

Decreased Matriptase/HAI-1 Ratio in Advanced Colorectal Adenocarcinoma of Chinese Patients

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Abstract

Matriptase is a serine protease expressed by tumors of surface epithelial origin. We tested the expressions of matriptase and hepatocyte growth factor activator inhibitor-1 (HAI-1) maybe associated with the progression of colorectal adenocarcinoma. Immunohistochemical analysis of matriptase and HAI-1 was performed in tissue microarray slides of 91 colorectal adenocarcinomas with various degrees. The matriptase scores in moderately (346.7 ± 10.6) and poorly differentiated (248.1 ± 12.9) were significantly lower than those in well differentiated (368.4 ± 9.6) colorectal adenocarcinomas. The matriptase/HAI-1 ratios in poorly (1.8 ± 0.4) and moderately differentiated (1.8 ± 0.3) were significantly lower than in well differentiated (2.2 ± 0.2) colorectal adenocarcinomas. Otherwise, the matriptase scores and matriptase/HAI-1 ratio showed significant reverse correlation with more advanced TNM stages of colorectal adenocarcinomas in Chinese patients. In conclusion, pharmacological inhibitors of matriptase may not be effective treatment for advanced colorectal adenocarcinomas.

Key Words: colorectal adenocarcinoma, HAI-1, immunohistochemistry, matriptase, microarray

Introduction

Colorectal adenocarcinoma is the most common histological type of primary colon cancer, accounting for 8.5% of all new malignancies (29). Histopathological differentiation, the depth of tumor invasion, and lymph node metastasis are associated with tumor prognosis (5, 6, 13, 30). Identification of mechanisms promoting tumor cell invasion may help direct creation of new therapies that can arrest local invasion and metastatic spread of disease.

Matriptase is a type II transmembrane serine protease that has been detected in several normal tissues rich in epithelium (25). Latent urokinase plasminogen activator (uPA) and pro-hepatocyte growth factor (pro-HGF) are substrates for matriptase (23, 31). Both substrates participate in neoplastic

invasion, most notably in the plasmin-mediated remodeling of the tumor extracellular matrix.

Besides, hepatocyte growth factor activator inhibitor-1 (HAI-1) is a membrane-bound serine protease and defined as an inhibitor of matriptase (22). Paradoxically, HAI-1 is also required for matriptase activation (22). Recently, a study concluded that HAI-1 might play an important role in regulating the carcinogenesis of cultured colorectal adenocarcinoma (18, 19, 27).

Our previous studies have demonstrated that the increase expression of matriptase is associated with more advanced TNM stage of ovary serous adenocarcinoma (15), breast infiltrating ductal carcinoma (16), hepatocellular carcinoma (33), renal cell carcinoma (17), and esophageal squamous cell carcinoma (7) in Chinese. However, the expression

profiles of matriptase and HAI-1 in colorectal adenocarcinomas are still vague.

In this study, we tested the hypothesis that the expression of matriptase, and its inhibitor, HAI-1, are associated with the pathological grading and clinical staging of colorectal adenocarcinoma. Our results demonstrated that significantly decreased matriptase/HAI-1 ratio and matriptase immunostaining score were associated with more advanced stages of colorectal adenocarcinoma.

Materials and Methods

Paraffin-embedded tumor tissues were obtained from the Department of Pathology, Tri-Service General Hospital, and tissue microarray slides were constructed. We selected blocks of 91 primary colorectal adenocarcinomas, including 32 well differentiated (glandular structure >95%), 33 moderately differentiated (glandular structure between 50%-95%), and 26 poorly differentiated tumors (glandular structure <50%). The histopathological differentiation of the colorectal adenocarcinoma was determined according to the criteria of WHO classification of tumor (2).

One core was taken from a selected area of each paraffin-embedded tumor tissue and tissue microarray slides were constructed according to a previously published method (14). Each representative core in the tissue microarray slide was 2 mm in diameter and the pathological diagnosis in these cases was reviewed by at least two experienced pathologists. All tumors were pathologically staged according to the 1997 American Joint Committee on Cancer (AJCC/TNM system). Normal colon tissues were obtained from 9 cases; tissues were taken 4 cm from the neoplasm. None of the cases had received radiation or chemotherapy before surgery. The histopathological diagnosis of colorectal adenocarcinomas was confirmed of by two experienced pathologists.

Immunohistochemistry

Tissue microarray sections were de-waxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 5 min to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating (100°C) each section for 30 min in 0.01 mol/L sodium citrate buffer (pH 6.0). After 3 rinses (each for 5 min in phosphate buffered saline [PBS]), the sections were incubated for 1 h at room temperature with a rabbit anti-human matriptase/ST14 antibody (1:100, Bethyl Laboratories, Montgomery, TX, USA), goat anti-human HAI-1 antibody (1:40, Santa Cruz, Inc., Santa Cruz, CA, USA) diluted in PBS. After 3 washes (each for 5 min in PBS), sections were incubated with biotin-labeled secondary immunoglobulin (1:100,

DAKO, Glostrup, Denmark) for 1 h at room temperature. After 3 additional washes, peroxidase activity was developed with AEC+ substrate chromogen (DAKO, Glostrup, Denmark) at room temperature. Sections of adenocarcinoma of the breast (known to stain positive for matriptase and HAI-1) were used as positive control (12) and colorectal tumor sections stained using the same procedure but omitting the primary antibody, *i.e.*, anti-matriptase or anti-HAI-1, were used as negative control.

For evaluation of immunoreactivity and histological appearance, all tissue microarray experiments were repeated three times and the slides were examined and scored by two authors concurrently. For assessment of matriptase and HAI-1 expression, the colorectal stroma was used as the negative control. In the matriptase and HAI-1 studies, the intensity of cytoplasmic and plasma membrane immunostaining was scored on a scale of 0 (no staining) to 4 (strongest intensity), and the percentage of cells with stained cytoplasm or plasma membrane was estimated at each intensity. Like our previous study (15), the percentage of cells (from 0 to 100) was multiplied by the corresponding immunostaining intensity (from 0 to 4) to obtain an immunostaining score ranging from 0 to 400.

Statistical Analysis

All results are expressed as mean \pm standard error of the mean (S.E.M.). The immunostaining scores of matriptase and HAI-1 of colorectal adenocarcinoma were compared with the score of normal colon epithelia. Statistical analysis was performed using the Student paired *t*-test between groups with a *P*-value smaller than 0.05 was considered to be statistically significant. SigmaState software (Jandel Scientific, San Rafael, CA, USA) was used to perform linear regression testing to analyze the relationship between matriptase scores or matriptase/HAI-1 ratio and clinicopathological parameters.

Results

Clinicopathological Characteristics

The study sample included 51 men and 40 women. The mean age was 65.5 years and ranged from 47 to 75 years. Table 1 shows the histopathological differentiation, tumor classification, and staging distributions.

Matriptase and HAI-1 Expression in Colorectal Adenocarcinoma

The immunostaining results of matriptase and

Table 1. Clinicopathologic characteristics of 91 patients with colorectal adenocarcinoma

Variable	n
Sex	
Male	51
Female	40
Histopathological differentiation	
Well differentiated	32
Moderately differentiated	33
Poorly differentiated	26
TNM category ^a	
T1	10
T2	4
T3	48
T4	29
N0	58
N1	26
N2	7
M0	78
M1	13
Tumor stage ^a	
I	13
II	39
III	26
IV	13

^aAJCC

HAI-1 expression in colorectal adenocarcinoma are revealed in Fig. 1 and Table 2. Non-neoplastic colonic glands (Fig. 1B) all had strong expression of matriptase and the average immunostaining score was 392.4 ± 4.6 . In all 91 colorectal adenocarcinoma specimens, matriptase immunoreactivities were present in the cell membrane and cytoplasm. The matriptase scores in well-differentiated (Fig. 1E, 368.4 ± 9.6), moderately-differentiated (Fig. 1H, 346.7 ± 10.6), and poorly-differentiated (Fig. 1K, 248.1 ± 12.9) colorectal adenocarcinomas were significantly lower than those in normal colon tissue. To find the relationship between matriptase expression and tumor progression, we compared the immunoscores of matriptase in moderately and poorly differentiated colorectal adenocarcinoma to well differentiated colorectal tumors. The matriptase scores in moderately and poorly differentiated colorectal adenocarcinoma were significantly lower than those in well-differentiated colorectal adenocarcinoma (Fig. 2A).

The HAI-1 scores in moderately differentiated (Fig. 1I, 210.0 ± 10.3) colorectal adenocarcinoma was significantly higher than in non-tumorous colonic glands (Fig. 1C, 156.7 ± 11.9), but not in well (Fig. 1F, 198.0 ± 12.5) and poorly-differentiated tumors

(Fig. 1L, 153.3 ± 11.8).

In Table 3, the matriptase/HAI-1 ratio in moderately-differentiated (1.8 ± 0.3) and poorly-differentiated (1.8 ± 0.4) colorectal adenocarcinomas were significantly lower than in normal colon tissue (2.8 ± 0.3). In addition, the matriptase/HAI-1 ratio in poorly-differentiated and moderately-differentiated colorectal adenocarcinoma was significantly lower than in well differentiated colorectal adenocarcinoma (Fig. 2B).

Expressions of Matriptase and HAI-1 Correlate with Clinical Stages

Linear regression testing was performed to analyze the relationship between matriptase or matriptase/HAI-1 ratio and clinical TNM stage. Matriptase had significant reverse correlation with the T, N and clinical TNM stages (data not shown). In addition, matriptase/HAI-1 ratio had a significant reverse correlation with the T and clinical TNM stages (data not shown).

Relationship Between Matriptase and HAI-1 Expression and Survival Time in Patients with Colorectal Adenocarcinoma

Among the 51 patients with colorectal adenocarcinoma at the 5-year follow-up periods, two groups were formed based on the matriptase and HAI-1 immunoscores. Accordingly, 27 cases with a higher expression of matriptase (immunostaining score > 330) were placed in one group and the remaining 24 cases with lower immunoreactivity (immunostaining score ≤ 330) comprised the other group. In addition, two groups were created based on HAI-1 immunoscores, with 29 cases having a higher expression of HAI-1 (immunostaining score > 180) and 22 cases having lower HAI-1 immunoreactivity (immunostaining score ≤ 180). The survival times in patients with colorectal adenocarcinoma were not significantly correlated with matriptase or HAI-1 immunoscores ($P > 0.05$, Figs. 3 and 4).

Discussion

Multiple factors (such as familial inherited disease, genetic mutation, cellular cycle regulator dysfunction, and imbalances in growth factors) increase the incidence and progression of colorectal cancer (9, 10, 13). However, the molecular events of early metastasis and rapid progression of colorectal cancer are still unknown. In some reports, the involvement of vascular endothelial growth factor-induced angiogenesis (19, 20), matriptase-caused extracellular matrix proteolysis (1, 4, 23, 27), dysregulation of cell

Table 2. Immunostaining scores of matriptase and HAI-1 in colorectal adenocarcinoma

Differentiation	No. of Cases	Intensity	% Staining	Total Score
Matriptase staining				
Normal colon tissue	9	3.9 ± 0.3	98.2 ± 0.4	392.4 ± 4.6
Well	32	3.8 ± 0.4	98.0 ± 0.9	$368.4 \pm 9.6^*$
Moderately	33	3.7 ± 0.3	93.6 ± 1.8	$346.7 \pm 10.6^*$
Poorly	26	2.8 ± 0.4	89.6 ± 1.8	$248.1 \pm 12.9^*$
HAI-1 staining				
Normal colon tissue	9	1.7 ± 0.2	92.2 ± 1.1	156.7 ± 11.9
Well	32	2.1 ± 0.3	95.5 ± 1.6	$198.0 \pm 12.5^*$
Moderately	33	2.2 ± 0.3	96.4 ± 1.1	$210.0 \pm 10.3^*$
Poorly	26	1.6 ± 0.4	96.1 ± 1.2	153.3 ± 11.8

Data are means \pm standard error of the mean (SEM) of immunostaining score of matriptase and HAI-1 in colorectal adenocarcinomas and non-tumor normal colon tissue. *Indicates statistical significance comparing with normal colon glands ($P < 0.05$).

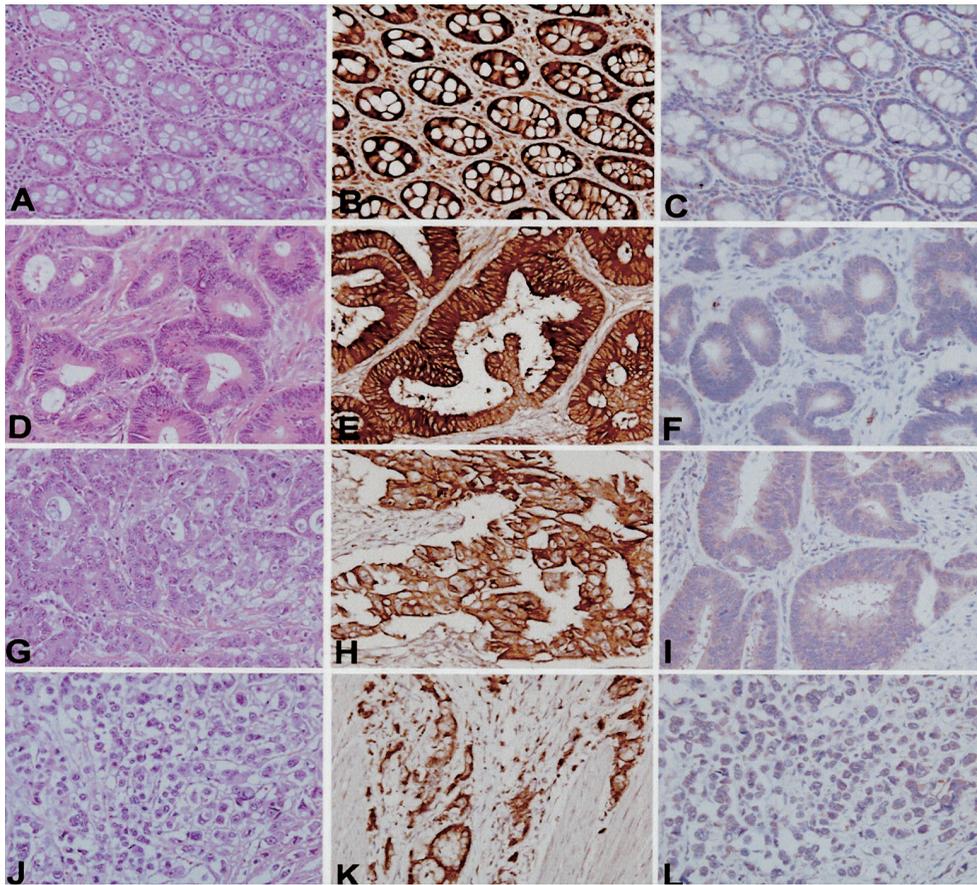


Fig. 1. Hematoxylin and eosin staining of non-neoplastic colon tissue (1A), well differentiated (1D), moderately differentiated (1G), and poorly differentiated (1J) colorectal adenocarcinomas; and immunohistochemical analysis of matriptase in non-neoplastic colon tissue (1B), well differentiated (1E), moderately differentiated (1H), and poorly differentiated (1K) colorectal adenocarcinomas; and immunohistochemical analysis of HAI-1 in non-neoplastic colon tissue (1C), well differentiated (1F), moderately differentiated (1I), and poorly differentiated (1L) colorectal adenocarcinomas. Original magnification $\times 400$.

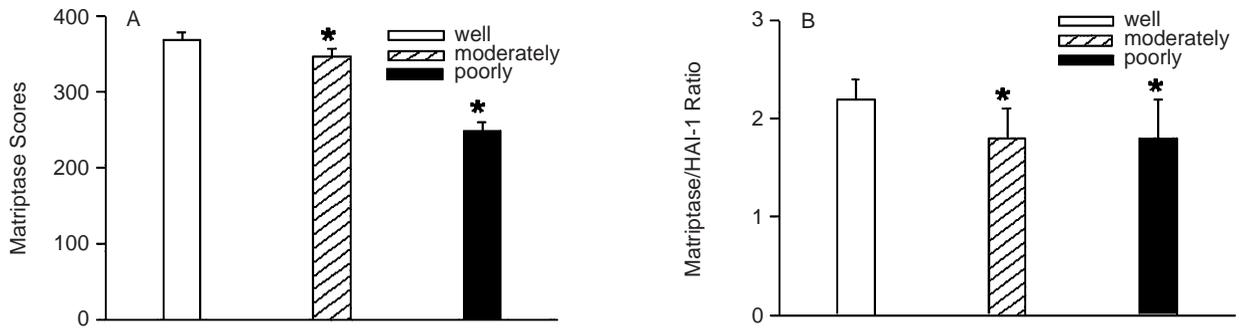


Fig. 2. Immunostaining scores of matriptase (A) and matriptase/HAI-1 ratio (B) in well differentiated, moderately differentiated, and poorly differentiated colorectal adenocarcinoma. *Indicates statistical significance comparing to well differentiated colorectal adenocarcinoma ($P < 0.05$).

Table 3. Matriptase/HAI-1 ratio in colorectal adenocarcinoma

Differentiation	No. of cases	Matriptase/HAI-1 Ratio
Normal colon tissue	9	2.8 ± 0.3
Well	32	2.2 ± 0.2
Moderately	3	1.8 ± 0.3*
Poorly	26	1.8 ± 0.4*

Data are means ± standard error of the matriptase/HAI-1 ratio in colorectal adenocarcinomas and non-tumor normal colon tissue. *Indicates statistical significance comparing with normal colon glands ($P < 0.05$).

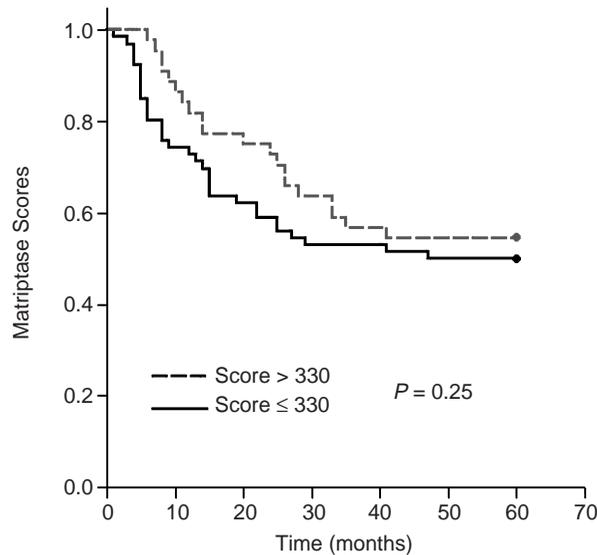


Fig. 3. Overall survival of 51 patients with colorectal adenocarcinomas. The immunostaining scores of matriptase did not significantly correlate with patient's survival rate. Survival rates were analyzed using the Kaplan-Meier survival test.

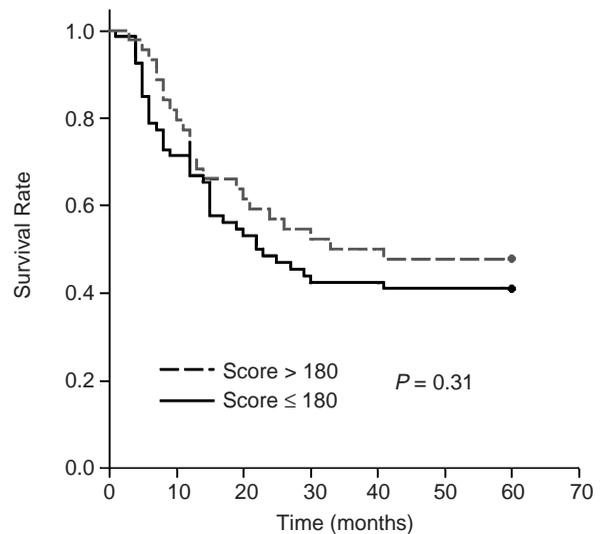


Fig. 4. Overall survival of 51 patients with colorectal adenocarcinomas. The immunostaining scores of matriptase did not significantly correlate with patient's survival rate. Survival rates were analyzed using the Kaplan-Meier survival test.

cycles (3, 24, 32), and mutation of tumor suppressor gene (2, 28) have been demonstrated by molecular and clinical studies. However, direct evidence for the relationship between these prognostic factors and clinicopathological grading and staging systems is still lacking.

In our study, all the tumor tissues were placed in a single tissue array slide. The tissue microarray technique is a powerful tool for simultaneous histological and immunohistochemical evaluation of tumors (20). Previous studies measuring immunohistochemical intensity of individual cases were limited because of the variability of the chemical signal generated under different environmental conditions (20). Recent results

support the reliability of immunohistochemistry conducted on tissue microarray slides (20). In our study, the clear cut difference in matriptase and HAI-1 staining between the colonic stromal and glandular components validated this use of tissue microarray slides.

Matriptase, a pro-invasion and metastasis factor, has the ability to cleave and activate growth factors and other serine proteases in several malignancies, such as ovarian and cervical cancers (21, 26). However, matriptase is also expressed on the cell membrane of some normal epithelia, especially in secretory organs and the gastrointestinal tract (25). Some published studies reveal an inconsistent relationship between matriptase expression and tumor progression. For example, matriptase expression increased in the advanced stage of breast and cervical cancer but decreased in the advanced stage of ovarian cancer (21, 26, 27).

Previous study showed that the matriptase gene is located within chromosome 11q24-q25 (6), and these regions are associated with poor survival of patients with ovary cancer (11, 21) and breast cancer (12). In contrast to patients with ovary cancer, the LOH of 11q24-q25 in colorectal adenocarcinoma is not correlated with adverse survival (8). In addition, a previous study has demonstrated that the cloning LOH in the region of the matriptase gene in colorectal adenocarcinoma is a putative tumor suppressor gene and may inhibit the growth of colorectal adenocarcinoma (34). These chromosomal findings may support our finding that the increased expression of matriptase in colorectal adenocarcinoma is not positively associated with advanced clinico-pathological stage of patients.

HAI-1 is a membranous protein that may suppress the growth of cancer cells by inhibiting the generation of active urokinase-type plasminogen activator (uPA) by matriptase or the activity of additional serine proteases (26). In our study, the more advanced stage of colorectal adenocarcinoma was significantly associated with a lower expression of matriptase and matriptase/HAI-1 ratio. Our results are inconsistent with those of a previously published study that measures the percent of matriptase-positive cases, instead of using an immunoscore system (19). In our study, not only intensity but also the percentage of matriptase- and HAI-1- stained cells was calculated in a single microarray slide. Thus, our study provides a more accurate evaluation of matriptase and HAI-1 expression in colorectal adenocarcinoma.

The relationship between matriptase and HAI-1 expressions and tumor progression and invasion have been well researched for some malignancies, such as breast (22), ovarian (15), cervical (21) and even colorectal carcinoma (19). However, up to now, the association of these biomarkers with clinical stage of colorectal adenocarcinoma has not been established.

Our study for the first time shows that lower matriptase expression and matriptase/HAI-1 ratio are associated with advanced stages of colorectal adenocarcinoma in Chinese patients. These results suggest that matriptase and HAI-1 may not involve in the progression of colorectal adenocarcinoma.

Decrease of matriptase in colorectal adenocarcinomas is associated with advanced differentiation of colorectal adenocarcinoma in Chinese patients. However, we cannot draw any conclusion because the immunoscores of matriptase and HAI-1 did not significantly correlate with advanced clinical stages and survival rates in colorectal adenocarcinoma. Thus, pharmacological inhibitors of matriptase may not be effective treatment for advanced colorectal adenocarcinoma.

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