Clinical Significance of Human Kallikrein Gene 6 Messenger RNA Expression in Colorectal Cancer

Kazuhiko Ogawa,[1,2] Tohru Utsunomiya,[1] Koshi Mimori,[1] Fumiaki Tanaka,[1] Hiroshi Inoue,[1] Hisashi Nagahara,[1] Sadayuki Murayama,[2] and Masaki Mori[1]

Abstract

Purpose: Human kallikrein gene 6 (*KLK6*) is a member of the human kallikrein gene family, and recent studies have found that many kallikreins have altered expression patterns in various malignancies. The purpose of the current study was to quantify the expression of *KLK6* in malignant and benign colorectal tissues and to statistically analyze whether *KLK6* expression levels correlate with clinicopathologic variables and prognosis in patients with colorectal cancer.

Experimental Designs: Paired colorectal tissue samples from cancerous and corresponding noncancerous tissues were obtained from 63 patients with colorectal cancer who underwent surgical resection. Quantitative analyses of *KLK6* mRNA expression were done using real-time quantitative reverse transcription-PCR.

Results: *KLK6* mRNA overexpression in cancerous tissues compared with normal counterparts was observed in 57 of 63 (90%) patients. The mean expression level of *KLK6* mRNA in cancerous tissues was significantly higher than that in noncancerous tissues ($P < 0.0001$). Elevated *KLK6* expression was significantly correlated with serosal invasion ($P < 0.05$), liver metastasis ($P < 0.05$), and advanced Duke's stage ($P < 0.01$). Furthermore, patients with high *KLK6* expression had a significantly poorer actuarial overall survival than patients with low *KLK6* expression (5-year overall survival rates: 54% and 73%, respectively, $P < 0.05$).

Conclusions: The results of this study indicated that *KLK6* mRNA expression was significantly higher in cancerous than in noncancerous colorectal tissues, and high expression of *KLK6* mRNA correlated with serosal invasion, liver metastasis, advanced Duke's stage, and a poor prognosis for patients with colorectal cancer.

The kallikrein gene family consists of 15 genes localized in tandem on chromosome 19q13.4, and all of the kallikrein genes encode for secreted serine proteases (1–4). Kallikreins have a similar genomic organization and show significant homology at both the nucleotide and the protein level (1–3, 5). The human kallikrein gene 6 (*KLK6*), which encodes for human kallikrein 6 protein (hK6), has been cloned independently by three groups. Using a differential display technique from primary and metastatic breast cancer cell lines, Anisowicz et al. (6) first isolated the cDNA of *KLK6*, which they named protease M. They showed that protease M is strongly expressed at the mRNA level in certain primary breast cancer cell lines and in ovarian cancer tissues and cell lines. Yamashiro et al. (7) cloned this same gene, which they named neurosin, from a cDNA library prepared from a human colorectal cancer cell line (COLO 201). Neurosin was found to be highly expressed in the brain. Finally, Little et al. (8) cloned the same cDNA, which they named zyme, by PCR amplification from the brain tissue of a patient with Alzheimer's disease. The predominant expression of *KLK6* in brain cells suggests the possible involvement of kallikrein 6 in the development and progression of Alzheimer's disease (6–8).

A growing body of evidence suggests that human kallikrein genes (*KLK*) are involved in human malignancies and that many *KLK*s are promising biomarkers of prostate, ovarian, breast, and testicular cancers (1–3). Prostate-specific antigen (hK3) has been used as a tumor marker for the early detection of prostate cancer as a result of studies which have identified elevated serum prostate-specific antigen levels in prostate cancer patients (9). An elevated level of prostate-specific antigens (hK3) is also recognized as a favorable prognostic factor for breast cancer (10). Recent reports have suggested that hK2 could be another useful diagnostic marker for prostate cancer (11). Several authors have reported that *KLK6* mRNA is highly expressed in ovarian cancer tissues (6, 12), and recent studies have indicated that hK6 could potentially be a biomarker for the diagnosis and monitoring of ovarian cancer (13, 14). In colorectal cancer, *KLK6*, *KLK8*, and *KLK10* have

Authors' Affiliations: [1]Department of Molecular and Surgical Oncology, Medical Institute of Bioregulation, Kyushu University, Beppu and [2]Department of Radiology, University of the Ryukyus, Okinawa, Japan

Received 11/8/04; revised 1/5/05; accepted 1/17/05.

Grant support: Grants-in-Aid for Scientific Research (B) (15390398 and 16390381) and for Scientific Research (C) (15591412 and 15591411), Japan Society for the Promotion of Science, and a Health and Labor Sciences Research Grant on Hepatitis and BSE (14230801), the Ministry of Health, Labor and Welfare of Japan, and by the Uehara Memorial Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: K. Ogawa and T. Utsunomiya contributed equally to this study.

Requests for reprints: Masaki Mori, Department of Molecular and Surgical Oncology, Medical Institute of Bioregulation, Kyushu University, Tsurumihara 4546, Beppu 874-0838, Japan. Phone: 81-977-27-1650; Fax: 81-977-27-1651; E-mail: mmori@tsurumi.beppu.kyushu-u.ac.jp.

been found to be up-regulated in cancer tissues compared with normal tissues (15, 16).

Because *KLK6* has been found to be highly expressed in colorectal cancer cells as well as in ovarian cancer cells (12, 15, 16), we hypothesize that *KLK6* may also have potential applications in the clinical diagnosis and monitoring of colorectal cancer. To the authors' knowledge, no information has been published concerning the clinical significance of *KLK6* mRNA expression in colorectal cancer. In the current study, we quantified the expression of *KLK6* in malignant and benign colorectal tissues. We then performed statistical analyses to determine whether *KLK6* expression levels correlate with clinicopathologic variables and prognosis in patients with colorectal cancer.

Materials and Methods

Patients and sample collection. Primary colorectal cancer specimens and adjacent normal colorectal mucosa were obtained from 63 patients who underwent surgery at the Medical Institute of Bioregulation Hospital, Kyushu University. Immediately after resection, the necrotic and ulcerated portions of the tumor were removed, and normal colonic mucosa was dissociated from muscle and connective tissue. All specimens were immediately frozen in liquid nitrogen and kept at −80°C until RNA extraction was done. Written informed consent was obtained from all patients. Whenever possible, specimens were also prepared for immunohistochemical studies. All 63 patients were clearly identified as having colorectal cancer based on the clinicopathologic findings. No patients received chemotherapy or radiotherapy prior to surgery. Fifteen of these 63 patients had synchronous liver metastases. All patients were closely followed after surgery at regular 1-month intervals. The follow-up periods ranged from 2 to 67 months with a mean of 30 months.

Total RNA extraction. Frozen tissue specimens were homogenized in guanidinium thiocyanate, and total RNA was obtained by ultracentrifugation through a cesium chloride cushion as described previously (17).

Real-time quantitative reverse transcription-PCR. The cDNA was synthesized from 8.0 μg of total RNA as described previously (18). Two gene-specific oligonucleotide primers were designed: (*KLK6*) sense, 5′-CATGGCGGACCCCTGCGACAAGAC-3′; antisense, 5′-TGGATCA-CAGCCCGGACAACAGAA-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense, 5′-TTGGTATCGTGGAAGGACTCA-3′; antisense, 5′-TGTCATCATATTTGGCAGGTT-3′.

These primers spanned more than one intron to avoid amplification of any contaminating DNA.

Real-time monitoring of PCR reactions was done using the Light-Cycler system (Roche Applied Science, Indianapolis, IN) and SYBR green I dye (Roche Diagnostics). Monitoring was done according to the manufacturer's instructions, as described previously (19). In brief, a master mixture was prepared on ice, containing 1 μL of cDNA, 2 μL of LC DNA Master SYBR Green I mix, 50 ng of primers, and 2.4 μL of 25 mmol/L $MgCl_2$. The final volume was adjusted to 20 μL with water. After being loaded into a glass capillary tube, the reaction mixture was exposed to the following cycling conditions: initial denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 62°C (60°C for GAPDH) for 10 seconds, and extension at 72°C for 10 seconds. After amplification, the products were subjected to a temperature gradient from 68°C to 95°C at 0.2°C/second under continuous fluorescence monitoring to produce a melting curve of the products. Only one peak for each sample was observed.

We determined the levels of *KLK6* and GAPDH mRNA expression by comparisons with cDNA from Human Universal Reference total RNA (Clontech, Palo Alto, CA). After proportional baseline adjustment, the fit point method was employed to determine the cycle in which the log-linear signal was distinguished from the baseline, and that cycle number (threshold cycle) was used as a crossing-point value. The standard curve was produced by measuring the crossing-point of each standard value (4-fold serially diluted cDNAs of Human Universal Reference total RNA) and plotting them against the logarithmic value of concentrations. The standard curve samples were included in each run. The concentrations of all samples were then calculated by plotting their crossing-points against the standard curve. All calculated concentrations were relative to the concentration of the cDNA of Human Universal Reference total RNA, and the amount of the target molecule was then divided by the amount of the endogenous reference (GAPDH) to obtain normalized *KLK6* expression values (20, 21). Each assay was done thrice to verify the results, and the mean mRNA expression was used for subsequent analysis.

Immunohistochemistry. Immunohistochemical studies of hK6 were done on surgical specimens from colorectal cancer patients using the avidin-biotin-peroxydase method (LSAB2 kit, Dako, Kyoto, Japan) on formalin-fixed, paraffin-embedded tissues. All sections were counter-stained with hematoxylin. The primary mouse monoclonal antibodies against hK6 (MCA2158, Serotec, Ltd., United Kingdom) were used at dilutions of 1:500.

Statistical analysis. Overall survival rates were calculated actuarially according to the Kaplan-Meier method (22), and were measured from the day of surgery. Differences between groups were estimated using the χ^2 test, Student's t test, and the log-rank test (23). A probability level of 0.05 was chosen for statistical significance. Statistical analysis was done with the SPSS software package (version 6.1, SPSS, Inc., Chicago, IL).

Results

With regard to *KLK6* mRNA expression in clinical samples, 57 of 63 patients (90%) showed a higher expression level of *KLK6* mRNA in cancerous tissues than in noncancerous tissues by real-time quantitative reverse transcription-PCR. The mean expression level of *KLK6* mRNA in tumor tissues, 0.309 ± 0.500 (mean ± SD), was significantly higher than 0.025 ± 0.092 in the corresponding normal tissues ($P < 0.0001$, Fig. 1). The median expression levels of *KLK6* mRNA in tumor tissues and normal tissues were 0.120 and 0.002, respectively. Immunohistochemical analysis revealed that hK6 was predominantly expressed in cancer cells (Fig. 2).

In the current study, patients with values less than the median expression level of 0.120 in tumor tissues were assigned to the low expression group ($n = 31$), whereas those with values ≥0.120 were assigned to the high expression group ($n = 32$). Table 1 shows the clinicopathologic data and *KLK6* mRNA expression in tumor specimens from the 63 colorectal cancer patients. The incidence of serosal invasion was significantly higher ($P = 0.020$) in the high expression group (27 of 32, 84%) than in the low expression group (18 of 31, 58%), and the incidence of liver metastasis was significantly higher ($P = 0.011$) in the high expression group (12 of 31, 39%) than in the low expression group (3 of 31, 10%). Moreover, the incidence of advanced stage cancer (according to Duke's classification) was significantly higher ($P = 0.003$) in the high expression group (20 of 32, 63%) than in the low expression group (9 of 31, 29%).

The 5-year actuarial overall survival rates in patients with high *KLK6* mRNA levels and patients with low *KLK6* mRNA levels were 54% and 73%, respectively (Fig. 3). The survival difference between these two groups was statistically significant ($P = 0.029$).

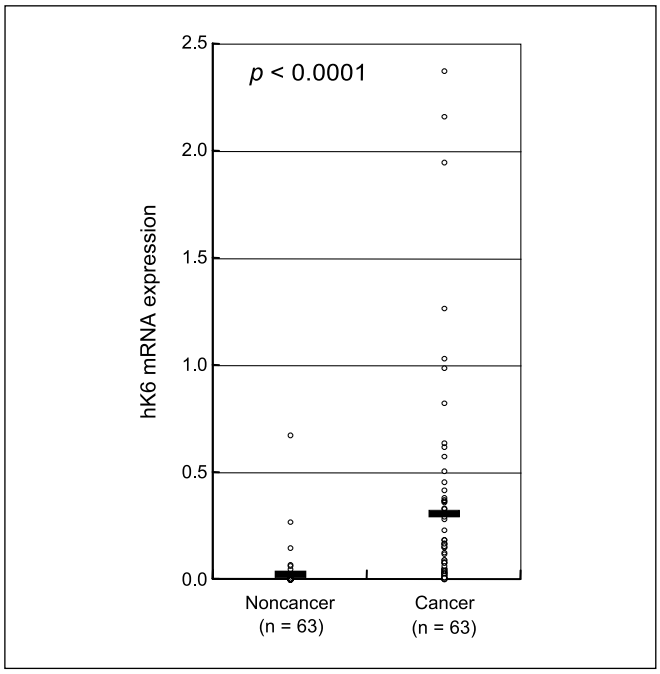

Fig. 1. *KLK6* mRNA expression in cancerous and noncancerous colorectal tissues. Horizontal lines indicate the means. Cancer tissues showed significantly higher *KLK6* mRNA expression levels compared to noncancerous tissues ($P < 0.0001$). The P value was calculated by Student's t test.

Discussion

The results of the current study indicated that in colorectal cancer patients, *KLK6* mRNA is more frequently overexpressed in cancerous tissues than in noncancerous tissues. Several authors have previously reported that *KLK6* mRNA is highly expressed in colorectal cancer tissues, whereas *KLK6* mRNA expression levels in normal tissues is quite low (15, 16). Schuster et al. (15) investigated mRNA levels of *KLK6* by real-time reverse transcription-PCR of colorectal cancer and normal colon mucosa tissues snap-frozen after surgical resection. They found that the mean *KLK6* expression level in normal samples was approximately 2-log lower than that of the cancer samples. Yousef et al. (16) also reported that *KLK6* was overexpressed in colorectal cancer tissues compared with normal colon tissues. These findings suggest that *KLK6* expression may play an important role in colorectal cancer development.

The current study found that high mRNA expression of *KLK6* was significantly associated with serosal invasion, liver metastasis, and advanced Duke's stage. Furthermore, the actuarial overall survival of patients with high expression of *KLK6* was significantly poorer than those with lower expression. To the authors' knowledge, this is the first report concerning the clinical significance of *KLK6* expression in colorectal cancer. With regard to ovarian cancer, several authors

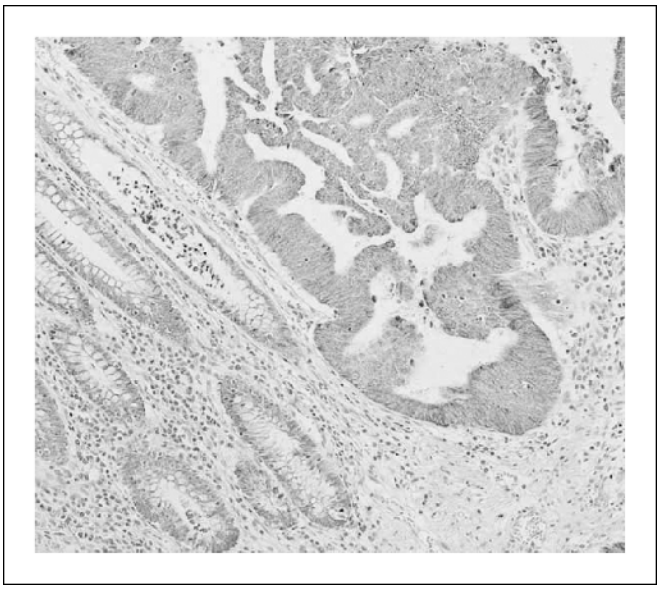

Fig. 2. Representative example of human hK6 expression in colorectal cancer. Immunohistochemical staining with anti-hK6 antibody in colorectal cancer cells (*right side*) and normal mucosa (*left side*) indicated that hK6 was predominantly expressed in the cancer cells.

Table 1. Clinicopathologic data and hK6 mRNA expression in the tumor specimens of 63 patients with colorectal carcinoma

Variable	High expression ($n = 32$)	Low expression ($n = 31$)	P
Age	64.6 ± 11.0	69.5 ± 9.8	0.066
Gender (male)			
Male	17	17	0.89
Female	15	14	
Site			
Colon	22	17	0.26
Rectum	10	14	
Histologic grade			
Well	7	5	0.840
Moderately	21	22	
Poorly	4	4	
Serosal invasion			
Absent	5	13	0.020
Present	27	18	
Lymph node metastasis			
Absent	15	22	0.052
Present	17	9	
Lymphatic permeation			
Absent	17	22	0.140
Present	15	9	
Venous permeation			
Absent	23	24	0.610
Present	9	7	
Liver metastasis			
Absent	21	28	0.011
Present	12	3	
Peritoneal dissemination			
Absent	1	0	0.320
Present	31	31	
Duke's classification			
A and B	12	22	0.003
C and D	20	9	

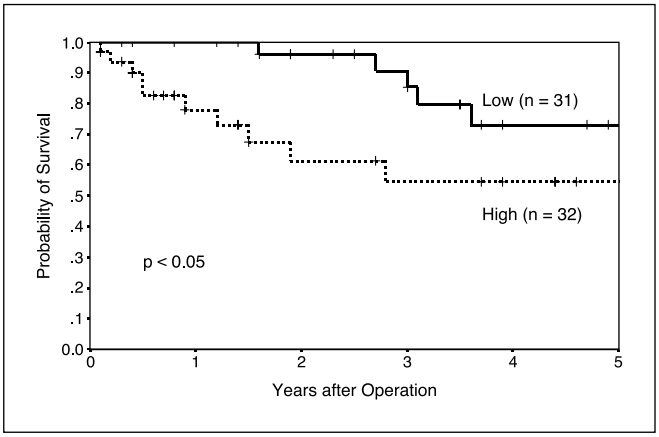

Fig. 3. Actuarial overall survival in patients with colorectal cancer as a function of *KLK6* expression level.

have reported that hK6 expression was higher in late stage disease and that tumor or serum hK6 expression was an adverse prognostic marker (13, 14).

*KLK*s are thought to possess serine protease catalytic activity, and the serine proteases comprise a family of protein-degrading enzymes that serve a variety of biological functions, including induction of blood coagulation, activation of growth and angiogenic factors, and degradation of extracellular matrix components (24–26). Expression levels of serine proteases (such as plasminogen activator) and other classes of proteinases have been shown to correlate positively with invasiveness and metastatic potential of many types of tumor cells (25, 27–31). This behavior is linked to the ability of these tumors to degrade extracellular matrix components, either directly, or indirectly through the proteolytic activation of other enzymes. Magklara et al. (32) reported that hK6 can degrade *in vitro* fibrinogen and collagen type I, basic constituents of the extracellular matrix, as well as collagen type IV, a major part of the basement membrane. Others have shown that hK6 can also digest laminin and fibronectin (33). These findings raise the possibility that *KLK6* may be an important factor in pericellular proteolysis and tumor invasion. Lysis of certain components of the extracellular matrix disrupts their dynamic interactions with cells and is linked with altered regulation of cell proliferation that can lead to tumor cell growth and malignant transformation. Such a role for *KLK6* is likely in colorectal cancer as well as in ovarian cancer, where elevated *KLK6* levels are associated with unfavorable prognosis (14). If serine proteases are involved in cancer progression, they may be suitable candidates for therapeutic targets.

Although *KLK6* seems to be a useful biomarker in colorectal cancer, the underlying biological mechanism of possible kallikrein involvement in the progression of colorectal cancer is currently unknown (16). It has been reported that in cancer cell lines, many *KLK*s, including *KLK6*, are under steroid hormone regulation (1–3, 34). Certain *KLK* genes are predominantly up-regulated by androgens and androgenic progestines (e.g., *KLK2*, *KLK3*, *KLK4*, *KLK13*, and *KLK15*), whereas others are primarily responsive to estrogens (e.g., *KLK5*, *KLK6*, *KLK7*, *KLK9*, *KLK10*, and *KLK11*; ref. 1). Recent studies also suggest that all of the *KLK*s may be able to activate each other as well as other molecules (e.g., growth factors and cytokines) in a cascade of events associated with tumorigenesis (1–3). Experimental evidence has shown that hK15 can activate hK3 (prostate-specific antigen), and hK4 has also been shown to activate hK3 (35, 36). In ovarian cancer, at least 10 of the 15 *KLK*s have prognostic value, and the vast majority of *KLK*s are coexpressed and presumably coordinately regulated (37). Because *KLK8* and *KLK10* as well as *KLK6* have been found to be overexpressed in colorectal cancer tissues (16), several *KLK*s may be also be able to activate each other in colorectal cancer. Therefore, it may be worthwhile to clarify the various mechanisms of *KLK* activation in colorectal cancer.

In conclusion, our results indicated that *KLK6* mRNA was overexpressed in colorectal cancer tissues and high *KLK6* expression levels were correlated with serosal invasion, liver metastasis, advanced Duke's stage, and poor prognosis. These findings suggest a possible role for *KLK6* expression level as a new diagnostic and prognostic biomarker for colorectal cancer. Furthermore, understanding the biological function of *KLK6* expression in colorectal tissue may help to delineate its role in colorectal physiology of colorectal cancer.

References

1. Borgono CA, Diamandis EP. The emerging roles of human kallikreins in cancer. Nat Rev Cancer 2004;4:876–90.
2. Borgono CA, Michael IP, Diamandis EP. Human tissue kallikreins: physiologic roles and applications in cancer. Mol Cancer Res 2004;2:257–80.
3. Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr Rev 2001;22:184–204.
4. Diamandis EP, Yousef GM. Human tissue kallikreins: a family of new cancer biomarkers. Clin Chem 2002;48:1198–205.
5. Yousef GM, Diamandis EP. Human tissue kallikreins: a new enzymatic cascade pathway? Biol Chem 2002;383:1045–57.
6. Anisowicz A, Sotiropoulou G, Stenman G, Mok SC, Sager R. A novel protease homolog differentially expressed in breast and ovarian cancer. Mol Med 1996;2:624–36.
7. Yamashiro K, Tsuruoka N, Kodama S, et al. Molecular cloning of a novel trypsin-like serine protease (neurosin) preferentially expressed in brain. Biochim Biophys Acta 1997;1350:11–4.
8. Little SP, Dixon EP, Norris F, et al. Zyme, a novel and potentially amyloidogenic enzyme cDNA isolated from Alzheimer's disease brain. J Biol Chem 1997;272:25135–42.
9. McCormack RT, Rittenhouse HG, Finlay JA, et al. Molecular forms of prostate-specific antigen and the human kallikrein gene family: a new era. Urology 1995;45:729–44.
10. Yu H, Giai M, Diamandis EP, et al. Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 1995;55:2104–10.
11. Magklara A, Scorilas A, Catalona WJ, Diamandis EP. The combination of human glandular kallikrein and free prostate-specific antigen (PSA) enhances discrimination between prostate cancer and benign prostatic hyperplasia in patients with moderately increased total PSA. Clin Chem 1999;45:1960–6.
12. Tanimoto H, Underwood LJ, Shigemasa K, Parmley TH, O'Brien TJ. Increased expression of protease M in ovarian tumors. Tumour Biol 2001;22:11–8.
13. Diamandis EP, Scorilas A, Fracchioli S, et al. Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and prognosis of ovarian carcinoma. J Clin Oncol 2003;21:1035–43.
14. Hoffman BR, Katsaros D, Scorilas A, et al. Immunofluorometric quantitation and histochemical localisation of kallikrein 6 protein in ovarian cancer tissue: a new independent unfavorable prognostic biomarker. Br J Cancer 2002;87:763–71.
15. Schuster R, Max N, Mann B, et al. Quantitative real-time RT-PCR for detection of disseminated tumor cells in peripheral blood of patients with colorectal cancer using different mRNA markers. Int J Cancer 2004;108:219–27.
16. Yousef GM, Borgono CA, Popalis C, et al. In silico analysis of kallikrein gene expression in pancreatic and colon cancers. Anticancer Res 2004;24:43–51.
17. Mori M, Barnard GF, Staniunas RJ, Jessup JM, Steele GD Jr, Chen LB. Prothymosin-α mRNA expression correlates with that of c-myc in human colon cancer. Oncogene 1993;8:2821–6.
18. Inoue H, Mori M, Honda M, et al. The expression of tumor-rejection antigen "MAGE" genes in human gastric carcinoma. Gastroenterology 1995;109:1522–5.

19. Ogawa K, Utsunomiya T, Mimori K, et al. Clinical significance of elongation factor-1δ mRNA expression in oesophageal carcinoma. Br J Cancer 2004;91:282–6.

20. Bieche I, Olivi M, Champeme MH, Vidaud D, Lidereau R, Vidaud M. Novel approach to quantitative polymerase chain reaction using real-time detection: application to the detection of gene amplification in breast cancer. Int J Cancer 1998;78:661–6.

21. Bieche I, Onody P, Laurendeau I, et al. Real time reverse transcription-PCR assay for future management of ERBB2-based clinical applications. Clin Chem 1999;45:1148–56.

22. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.

23. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163–70.

24. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991;64:327–36.

25. Duffy MJ. The role of proteolytic enzymes in cancer invasion and metastasis. Clin Exp Metastasis 1992;10:145–55.

26. Tryggvason K, Hoyhtya M, Salo T. Proteolytic degradation of extracellular matrix in tumor invasion. Biochim Biophys Acta 1987;907:191–217.

27. Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation and cancer. Adv Cancer Res 1985;44:139–266.

28. Aznavoorian S, Murphy AN, Stetler-Stevenson WG, Liotta LA. Molecular aspects of tumor cell invasion and metastasis. Cancer 1993;71:1368–83.

29. Matrisian LM. Cancer biology: extracellular proteinases in malignancy. Curr Biol 1999;9:R776–8.

30. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000;18:1135–49.

31. McCawley LJ, Matrisian LM. Tumor progression: defining the soil round the tumor seed. Curr Biol 2001;11:R25–7.

32. Magklara A, Mellati AA, Wasney GA, et al. Characterization of the enzymatic activity of human kallikrein 6: autoactivation, substrate specificity, and regulation by inhibitors. Biochem Biophys Res Commun 2003;307:948–55.

33. Bernett MJ, Blaber SI, Scarisbrick IA, Dhanarajan P, Thompson SM, Blaber M. Crystal structure and biochemical characterization of human kallikrein 6 reveals that a trypsin-like kallikrein is expressed in the central nervous system. J Biol Chem 2002;277:24562–70.

34. Yousef GM, Luo LY, Scherer SW, Sotiropoulou G, Diamandis EP. Molecular characterization of Zyme/Protease M/neurosin (PRSS9), a hormonally regulated kallikrein-like serine protease. Genomics 1999;62:251–9.

35. Takayama TK, Carter CA, Deng T. Activation of prostate-specific antigen precursor (pro-PSA) by prostin, a novel human prostatic serine protease identified by degenerate PCR. Biochemistry 2001;40:1679–87.

36. Takayama TK, McMullen BA, Nelson PS, Matsumura M, Fujikawa K. Characterization of hK4 (prostase), a prostate-specific serine protease: activation of the precursor of prostate specific antigen (pro-PSA) and single-chain urokinase-type plasminogen activator and degradation of prostatic acid phosphatase. Biochemistry 2001;40:15341–8.

37. Yousef GM, Diamandis EP. Kallikreins, steroid hormones and ovarian cancer: is there a link? Minerva Endocrinol 2002;27:157–66.