation of the components of the extracellular matrix and the basement membrane. Many proteolytic enzymes such as metalloproteinases, serine or aspartic proteinases, have been reported to play an essential part in such matrix degradation. Among the proteolytic enzymes, the matrix metalloproteinases (MMPs) are con-

sidered to have an important role in this event. The MMPs have been categorised into three groups consisting of: the collagenases, the stromelysins, and the gelatinases.² MMP-7 is a member of the stromelysin subclass and is the smallest member of the MMP family.

We have used the technique of subtractive complementary DNA (cDNA) cloning to determine potential new markers for human gastrointestinal carcinomas.³⁻⁶ From a subtracted cDNA library constructed from human gastric cancer tissue minus normal gastric mucosa, we determined one overexpressed clone that showed a 96% identity with MMP-7.⁷ This thus prompted us to study MMP-7 expression in clinical samples of human gastric carcinoma. MMP-7 has been reported to be overexpressed in several kinds of carcinomas, however, there is still little information on the significance of MMP-7 in human gastric carcinomas.⁷⁻¹¹ We report on the results of the expression of MMP-7 in human gastric carcinomas and also discuss the significance of its overexpression in patients with gastric carcinoma.

Methods

Patients and tissue sampling

The fresh surgical specimens included 47 primary gastric carcinomas and their paired adjacent normal gastric mucosa. Immediately after resection, the necrotic and ulcerated parts of the tumour were removed and the normal gastric mucosa was dissociated from the muscle and connective tissue. All tissue specimens were then frozen in liquid nitrogen and kept at -90°C until the extraction of RNA.

Extraction of total RNA

The total cellular RNA was extracted from specimens according to the method of Chirgwin *et al.*¹² Briefly, each specimen was homogenised in guanidinium isothiocyanate, the total RNA was extracted, and ultracentrifuged through a cesium chloride solution at 32 000 rpm for 20 hours. The concentration of RNA was measured using a spectrophotometer (DU-70; Beckman, Fullerton, CA).

RT-PCR and analysis

A reverse transcriptase-polymerase chain reaction (RT-PCR) was done as follows: oligonucleotide primer pairs for MMP-7 and GAPDH were synthesised on a DNA synthesiser (Applied Biosystems, Foster City,

Department of Surgery, Medical Institute of Bioregulation, Kyushu University, Beppu, Japan
M Honda
M Mori
T Akiyoshi

Department of Surgery, Oita Prefectural Hospital, Oita, Japan
H Ueo

Department of Surgery II, Kyushu University, Fukuoka, Japan
K Sugimachi

Correspondence to:
Dr M Mori,
Department of Surgery,
Medical Institute of Bioregulation,
Kyushu University,
4546 Tsurumibaru,
Beppu 874, Japan.

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CA) (sense MMP-7, 5'-AGATGTGGAGTG-CCAGATGT-3'; anti-sense MMP-7, 5'-TACTGCTACCATCCGTCC-3'^{10 13}; sense GAPDH, 5'-GTCAACGGDTTGGFCGT-ATT-3'; anti-sense GAPDH, 5'-AGTCTTCTGGGTGGCAGTGAT-3'¹⁴). The oligonucleotide primer was end labelled with ³²P-adenosine 5'-triphosphates (Amersham, Tokyo, Japan) at 3000 Ci/mmol using T4 polynucleotide kinase (New England Biolabs, Beverly, MA), followed by the removal of unincorporated ³²P using a spin column. A polymerase chain reaction was carried out in a 25 µl volume containing 20–30 ng of cDNA template, 10 pmol each of oligodeoxynucleotide primer, 200 mM each of deoxynucleotide triphosphate, 1.5 mM magnesium chloride, 0.01% gelatin, and 1.5 U of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). The samples were overlaid with mineral oil and processed through 24 cycles consisting of one minute at 94°C (denaturation), two minutes at 57°C (annealing), and two minutes at 72°C (elongation). The aliquots of the amplified DNA by polymerase chain reaction were mixed with a formamide gel loading buffer and then were electrophoresed on 2% agarose gels. The gels were dried, and exposed to an imaging plate. The signals were then quantified using a Bio-Image analyser BAS 1000 (Fuji Photo Coop, Tokyo, Japan). The mRNA expression in tumour (T) and normal (N) tissue in each pair was then estimated based on the counts obtained. The tumour-normal ratio of MMP-7 expression (T/N ratio) was calculated after a correction for that of GAPDH expression.

We determined the nucleotide sequence of each PCR product and confirmed that both were identical to the expected fragments of the cDNAs of MMP-7 and GAPDH. A GenBank-UPD New sequences library nucleotide database search demonstrated the sequence to be specific for MMP-7. We also confirmed that the normal tissue examined for the DNA sequence did not contain any carcinoma tissue at all by studying a frozen section.

To check for any possible artefacts based on the possible contamination of RNA by

genomic DNA during RT-PCR, a few RT-PCR reactions were done using a truly positive sample under the same conditions but with no reverse transcription step.

Immunohistochemistry

To identify the localisation of MMP-7 in the gastric cancer tissue specimens, an immunohistochemical analysis was performed in 10 cases by using a method described previously.⁵ Briefly, five micron thin sections were cut from the AMeX fixed, paraffin wax embedded block. The primary polyclonal antibody used was kindly provided by Professor Miyazaki of the Kihara Institute for Biological Research, Yokohama City University, Japan. The recommended dilution was 1:100. Immunostaining was done by the avidin-biotin-peroxidase method.

Clinicopathological findings

The criteria for pathological diagnosis, such as gross type, depth of tumour invasion, and vascular vessel invasion of the gastric carcinoma tissue, were based on the general rules for gastric cancer outlined by the Japanese Research Society for Gastric Cancer.¹⁵ The histological type was determined according to Lauren's classification.¹⁶

Statistical methods

The associations between the variables were tested by Fisher's exact probability test.

Results

To approve the quantitative analysis for MMP-7 expression using our RT-PCR method, the values of the radioactivity of the specific band for either MMP-7 or GAPDH were examined based on the various numbers of PCR cycles. Figure 1 shows the values of radioactivity increased linearly at the point of 24 cycles, and thus indicated the quantitative assessment in this assay. The present analysis showed that gastric carcinoma and normal mucosa showed variable levels of MMP-7 mRNA signal. The T/N ratio of MMP-7 mRNA, which was corrected for that of GAPDH mRNA, ranged from 0.6 to 3.0, and was less than 1.0 in six cases (13%). In 87% of the cases, the expression of MMP-7 mRNA was greater in T than in N. Figure 2 shows representative cases whose T/N ratio ranged from 0.85 to 2.92.

When used in practical evaluations, it is desirable to establish the cut off value of the T/N ratio to determine the malignant potentiality in each case. We therefore tried several cut off values to select the best one. In fact we tried T/N ratios of 1.3, 1.6, 2.0, and 2.5. The mean value of T/N ratio of all 47 cases was 1.6. For 1.3 or 1.6, no significant differences in the known prognostic factors between the low T/N group and the high T/N group were seen. With 2.5, several pathological factors showed a significant difference between

Figure 1: Correlation between the radioactivity of PCR products and the number of PCR cycles. In both MMP-7 and GAPDH, 24 cycles of PCR are performed according to the conditions mentioned in Methods.

Figure 2: MMP-7 mRNA expression is seen in six representative cases of gastric carcinoma detected by RT-PCR. T=tumour tissue and N=normal tissue. GAPDH is used as an internal control. The T/N ratio of MMP-7 mRNA corrected for that of GAPDH mRNA is 1.51, 2.71, 2.92, 0.98, and 0.85 in cases 1, 2, 3, 4, 5, and 6, respectively.

the low and the high group, however, the high group number was small (n=8). With 2.0, the high group (group I) number was 13 while the low group (group II) was 34, and several factors showing significant differences between the two were seen. We thus selected 2.0 as the cut off value of T/N ratio. As the Table shows group I cases showed a significantly deeper invasion of cancer cells (p<0.01), and higher frequency of lymphatic or vascular vessel permeation (p<0.05, p<0.01, respectively) than those in group II cases (Table). Although no significant differences were seen, group I cases tended to metastasise to the lymph nodes more frequently and showed a higher stage of disease than the group II cases. On the other hand, there were no significant differences regarding age, sex, tumour location, tumour size, on histological type of the tumours between groups I and II.

An immunohistochemical study was performed on 10 cases. In the normal mucosal tissue, positive staining was seen in the antral gland epithelium and was very weakly seen in the foveolar epithelium (Fig 3A). In tumour tissue, positive staining was strongly seen in carcinoma cells in all 10 cases (Fig 3B). The stromal mesenchymal cells stained negatively in most areas or very weakly positive in some area.

Clinicopathological data and MMP-7 expression

Variables	T/N<2	2≤T/N	p Value
	(n=34)	(n=13)	
Age (mean, age)	68.1	63.3	NS
Sex			
male	25	6	NS
female	9	7	
Histology			
intestinal	16	8	NS
diffuse	18	5	
Depth of invasion			
within the wall	20	2	0.0098
beyond the wall	14	11	
Lymph node metastasis			
absent	17	3	NS
present	17	10	
Lymphatic invasion			
absent	12	0	0.0209
present	22	13	
Vascular vessel invasion			
absent	24	0	0.0001
present	10	13	
Stage of disease			
1, 2	19	3	NS (0.0561)
3, 4	15	10	

Discussion

MMP-7 has a wide range of substrates such as gelatin, proteoglycans, fibronectin, elastin, and casein.^{17 18} Its expression is reported to be regulated by oncogenes, growth factors, or hormones.² MMP-7 is structurally different from the other MMPs: it shares a common domain structure such as N-terminal and zinc binding catalytic domain required for proteolytic activity but lacks a conserved carboxyl-terminal protein domain.¹¹ Another different feature of MMP-7 is that it is expressed almost exclusively by malignant or normal epithelial cells. The other MMPs except for MMP-7 and membrane-type MMP,¹⁸ are expressed by the stromal components of the tissue.^{19 20}

MMPs, including MMP-7, have been shown to be overexpressed in several kinds of carcinomas, and to be associated with tumour invasion, metastasis or progression.²¹ The activity of MMPs are regulated by tissue inhibitors of metalloproteinases (TIMP).^{22 23} Several experimental studies using TIMP showed that an inhibition of metastasis could be achieved by specific inhibitors of the MMPs,²³⁻²⁶ thereby supporting the important part that MMPs play in tumour invasion or metastasis. There have been, however, few experiments that have directly determined the contribution of an individual MMP. With respect to MMP-7, Powell *et al* reported that a human prostate cancer cell line (DU-145), when transfected with MMP-7 cDNA, gained dramatic invasive potential into the diaphragm after an intraperitoneal injection into SCID mice.⁸ Witty *et al* also observed differences in invasive potential between MMP-7-transfected human colon cancer cells and control cells when they were injected into mice.²⁷ They also

Figure 3: Immunohistochemical staining for MMP-7. (A) Very weak staining is seen mainly in the antral gland epithelium. (B) Moderately differentiated carcinoma cells show strong positive staining. No staining is seen in the stromal cells.

emphasised the significant contribution of MMP-7 to the tumorigenicity and progression of colorectal tumours *in vitro*.

There have been few reports studying MMP-7 expression in clinical samples. McDonnell *et al* reported that MMP-7 was overexpressed in six of eight colon carcinomas and in eight of 10 gastric carcinomas by means of a northern blot analysis. They described that there was some correlation between MMP-7 expression and lymph node metastasis, although it was not significant.⁹ Yoshimoto *et al* studied the expression of MMP-7 in 10 cases of colon carcinoma by means of RT-PCR; nine of 10 cases overexpressed MMP-7 gene, but no detailed clinicopathological analysis was reported.¹⁰ We previously studied 47 cases of colorectal carcinoma by means of a northern blot analysis and found that MMP-7 was overexpressed in 39 of 47 tumours compared with their normal counterparts. The expression of MMP-7 mRNA in colorectal tumour tissue increased with increasing Dukes's stage.⁷ Newell *et al*²⁸ and Yamamoto *et al*²⁹ reported that the MMP-7 gene was recognised in colon adenomas, which thus suggested that MMP-7 might play a part in the early events of colon tumour progression.

This study showed that MMP-7 expression in gastric carcinoma tissue was greater than that in the normal mucosal tissue of the stomach in 41 (87%) of 48 cases. In addition, the overexpression of MMP-7 in carcinoma tissue correlated significantly with a deeper invasion of the gastric wall, and more frequent lymphatic or vascular vessel permeations. The findings strongly support the hypothesis that MMP-7 expression plays an important part in tumour invasion. The cases with lymph node metastasis were more frequently seen in group I with a high T/N ratio of MMP-7 expression than in group II with a low T/N ratio, however, the difference was not significant. As the method of RT-PCR used in this study can be done using biopsy specimens,³⁰ the determination of the MMP-7 mRNA expression can be done preoperatively and objectively. Therefore, it will provide useful information that will help in determining whether to use chemotherapy or not. In addition, it will also help surgeons to determine what level of lymph node dissection should be done.

MMP-7 expression has been reported to be localised to tumour cells not in stromal cells as mentioned above. Its expression has been seen not only in tumour cells but also in normal epithelial cells of the head and neck, breast, or prostate.³¹⁻³³ Its expression has also been detected in such tissues as the glomerular mesangium, normal kidney cells, or the endometrial epithelium.^{20 34 35} McDonnell *et al* showed that MMP-7 mRNA was not detectable by a northern blot in any of the 10 normal tissue samples of the stomach.⁹ Our study, however, disclosed that RT-PCR showed MMP-7 mRNA in the normal gastric mucosa of all 47 cases. We confirmed that the amplified DNA product from normal mucosal tissue and that from carcinoma tissue were

both identical to the MMP-7 gene. In addition, the normal tissue did not contain any malignant tissue histologically. To exclude the possibility of contaminating the genomic DNAs, we did the RT-PCR with the omission of the RT enzyme and confirmed there was no amplified DNA product, which thus suggested that there was no contamination of the genomic DNA. The polyclonal antibody for MMP-7 certainly reacted with the normal epithelium of the gastric glands. We therefore consider that MMP-7 was expressed not only in gastric carcinoma cells but also in the normal gastric epithelium, although the expression was weak in the normal epithelium. In conclusion, the overexpression of MMP-7 was recognised in most gastric carcinomas and may therefore contribute to tumour invasion in human gastric carcinomas.

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