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World J Gastroenterol 2005;11(39):6202-6207
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Expression of serine protease SNC19/matriptase and its inhibitor hepatocyte growth factor activator inhibitor type 1 in normal and malignant tissues of gastrointestinal tract

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Received: 2005-03-01 Accepted: 2005-04-18

Abstract

AIM: To provide the expression profile of serine protease SNC19/matriptase and its inhibitor hepatocyte growth factor activator inhibitor type 1 (HAI-1) in normal and malignant tissues of gastrointestinal tract at mRNA level for further study on their correlations with tumor progression and metastasis.

METHODS: Total RNAs were prepared from 37 samples of colorectal cancer tissues, 40 samples of gastric cancer tissues, and their adjacent normal tissues. The expression of SNC19/matriptase and HAI-1 in these samples was detected by real-time fluorescent quantitative PCR using glyceraldehyde-3-phosphate dehydrogenase as internal standard, and the clinical significance for the correlation with clinicopathological parameters was evaluated.

RESULTS: In gastric cancer tissues the expression of HAI-1 and SNC19/matriptase was significantly lower than that in the corresponding adjacent normal tissues (Z = -3.280, P = 0.006; Z = -4.651, P = 0.000). HAI-1:SNC19/matriptase ratio showed no difference between normal and malignant tissues (P>0.05). Analysis of clinicopathological parameters showed decreased expression of HAI-1 and HAI-1:SNC19/matriptase ratio associated with stage III/IV gastric tumors as compared to stage I/II ones (Z = -2.140, P = 0.031; Z = -2.155, P = 0.031), and with lymph node-positive gastric cancer tissues as compared to lymph node-negative ones (Z = -2.081, P = 0.036; Z = -2.686, P = 0.006). The expression of SNC19/matriptase had no relationship with stages and lymph node metastasis (P>0.05). The expression of HAI-1 and HAI-1:SNC19/matriptase ratio increased in well-differentiated gastric cancer tissues, but there was no statistical significance (P>0.05). The difference of SNC19/matriptase expression was not significant in gastric cancer tissues of different histological differentiation status (P>0.05). In colorectal cancer tissues, the expression of HAI-1 and SNC19/matriptase was also markedly lower than that in their adjacent normal tissues (Z = -3.100, P = 0.002; Z = -2.731, P = 0.006), whereas HAI-1:SNC19/matriptase ratio showed no difference. Decreased expression of HAI-1 was associated with increased invasive depth and lymph node metastasis, but there was no statistical significance (P>0.05). The difference of SNC19/matriptase expression and HAI-1:SNC19/matriptase ratio was not significant in different stages and different lymph node metastasis status (P>0.05). The expression of SNC19/matriptase, HAI-1 or HAI-1:SNC19/matriptase ratio showed no difference in colorectal cancer tissues of different histological differentiation status (P>0.05).

CONCLUSION: The expressions of SNC19/matriptase and its inhibitor HAI-1 are decreased in gastrointestinal cancer tissues compared to their normal counterparts, and the decreased expression of HAI-1 may correlate with invasion and lymph node metastasis. The possible mechanisms involved need to be further investigated.

Key words: Matriptase; Hepatocyte growth factor activator inhibitor type 1; Expression; Metastasis

Zeng L, Cao J, Zhang X. Expression of serine protease SNC19/matriptase and its inhibitor hepatocyte growth factor activator inhibitor type 1 in normal and malignant tissues of gastrointestinal tract. *World J Gastroenterol* 2005; 11(39): 6202-6207
http://www.wjgnet.com/1007-9327/11/6202.asp

INTRODUCTION

Hepatocyte growth factor (HGF), also known as scatter factor (SF)[1], is a pleiotropic factor which functions as a mitogen, a morphogen, and a motogen for a variety of cells bearing Met receptor tyrosine kinase, particularly for epithelial cells and vascular endothelial cells[2-4]. To date, a significant body of evidence is accumulating in favor of the notion that HGF/SF and Met play an important role in tumorigenesis, invasiveness, differentiation and angiogenesis of tumor cells[5,6]. HGF/SF is secreted by mesenchymal cells as an inactive precursor form, which lacks biological activity. The proteolytic activation of a single-chain precursor

form to a two-chain heterodimeric active form is a critical limiting step in the HGF/SF-induced Met signaling pathway[7]. Five proteases have been supposed to be responsible for the activation of HGF/SF: hepatocyte growth factor activator (HGFA), which exhibits the most potent activity, urokinase-type plasminogen activator (uPA), tissue-type plasminogen activator, blood coagulation factor XIIa[8,9], and SNC19/matriptase[10,11]. SNC19 initially identified from subtractive hybridization screening[12], has been confirmed to be indistinguishable from serine protease matriptase, which was first found in T47-D human breast cancer cells[13]. In addition to activation of HGF/SF, SNC19/matriptase can also degrade extracellular matrix (ECM), and activate another ECM-degrading protease uPA which is involved in tumor invasion[13-15]. SNC19/matriptase contributes to ECM degradation, cellular motility, epithelial migration and turnover, tissue remodeling, and it is supposed to be involved in tumor cell invasion and metastasis[14,16].

Hepatocyte growth factor activator inhibitor type 1 (HAI-1) is a Kunitz-type serine protease inhibitor which was initially purified from the conditioned medium of a human MKN45 stomach carcinoma cell line in 1997[17]. HAI-1 can specifically inhibit serine proteases HGFA and SNC19/matriptase, and they both are activators of HGF/SF[17-19]. Although it is supposed that HAI-1 and SNC19/matriptase may be involved in tumorigenesis, progression, and metastasis[16,20-22], more evidence is needed to draw a definite conclusion. This paper presents new evidence for the expression of HAI-1 and SNC19/matriptase in normal and malignant tissues of gastrointestinal tract at mRNA level, and their correlations with tumor progression and metastasis.

MATERIALS AND METHODS

Patients and tissues

A total of 40 samples from gastric cancer patients (median age, 58.95±12.43 years; range, 29-82 years; 28 men and 12 women) and 37 samples from colorectal cancer patients (median age, 61.64±13.54 years; range, 32-89 years; 13 men and 24 women), after surgical treatment in Second Affiliated Hospital, Zhejiang University College of Medicine, were studied retrospectively. The stage of gastric cancer was classified according to UICC guidelines (1985). Dukes' stage of colorectal cancer was classified according to the diagnosis and treatment criteria for common malignancy in China (1990). Tumor location, tumor histology, and histological grade were determined by pathological examination.

Specimens and RNA preparation

Cancer tissues and their adjacent normal tissues at least 15 cm away from cancer were collected immediately after excision, snap-frozen in liquid nitrogen, and stored at -80 ℃ for RNA extraction. Total RNAs were extracted from the tissue samples by TRIzol reagent (Gibco BRL Co., USA) according to the manufacturer's instructions in 0.1% RNase-free DEPC H_2O. RNA samples were quantified by ultraviolet spectrophotometry (Hewlett-Packard Co., USA).

Primers and probes

Primers and probes used for real-time fluorescent quantitative PCR were designed with the software PrimerExpress (Applied Biosystems Inc., USA). To avoid possible amplification of contaminating genomic DNA, primers were designed, so that each PCR product covered at least one intron. The sequences of primers and probes are listed in Table 1.

Table 1 Sequences of primers and probes

Target gene (amplicon size)		Sequence (5′–3′)
HAI-1	Upstream primer	TGA GGA AGA GCA GCA GTG CC
(151 bp)	Downstream primer	GGC TAC CAC CAC CAC AAT GC
	Probe	*FAM*-CCA GCA CAG GCT CTG TGG AGA TGG C -*TAMRA*
SNC19/ matriptase	Upstream primer	GGG ACA CAC CCA GTA TGG AGG
(168 bp)	Downstream primer	CCG GAA TCA CCC TGG CAG GA
	Probe	*FAM*-TCC TGC AAA AGG GTG AGA TCC GCG -*TAMRA*
GAPDH	Upstream primer	CTT AGC ACC CCT GGC CAA G
(151 bp)	Downstream primer	GAT GTT CTG GAG AGC CCC G
	Probe	*FAM*-CAT GCC ATC ACT GCC ACC CAG AAG A -*TAMRA*

PCR standards

Plasmids containing HAI-1, SNC19/matriptase or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs were constructed and maintained in our laboratory. Purified plasmid DNAs were quantified by absorbance at 260 nm and serially diluted as PCR standards.

Real-time RT-PCR

Two micrograms of total RNA was used in a 25 μL reverse transcription reaction system containing oligo $(dT)_{15}$ and M-MLV reverse transcriptase (Promega Co., USA). For real-time PCR, the cDNA samples were brought to a final concentration of 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 9.0), 1.5 mmol/L $MgCl_2$, 0.1% Triton-X 100, 200 μmol/L dNTPs, 0.2 μmol/L each primer, 0.1 μmol/L probe, and 5 U/μL Taq DNA polymerase (Promega Co., USA). The reactions were carried out under pre-optimized conditions. Samples were amplified with a hold at 95 ℃ for 5 min, followed by 40 cycles of denaturation at 95 ℃ for 20 s, annealing at 60 ℃ for 20 s and extension at 72 ℃ for 20 s in the ABI Prism 7700 sequence detector (Perkin Elmer Co., USA). Serially diluted standard specimens with the concentration of 10^6-10^{10} copies/mL were used to plot the standard curve. No template control and no reverse transcription control were included; 2% agarose gel electrophoresis was used in optimizing procedure.

Statistical analysis

All statistical analyses were done by the SPSS 11.0 software package. Non-parametric data were analyzed with Wilcoxon, Mann-Whitney, and Kruskal-Wallis rank sum tests to evaluate the association between SNC19/matriptase and HAI-1 mRNA expression and clinicopathological parameters. $M(Q_R)$ was computed as summary statistics.

RESULTS

Real-time RT-PCR of SNC19/matriptase and HAI-1

Real-time fluorescent quantitative RT-PCR was performed to determine the expression of SNC19/matriptase and HAI-1 at mRNA level using GAPDH as internal control. Figure 1 shows the detection and plotting of the GAPDH standard curve. The PCR products were subjected to 2% agarose gel electrophoresis in optimized procedure, as shown in Figure 2. The products were specific, with anticipated sizes confirmed by DNA sequencing.

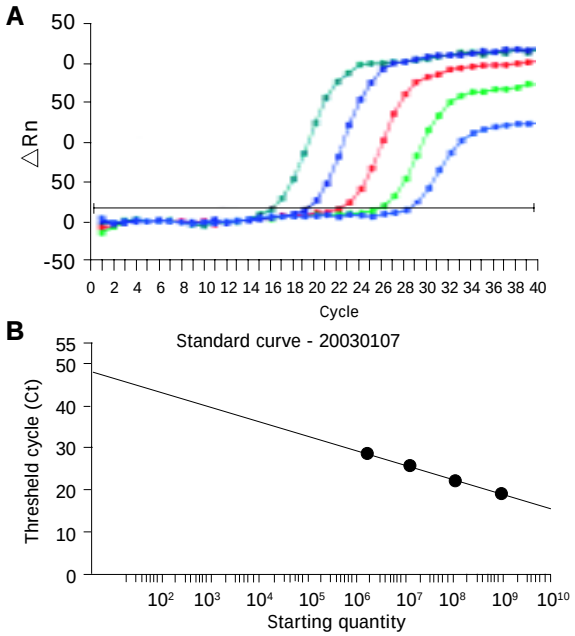

Figure 1 Representative real-time RT-PCR analysis for GAPDH mRNA copy levels. A: Amplification plots of pGEM-T Easy/GAPDH cDNA; B: standard curve plotted against pGEM-T Easy/GAPDH cDNA copy number.

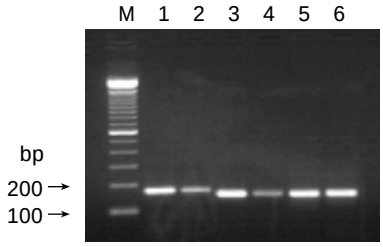

Figure 2 Expression of SNC19/matriptase and HAI-1 in gastric cancer tissue and its corresponding normal tissue (real-time RT-PCR products assay). M: Marker (100-bp DNA ladder); lanes 1 and 2: SNC19/matriptase (168 bp); lanes 3 and 4: HAI-1 (151 bp); lanes 5 and 6: GAPDH (151 bp); lanes 1, 3 and 5: gastric normal tissues; lanes 2, 4, and 6: gastric cancer tissues. Figures in brackets indicate the sizes of real-time RT-PCR products.

Expression of HAI-1 and SNC19/matriptase in normal and malignant tissues of gastrointestinal tract and correlation with clinicopathological parameters

The mRNA copy numbers of HAI-1, SNC19/matriptase and internal control GAPDH in each of normal and malignant tissues of gastrointestinal tract were detected by real-time fluorescent quantitative RT-PCR technique. The ratios of HAI-1:GAPDH and SNC19/matriptase: GAPDH were used as the relative expression level of HAI-1 and SNC19/matriptase, respectively. As shown in Table 2, the expression of HAI-1 and SNC19/matriptase in both gastric and colorectal cancer tissues was markedly lower than that in their adjacent normal tissues ($P<0.01$), while HAI-1:SNC19/matriptase ratio showed no significant difference between normal and malignant tissues of gastrointestinal tract ($P>0.05$).

The correlations between the expression of HAI-1 and SNC19/matriptase and clinicopathological parameters were evaluated (Tables 3 and 4). In gastric cancer tissues (Table 3), decreased expression of HAI-1 and HAI-1: SNC19/matriptase ratio were associated with stage III/IV cancer tissues compared to stage I/II ones ($Z = -2.140$, $P = 0.031$; $Z = -2.155$, $P = 0.031$). The expression of SNC19/matriptase had no relationship with clinical stages ($P>0.05$). The expression of HAI-1 and HAI-1:SNC19/matriptase ratio in lymph node-positive cancer tissues were lower than those in lymph node-negative ones ($Z = -2.081$, $P = 0.036$; $Z = -2.686$, $P = 0.006$). The expression of HAI-1 and HAI-1:SNC19/matriptase ratio increased in well-differentiated gastric cancer tissues, but there was no statistical significance ($P>0.05$). The differences of SNC19/matriptase were not significant in gastric cancer tissues with different histological differentiation status ($P>0.05$). In colorectal cancer tissues, the expression of HAI-1 was lower in Dukes' C/D cancer tissues than in Dukes' B ones. HAI-1 expression in lymph node-positive cancer tissues was lower than that in lymph node-negative ones. Decreased expression of HAI-1 was associated with increased invasive depth and lymph node metastasis, although there was no statistical significance ($P>0.05$). However, the differences of SNC19/matriptase expression and HAI-1:SNC19/matriptase ratio were not significant in different stages and different lymph node metastasis status ($P>0.05$). All the expression of HAI-1 and SNC19/matriptase and their ratio showed no statistical significance in different histological differentiation status ($P>0.05$). In gastrointestinal cancers, the expression of HAI-1 and SNC19/matriptase and HAI-1:SNC19/matriptase ratio showed no difference in patients of different ages, genders, location of tumors, and histological types ($P>0.05$).

Table 2 Expression of HAI-1 and SNC19/matriptase in normal and malignant tissues of gastrointestinal tract [$M(Q_R)$]

	Gastric tissues			Colorectal tissues		
	HAI-1	SNC19	HAI-1:SNC19	HAI-1	SNC19	HAI-1:SNC19
Normal tissues	0.012 (0.021)	0.010 (0.014)	1.420 (1.662)	0.014 (0.022)	0.010 (0.014)	0.971 (1.415)
Malignant tissues	0.005 (0.013)	0.004 (0.005)	1.365 (1.820)	0.009 (0.007)	0.005 (0.005)	1.520 (1.215)
Z	-3.280	-4.651	-0.121	-3.100	-2.731	-0.999
P	0.001	0.000	0.904	0.002	0.006	0.318

Table 3 Correlation between HAI-1 and SNC19/matriptase expression and clinicopathology in gastric cancer tissues [$M(Q_R)$]

	Patients	HAI-1	SNC19	HAI-1:SNC19
Age (yr)				
<40 yr	3	0.005 (0.011)	0.003 (0.002)	1.415 (1.416)
40–60 yr	17	0.005 (0.016)	0.005 (0.006)	1.680 (2.022)
>60 yr	20	0.005 (0.001)	0.003 (0.003)	0.882 (0.144)
Gender				
Men	28	0.005 (0.013)	0.004 (0.004)	1.220 (1.631)
Women	12	0.005 (0.020)	0.003 (0.005)	1.680 (1.571)
Location				
Cardia	7	0.005 (0.004)	0.003 (0.002)	1.430 (0.210)
Body	7	0.007 (0.023)	0.004 (0.005)	2.620 (2.846)
Pylorus	17	0.005 (0.015)	0.005 (0.005)	1.080 (1.631)
Unknown	9	0.005 (0.110)	0.003 (0.004)	1.220 (1.219)
Tumor histology				
Adenocarcinoma	18	0.005 (0.008)	0.004 (0.004)	1.365 (1.442)
Mucoid carcinoma	7	0.005 (0.021)	0.003 (0.008)	1.740 (1.626)
Poorly differentiated carcinoma	11	0.003 (0.016)	0.003 (0.004)	0.882 (2.377)
Unknown	4	0.005 (0.114)	0.005 (0.004)	1.555 (1.386)
N factor				
N0	12	0.017 (0.032)[a]	0.004 (0.005)	2.280 (3.148)[e]
N1/N2	28	0.004 (0.005)	0.004 (0.005)	1.080 (1.489)
UICC stage				
I/II	10	0.017 (0.020)[c]	0.004 (0.005)	2.280 (2.718)[g]
III/IV	30	0.004 (0.007)	0.003 (0.004)	1.245 (1.541)
Histological grade				
Well	3	0.011 (0.008)	0.003 (0.002)	2.870 (1.753)
Moderate	15	0.005 (0.004)	0.004 (0.004)	1.400 (1.121)
Poor	22	0.004 (0.017)	0.003 (0.007)	1.150 (0.470)

[a]P<0.05, [c]P <0.05 *vs* lymph node-positive group (N1/N2); [e]P <0.05, [g]P<0.05 *vs* stage III/IV group.

Table 4 Correlation between HAI-1 and SNC19/matriptase expression and clinicopathology in colorectal cancer tissues [$M(Q_R)$]

	Patients	HAI-1	SNC19	HAI-1:SNC19
Age (yr)				
<40 yr	3	0.004 (0.003)	0.005 (0.002)	0.778 (0.349)
40–60 yr	10	0.007 (0.102)	0.003 (0.003)	0.798 (1.096)
>60 yr	23	0.009 (0.104)	0.005 (0.005)	0.677 (0.459)
Unknown	1			
Gender				
Men	13	0.005 (0.109)	0.005 (0.006)	0.944 (0.728)
Women	24	0.009 (0.100)	0.005 (0.004)	0.667 (0.592)
Location				
Cecum/ascending colon	9	0.009 (0.101)	0.005 (0.006)	0.660 (0.482)
Transverse colon	2	0.003 (0.003)	0.002 (0.002)	0.862 (0.377)
Descending/ sigmoid colon	6	0.010 (0.004)	0.006 (0.002)	0.663 (0.715)
Rectum	19	0.005 (0.009)	0.004 (0.005)	0.778 (1.158)
Unknown	1			
Tumor histology				
Adenocarcinoma	32	0.005 (0.009)	0.004 (0.005)	0.734 (0.788)
Mucoid carcinoma	4	0.009 (0.012)	0.005 (0.004)	0.511 (0.194)
Poorly differentiated carcinoma	1			
N factor				
N0	18	0.010 (0.015)	0.005 (0.009)	1.470 (0.699)
N1/N2	19	0.007 (0.005)	0.005 (0.004)	1.480 (0.980)
Dukes' stage				
A	7	0.011 (0.015)	0.004 (0.008)	1.790 (3.860)
B	10	0.010 (0.016)	0.006 (0.015)	1.290 (1.243)
C/D	20	0.006 (0.006)	0.005 (0.005)	1.385 (0.955)
Histological grade				
Well	8	0.005 (0.010)	0.006 (0.024)	0.891 (1.482)
Moderately	24	0.009 (0.009)	0.004 (0.005)	1.485 (1.250)
Poorly	5	0.009 (0.012)	0.005 (0.005)	1.790 (1.000)

DISCUSSION

The inhibitors of serine proteases are important regulators of enzyme activities. They are classified into at least 10 families[23]. Among them, serpins (serine protease inhibitor) such as Kunitz-type inhibitors are correlated with suppression of tumor invasion[24].

Serine protease SNC19/matriptase is a newly characterized ECM-degrading protease, which may also function as an activator for HGF/SF and other proteases. HAI-1, the cognate Kunitz type inhibitor of SNC19/matriptase, was first found in the stomach carcinoma cell line. Although the expression of HAI-1 has been studied in some types of cancer, the expression profile in normal and malignant gastric tissues is still absent.

As was found in epithelial ovarian cancers that all HAI-1-positive tumors are also SNC19/matriptase positive[16], the imbalance between the protease and the inhibitor may play an important role in the development of tumors. In this study, HAI-1 and SNC19/matriptase showed a remarkable decrease in cancer tissues compared to their adjacent normal tissues in gastrointestinal tract, but the ratio of HAI-1: SNC19/matriptase showed no significant difference between normal and malignant gastrointestinal tissues. Whether the ratio is critical to tumor development or progression needs to be further investigated. Since a novel function of the integral membrane Kunitz-type inhibitor in the regulation of pericellular protease activity has been suggested, HAI-1 may act not only as an inhibitor but also as a reservoir of this enzyme on cell surface[25,26]. With this possibility and other lines of evidence, the situation in cancer may be more complicated. HAI-1 has a strong affinity for both HGFA and SNC19/matriptase to form a protease/inhibitor complex and its specific Kunitz domains are responsible for the inhibitory activity[27]. It was reported that HAI-1 may participate in the activation of SNC19/matriptase through their low-density lipoprotein receptor class A domains interacting with each other, and HAI-1-mediated activation and inhibition of SNC19/ matriptase occur in human mammary epithelial cells[28]. At the same time, HAI-1 not only inhibits the activity of SNC19/matriptase but also prevents it from being hydrolyzed by other proteases[29]. It was reported that decreased immunoreactivity of membrane-form HAI-1 and enhanced ectodomain shedding of HAI-1 are found in colorectal adenocarcinoma cells[30]. There might be a more complicated interaction between HAI-1 and SNC19/matriptase rather than simple activity inhibition. In breast cancer cells, SNC19/matriptase is detected mainly as an uncomplexed form, whereas in human milk a complex containing matriptase and HAI-1 is detectable[18]. The active form of SNC19/matriptase may release from HAI-1 fragment into extracellular milieu, thus playing a role in tumor cells as ectodomain shedding of HAI-1 enhances, which may provide SNC19/matriptase opportunities to actively down-stream biological factors.

HAI-1 expression and HAI-1: SNC19/matriptase ratio decrease in gastric cancer tissues where invasion and lymph node metastasis occur. HAI-1 may play a role in progression and metastasis of gastric cancer by inhibiting HGF. SNC19/ matriptase showed no significant difference in invasive tumors in this study. Lower ratio of HAI-1:SNC19/

matriptase in stage III/IV cancers compared to stage I/II ones may be due to the down-regulated expression of HAI-1. The expression of HAI-1 tends to increase in well-differentiated gastric cancer tissues. It was reported that the expression of HAI-1 increases as the histological grade decreases in hepatocellular carcinoma [31], and decreases in poorly differentiated breast tumors [32]. HAI-1 displays different patterns of expression in different tissues[33].

In colorectal cancer tissues, HAI-1 expression is inverse correlated tumor invasion and lymph node metastasis. In our study, the expression of HAI-1, SNC19/matriptase and their ratio did not show differences in colorectal cancer tissues with different differentiation status, but the expression of HAI-1 seemed to be related to histological grades. Further analysis regarding the patients' age, gender, location, and histological type of tumor showed no correlation with HAI-1 expression.

In conclusion, both SNC19/matriptase and HAI-1 are downregulated in gastrointestinal cancer tissues, and the underlying mechanisms need to be extensively investigated. A larger scale study both in case number and disease spectrum needs to be carried out to elucidate the exact role of SNC19/matriptase and HAI-1 in tumor development and progression.

REFERENCES

1 **Weidner KM,** Arakaki N, Hartmann G, Vandekerckhove J, Weingart S, Rieder H, Fonatsch C, Tsubouchi H, Hishida T, Daikuhara Y. Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc Natl Acad Sci USA* 1991; **88**: 7001-7005

2 **Naldini L,** Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, Narsimhan RP, Hartmann G, Zarnegar R, Michalopoulos GK. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *Embo J* 1991; **10**: 2867-2878

3 **Boros P,** Miller CM. Hepatocyte growth factor: a multifunctional cytokine. *Lancet* 1995; **345**: 293-295

4 **Matsumoto K,** Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. *J Biochem* 1996; **119**: 591-600

5 **Bellusci S,** Moens G, Gaudino G, Comoglio P, Nakamura T, Thiery JP, Jouanneau J. Creation of an hepatocyte growth factor/scatter factor autocrine loop in carcinoma cells induces invasive properties associated with increased tumorigenicity. *Oncogene* 1994; **9**: 1091-1099

6 **Nakamura T,** Matsumoto K, Kiritoshi A, Tano Y. Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: *in vitro* analysis of tumor-stromal interactions. *Cancer Res* 1997; **57**: 3305-3313

7 **Gak E,** Taylor WG, Chan AM, Rubin JS. Processing of hepatocyte growth factor to the heterodimeric form is required for biological activity. *FEBS Lett* 1992; **311**: 17-21

8 **Shimomura T,** Miyazawa K, Komiyama Y, Hiraoka H, Naka D, Morimoto Y, Kitamura N. Activation of hepatocyte growth factor by two homologous proteases, blood-coagulation factor XIIa and hepatocyte growth factor activator. *Eur J Biochem* 1995; **229**: 257-261

9 **Mars WM,** Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am J Pathol* 1993; **143**: 949-958

10 **Cao J,** Zheng S, Zheng L, Cai X, Zhang Y, Geng L, Fang Y. A novel serine protease SNC19 associated with human colorectal cancer. *Chin Med J* 2001; **114**: 726-730

11 **Lee SL,** Dickson RB, Lin CY. Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. *J Biol Chem* 2000; **275**: 36720-36725

12 **Cao J,** Cai X, Zheng L, Geng L, Shi Z, Pao CC, Zheng S. Characterization of colorectal-cancer-related cDNA clones obtained by subtractive hybridization screening. *J Cancer Res Clin Oncol* 1997; **123**: 447-451

13 **Lin CY,** Anders J, Johnson M, Sang QA, Dickson RB. Molecular cloning of cDNA for matriptase, a matrix-degrading serine protease with trypsin-like activity. *J Biol Chem* 1999; **274**: 18231-18236

14 **Satomi S,** Yamasaki Y, Tsuzuki S, Hitomi Y, Iwanaga T, Fushiki T. A role for membrane-type serine protease (MT-SP1) in intestinal epithelial turnover. *Biochem Biophys Res Commun* 2001; **287**: 995-1002

15 **Mignatti P,** Rifkin DB. Biology and biochemistry of proteinases in tumor invasion. *Physiol Rev* 1993; **73**: 161-195

16 **Oberst MD,** Johnson MD, Dickson RB, Lin CY, Singh B, Stewart M, Williams A, al-Nafussi A, Smyth JF, Gabra H, Sellar GC. Expression of the serine protease matriptase and its inhibitor HAI-1 in epithelial ovarian cancer: correlation with clinical outcome and tumor clinicopathological parameters. *Clin Cancer Res* 2002; **8**: 1101-1107

17 **Shimomura T,** Denda K, Kitamura A, Kawaguchi T, Kito M, Kondo J, Kagaya S, Qin L, Takata H, Miyazawa K, Kitamura N. Hepatocyte growth factor activator inhibitor, a novel Kunitz-type serine protease inhibitor. *J Biol Chem* 1997; **272**: 6370-6376

18 **Lin CY,** Anders J, Johnson M, Dickson RB. Purification and characterization of a complex containing matriptase and a Kunitz-type serine protease inhibitor from human milk. *J Biol Chem* 1999; **274**: 18237-18242

19 **Kataoka H,** Miyata S, Uchinokura S, Itoh H. Roles of hepatocyte growth factor (HGF) activator and HGF activator inhibitor in the pericellular activation of HGF/scatter factor. *Cancer Metastasis Rev* 2003; **22**: 223-236

20 **Oberst M,** Anders J, Xie B, Singh B, Ossandon M, Johnson M, Dickson RB, Lin CY. Matriptase and HAI-1 are expressed by normal and malignant epithelial cells *in vitro* and *in vivo*. *Am J Pathol* 2001; **158**: 1301-1311

21 **Johnson MD,** Oberst MD, Lin CY, Dickson RB. Possible role of matriptase in the diagnosis of ovarian cancer. *Expert Rev Mol Diagn* 2003; **3**: 331-338

22 **Kang JY,** Dolled-Filhart M, Ocal IT, Singh B, Lin CY, Dickson RB, Rimm DL, Camp RL. Tissue microarray analysis of hepatocyte growth factor/Met pathway components reveals a role for Met, matriptase, and hepatocyte growth factor activator inhibitor 1 in the progression of node-negative breast cancer. *Cancer Res* 2003; **63**: 1101-1105

23 **Laskowski M Jr,** Kato I. Protein inhibitors of proteinases. *Annu Rev Biochem* 1980; **49**: 593-626

24 **Kobayashi H,** Shinohara H, Takeuchi K, Itoh M, Fujie M, Saitoh M, Terao T. Inhibition of the soluble and the tumor cell receptor-bound plasmin by urinary trypsin inhibitor and subsequent effects on tumor cell invasion and metastasis. *Cancer Res* 1994; **54**: 844-849

25 **Kataoka H,** Shimomura T, Kawaguchi T, Hamasuna R, Itoh H, Kitamura N, Miyazawa K, Koono M. Hepatocyte growth factor activator inhibitor type 1 is a specific cell surface binding protein of hepatocyte growth factor activator (HGFA) and regulates HGFA activity in the pericellular microenvironment. *J Biol Chem* 2000; **275**: 40453-40462

26 **Kataoka H,** Itoh H, Hamasuna R, Meng JY, Koono M. Pericellular activation of hepatocyte growth factor/scatter factor (HGF/SF) in colorectal carcinomas: roles of HGF activator (HGFA) and HGFA inhibitor type 1 (HAI-1). *Hum Cell* 2001; **14**: 83-93

27 **Denda K,** Shimomura T, Kawaguchi T, Miyazawa K, Kitamura N. Functional characterization of Kunitz domains in hepatocyte growth factor activator inhibitor type 1. *J Biol Chem* 2002; **277**: 14053-14059

28 **Lee MS,** Kiyomiya KI, Benaud C, Dickson RB, Lin CY. Simultaneous activation and HAI-1-mediated inhibition of matriptase induced at activation foci in human mammary epithelial cells. *Am J Physiol Cell Physiol* 2004; **288**: C932-C941
29 **Oberst MD,** Williams CA, Dickson RB, Johnson MD, Lin CY. The activation of matriptase requires its noncatalytic domains, serine protease domain, and its cognate inhibitor. *J Biol Chem* 2003; **278**: 26773-26779
30 **Nagaike K,** Kohama K, Uchiyama S, Tanaka H, Chijiiwa K, Itoh H, Kataoka H. Paradoxically enhanced immunoreactivity of hepatocyte growth factor activator inhibitor type 1 (HAI-1) in cancer cells at the invasion front. *Cancer Sci* 2004; **95**: 728-735
31 **Nagata K,** Hirono S, Ido A, Kataoka H, Moriuchi A, Shimomura T, Hori T, Hayashi K, Koono M, Kitamura N, Tsubouchi H. Expression of hepatocyte growth factor activator and hepatocyte growth factor activator inhibitor type 1 in human hepatocellular carcinoma. *Biochem Biophys Res Commun* 2001; **289**: 205-211
32 **Parr C,** Watkins G, Mansel RE, Jiang WG. The hepatocyte growth factor regulatory factors in human breast cancer. *Clin Cancer Res* 2004; **10**: 202-211
33 **Parr C,** Jiang WG. Expression of hepatocyte growth factor/scatter factor, its activator, inhibitors and the c-Met receptor in human cancer cells. *Int J Oncol* 2001; **19**: 857-863

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