Human Kallikrein 8 Protein Is a Favorable Prognostic Marker in Ovarian Cancer

Carla A. Borgoño,[1,2] Tadaaki Kishi,[1,2] Andreas Scorilas,[3] Nadia Harbeck,[4] Julia Dorn,[4] Barbara Schmalfeldt,[4] Manfred Schmitt,[4] and Eleftherios P. Diamandis[1,2]

Abstract

Human kallikrein 8 (hK8/neuropsin/ovasin; encoded by *KLK8*) is a steroid hormone – regulated secreted serine protease differentially expressed in ovarian carcinoma. *KLK8* mRNA levels are associated with a favorable patient prognosis and hK8 protein levels are elevated in the sera of 62% ovarian cancer patients, suggesting that *KLK8*/hK8 is a prospective biomarker. Given the above, the aim of the present study was to determine if tissue hK8 bears any prognostic significance in ovarian cancer. Using a newly developed ELISA, hK8 was quantified in 136 ovarian tumor extracts and correlated with clinicopathologic variables and outcome [progression-free survival (PFS); overall survival (OS)] over a median follow-up period of 42 months. hK8 levels in ovarian tumor cytosols ranged from 0 to 478 ng/mg total protein, with a median of 30 ng/mg. An optimal cutoff value of 25.8 ng/mg total protein (74th percentile) was selected based on the ability of hK8 values to predict the PFS of the study population and to categorize tumors as hK8 positive or negative. Women with hK8-positive tumors most often had lower-grade tumors (G1), no residual tumor after surgery, and optimal debulking success ($P < 0.05$). Univariate and multivariate analyses revealed that patients with hK8-positive tumors had a significantly longer PFS and OS than hK8-negative patients ($P < 0.05$). Kaplan-Meier survival curves further confirmed a reduced risk of relapse and death in women with hK8-positive tumors ($P = 0.001$ and $P = 0.014$, respectively). These results indicate that hK8 is an independent marker of favorable prognosis in ovarian cancer.

Epithelial ovarian cancer, comprising ∼90% of all ovarian cancer cases, continues to be the fourth leading cause of cancer-related death and the most lethal gynecologic malignancy among women in the United States (1). High mortality is attributed to delays in diagnosis, a result of the late clinical manifestation of ovarian tumors. In fact, over two thirds of patients are diagnosed at late Fédération Internationale des Gynaecologistes et Obstetristes stage III or IV disease and have lower long-term survival rates (10-30%) compared with the 80% to 95% survival rate of patients diagnosed at early Fédération Internationale des Gynaecologistes et Obstetristes stage I or II (2). Survival rates have remained largely unchanged over the past two decades despite the availability of new cytotoxic treatments (3). Hence, early detection remains the most important approach to improve long-term survival of patients with ovarian cancer. Novel high-throughput technologies, such as microarrays and proteomics, hold promise for the identification of new molecular signatures of early disease and the discovery of novel screening/diagnostic biomarkers for ovarian cancer (4). However, until reliable screening or diagnostic strategies become available, identification of new prognosticators will contribute to the optimal management of ovarian cancer patients.

Prognostic indicators, defined as factors that correlate with patient survival, improve the accuracy of medical prediction. Traditional clinicopathologic variables of prognosis in ovarian cancer (e.g., Fédération Internationale des Gynaecologistes et Obstetristes stage, tumor grade, tumor size, residual tumor size after surgery, age, and presence/absence of ascites) have limitations in predicting the outcome of an individual patient due to the heterogeneity of the disease and its relatively undefined etiology. Thus, there exists a need to discover biomarkers that provide independent prognostic information from that of traditional criteria to tailor treatment strategies to individual patients and even provide insight into the biology of ovarian tumors. In recent years, a plethora of individual biomarkers with prognostic potential have been discovered (e.g., cell cycle control proteins, growth factor receptors, proteases, and protease inhibitors; refs. 5–8) and a 115-gene prognostic signature denoted the "Ovarian Cancer Prognostic

Profile" with independent prognostic value was identified by microarray analysis (9). Many members of the human tissue kallikrein family are among the recently identified prognostic factors of clinical interest in ovarian cancer.

Human tissue kallikreins (hK) constitute a group of 15 trypsin and chymotrypsin-like secreted serine proteases, encoded by a contiguous cluster of structurally similar genes (*KLK*) on chromosome 19q13.4 (10–12). The most prominent hK is human kallikrein 3 (hK3; also known as prostate-specific antigen), the best known cancer biomarker in clinical medicine for the early detection and management of prostate cancer (13). Recent data suggest that many other tissue kallikreins, in addition to hK3/prostate-specific antigen, represent promising biomarkers for several cancer types, particularly ovarian carcinoma (14). To date, 12 *KLK* genes are reportedly up-regulated in ovarian cancer as evidenced by numerous studies of altered *KLK* transcript and hK protein levels in the tumor tissues, cell lines, ascites fluid, and/or serum of patients with this malignancy (12, 15). Recently, with the advent of more inclusive DNA chips, many microarray gene expression profiling studies have found *KLK* gene up-regulation in ovarian cancer tissues (16–21). Moreover, preliminary clinical studies indicate that the overexpression of 11 kallikreins in ovarian cancer correlates with patient prognosis (12, 15). Interestingly, *KLK7* is part of the aforementioned Ovarian Cancer Prognostic Profile signature (9). Furthermore, elevated serum levels of hK5 (22), hK6 (23), hK8 (24), hK10 (25), hK11 (26), and hK14 (27) in ovarian cancer patients may aid in the early detection of this malignancy.

Human kallikrein gene 8 [*KLK8*, also known as *neuropsin, ovasin, tumor-associated differentially expressed gene-14* (*TADG-14*); encoding the hK8 protein] was originally cloned from hippocampus cDNA as the human orthologue of mouse *neuropsin* (28), a brain-related trypsin–like serine protease implicated in various neurologic processes including neural plasticity, memory formation, and some forms of epilepsy (29–32). Human *KLK8* is expressed in multiple brain regions at the mRNA (28, 33, 34) but not at the protein level (35), suggesting that the human hK8 protein, unlike its mouse orthologue, may not have a principal role in normal brain physiology. However, *KLK8* mRNA was shown to be significantly elevated in the hippocampus of patients with Alzheimer's disease (34), indicating that the hK8 protein may have a causal role in the pathology of Alzheimer's disease. In addition to its implied role in the brain, an increasing body of evidence links *KLK8*/hK8 with ovarian cancer. We, among other groups, have previously reported overexpression of the *KLK8* gene in ovarian carcinoma tissues at both the mRNA and protein levels by a variety of technologies including

bioinformatics (36), Northern blotting (37), reverse transcription-PCR (37–39), microarray (19, 20), immunoassay (24), and immunohistochemistry (37, 39). *KLK8* overexpression was found to correlate with a favorable patient prognosis (i.e., early-stage disease, low-grade tumors, and a longer disease-free and overall patient survival; refs. 38, 39). Because hK8 is a secreted protein, we have also observed elevated levels in the tissues, serum, and ascites fluid of a proportion of women with ovarian cancer (24). Taken together, *KLK8*/hK8 represents a promising diagnostic and prognostic biomarker for ovarian carcinoma.

Given the heterogeneity of ovarian carcinomas, the pressing need for ovarian cancer prognosticators, and the repeatedly reported association of hK8 with this malignancy, the aim of the present study was to further assess the prognostic value of hK8 expression in ovarian carcinoma by quantifying and correlating hK8 levels in epithelial ovarian tumor cytosolic extracts to clinicopathologic variables and patient survival.

Materials and Methods

Ovarian cancer patients and specimens. One hundred thirty-six patients with primary epithelial ovarian cancer were examined in this study, ranging in age from 20 to 85 years, with a median age of 57 (Table 1). Patients were monitored for survival and disease progression (no apparent progression or progression) for a median duration of 42 months. Follow-up information was available for all 136 patients, among which 78 (57%) had relapsed and 59 (43%) had died.

Histologic examination, done during intrasurgery frozen section analysis, allowed representative portions of each tumor containing >80% tumor cells to be selected for storage until analysis. Clinical and pathologic information documented at the time of surgery included disease stage, tumor grade and histotype, residual tumor size, debulking success, and volume of ascites fluid (Table 2). The staging of tumors was in accordance with the Fédération Internationale des Gynaecologistes et Obstetristes criteria (40), grading was established according to Day et al. (41), and the classification of histotypes was based on both the WHO and Fédération Internationale des Gynaecologistes et Obstetristes recommendations (42). CA125 levels (KU/mg) in tumors were measured using the Immulite 2000 assay (Diagnostic Products Corporation, Los Angeles, CA).

Patients with disease of clinical stages I to IV and tumor grades 1 to 3 were represented in this study. Of the 136 ovarian tumors, the majority (97; 71%) were of the serous papillary histotype, followed by mucinous (12; 9%), undifferentiated (12; 9%), endometrioid (6; 4%), clear cell (4; 3%), or were unclassified (5; 4%). Residual tumor size ranged from 0 to 6 cm.

Investigations were carried out in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983, and were approved by the Institutional Review Boards of Mount Sinai Hospital and the Technical University of Munich.

Table 1. Descriptive statistics of the continuous variables in the ovarian cancer study population

Variable	Mean ± SE	Range	Percentiles						
			10	25	40	50 (median)	60	75	90
hK8 (ng/mg)	30.0 ± 5.1	0.00-478	0.00	1.5	6.8	10.1	14.2	25.7	94.3
CA 125 (KU/mg)	2.6 ± 0.4	0.00-32.7	0.02	0.1	0.4	1.00	1.5	2.9	7.2
Age (y)	58.5 ± 1.1	20.00-85.0	42.7	51.0	55.0	57.0	62.0	67.0	75.0

Table 2. Relationship between hK8 status and other variables in ovarian cancer patients

Variable	Patients	No. patients (%)		*P*
		hK8 negative	hK8 positive	
Stage				
I	25	14 (56.0)	11 (44.0)	
II	8	6 (75.0)	2 (25.0)	0.093*
III	75	57 (76.0)	18 (24.0)	
IV	28	24 (85.7)	4 (14.3)	
Grade				
G1	13	6 (46.2)	7 (53.8)	
G2	39	27 (69.2)	12 (30.8)	0.014*
G3	83	68 (81.9)	15 (18.1)	
Histotype				
Serous	97	73 (75.3)	24 (24.7)	
Mucinous	12	8 (66.7)	4 (33.3)	
Endometrioid	6	6 (100.0)	0 (0.00)	0.39*
Clear cell	4	3 (75.0)	1 (25.0)	
Undifferentiated	12	7 (58.3)	5 (41.7)	
Residual tumor (cm)				
0	70	46 (65.7)	24 (34.3)	
≤2	38	31 (81.6)	7 (18.4)	0.051*
›2	24	21 (87.5)	3 (12.5)	
Debulking success†				
SD	62	52 (83.9)	10 (16.1)	0.027‡
OD	70	46 (65.7)	24 (34.3)	
Ascites fluid (mL)				
0	41	29 (70.7)	12 (29.3)	
≤500	45	30 (66.7)	15 (33.3)	0.12*
›500	46	39 (84.8)	7 (15.2)	

NOTE: The optimal cutoff value 25.8 ng/mg total protein (74th percentile) was used to classify tumors as hK8 positive and hK8 negative.
*χ^2 test.
†Fisher's exact test.
‡OD, optimal debulking (0-1 cm); SO, suboptimal debulking (›1 cm).

Preparation of cytosolic extracts. Tumor specimens were snap-frozen in liquid nitrogen immediately after surgery and stored at −80°C until extraction. Frozen tissues (20-100 mg) were pulverized on dry ice to a fine powder and added to 10 volumes of extraction buffer [50 mmol/L Tris (pH 8.0), 150 mmol/L NaCl, 5 mmol/L EDTA, 10 g/L NP40 surfactant, 1 mmol/L phenylmethylsulfonyl fluoride, 1 g/L aprotinin, 1 g/L leupeptin]. The resulting suspensions were incubated on ice for 30 minutes with repeated shaking and vortexing every 10 minutes. The mixtures were then centrifuged at 14,000 rpm at 4°C for 30 minutes and the supernatant (cytosolic extract) was collected and stored at −80°C until further analysis. Protein concentration of the extracts was determined using the bicinchoninic acid method with bovine serum albumin as standard (Pierce Chemical Co., Rockford, IL).

Measurement of hK8 in ovarian cytosolic extracts. The concentration of hK8 in cytosolic extracts was quantified using a highly sensitive and specific noncompetitive "sandwich-type" ELISA previously described and evaluated (35). The hK8-ELISA includes two mouse monoclonal anti-hK8 antibodies and recombinant hK8 produced in baculovirus as a standard. The dynamic range of this assay is 0.1 to 20 μg/L. hK8 measurements in micrograms per liter were converted to nanograms of hK8 per milligram of total protein to adjust for the amount of tumor tissue extracted. Please note that the

inclusion of surfactant and protease inhibitors in the extraction buffer did not perturb the measurement of hK8 with our ELISA as consistent correlations between hK8 levels from tissues extracted with and without surfactant and protease inhibitors were observed (data not shown).

Statistical analysis. Statistical analyses were done with SPSS software (SPSS, Inc., Richmond, CA). Ovarian tumor extracts were categorized as either hK8 positive or hK8 negative. The relationship between hK8 status and various clinicopathologic variables was analyzed with the χ^2 test and the Fisher's exact test as appropriate.

For survival analysis, two different end points—cancer relapse (either local recurrence or distant metastasis) and death—were used to calculate progression-free survival (PFS) and overall survival (OS), respectively. PFS was defined as the time interval between the date of surgery and the date of identification of recurrent of metastatic disease. OS was defined as the time interval between the date of surgery and the date of death. The effect of hK8 on patient survival (PFS and OS) was assessed with the hazard ratio (HR; relative risk of relapse or death in the hK8-positive group) calculated with the Cox univariate and multivariate proportional hazard regression model (43). Only patients for whom the status of all variables was known were included in the multivariate regression models. The multivariate models were adjusted for hK8 expression in tumors and other clinical and pathologic variables that may affect survival, including age, stage of disease, tumor grade, CA125, and age. Kaplan-Meier PFS and OS curves (44) were also constructed to show survival differences between the hK8-positive and hK8-negative patients. The differences between the survival curves were tested for statistical significance using the log-rank test (45).

Results

Distribution of hK8 concentration in ovarian tumor tissues. hK8 concentration in ovarian tumor cytosols from 136 patients ranged from 0 to 478 ng/mg total protein, with a mean of 30 ng/mg total protein and a median of 10 ng/mg total protein (Table 1). An optimal cutoff value of 25.8 ng/mg total protein
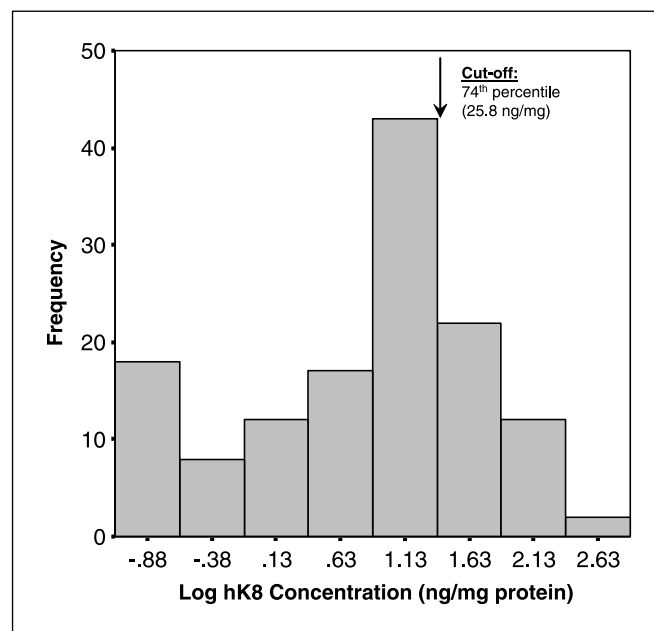

Fig. 1. Frequency distribution of hK8 concentration in ovarian tumor cytosols. The optimal cutoff value 25.8 ng/mg total protein (74th percentile) was used to classify tumors as hK8 positive and hK8 negative.
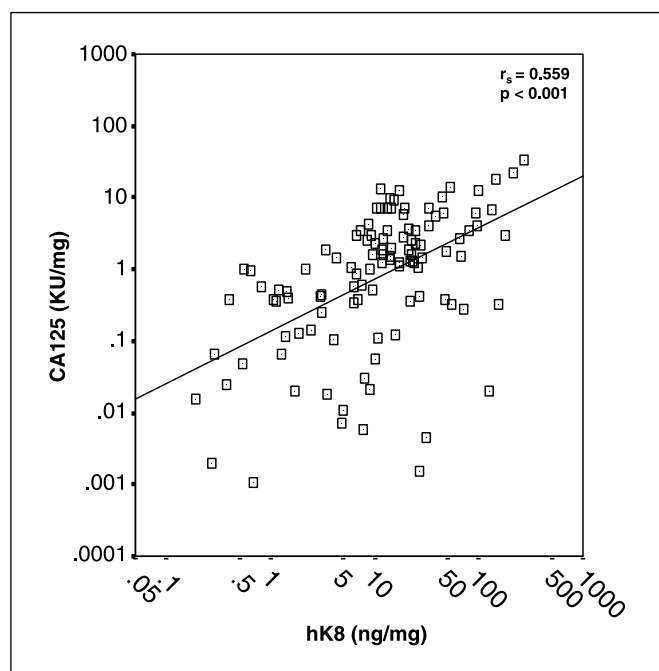
Fig. 2. Correlation between tissue CA125 and hK8 levels.

Relationships between hK8 status and other clinicopathologic variables. The distributions of various clinicopathologic variables between hK8-positive and hK8-negative patients are summarized in Table 2. The relationships between hK8 and these variables were examined with either the χ^2 or Fisher's exact test. Patients with hK8-positive ovarian tumors were more likely to have low-grade tumors (G1), no residual tumor after surgery, and optimal debulking success ($P < 0.05$). Although marginally significant, patients with hK8-positive tumors mainly had early-stage disease (stage I/II; $P = 0.093$). No relationship was observed between hK8 status and tumor histotype or volume of ascites fluid.

The correlation between tissue CA125 and hK8 levels (Spearman correlation $r_s = 0.559$) is shown in Fig. 2. Although the correlation is significant ($P < 0.001$), many samples display variable values.

Univariate and multivariate survival analysis. The strength of association between hK8-positive tumors and survival outcome is presented in Table 3. In univariate Cox regression analysis, hK8-positive patients had a lower risk of relapse (HR, 0.36; $P = 0.002$) and death (HR, 0.41; $P = 0.018$). Similarly, in multivariate Cox regression analysis, hK8 positivity was found to be significantly associated with a longer PFS and OS (HR, 0.48 and 0.47; $P = 0.037$ and $P = 0.076$, respectively). This regression model suggests that there is an ~48% reduction in either the risk of relapse or death in patients with hK8-positive tumors compared with those who are hK8 negative. Kaplan-Meier survival curves (Fig. 3) further show that women with hK8-positive ovarian tumors have substantially longer PFS and OS ($P = 0.001$ and $P = 0.014$, respectively) compared with those with hK8-negative tumors.

was identified by χ^2 analysis based on the ability of hK8 to predict the PFS of the study population. Based on this cutoff (74th percentile), 25.7% of the ovarian tumors were categorized as hK8 positive (Fig. 1).

Table 3. Univariate and multivariate analysis of hK8 with respect to PFS and OS

Variable	PFS			OS		
	HR*	95% CI[†]	*P*	HR*	95% CI[†]	*P*
Univariate analysis						
hK8 (*n* = 132)						
Negative	1.00			1.00		
Positive	0.36	0.19-0.69	0.002	0.41	0.19-0.85	0.018
As continuous variable	0.990	0.982-0.998	0.021	0.988	0.978-0.999	0.037
Stage of disease (ordinal)	2.51	1.53-3.02	<0.001	2.97	1.96-4.52	<0.001
Grading (ordinal)	1.48	1.08-2.029	0.014	1.63	1.14-2.31	0.006
CA125	0.96	0.89-1.03	0.23	0.96	0.89-1.04	0.35
Age (y)	1.01	0.99-1.03	0.14	1.02	1.00-1.04	0.079
Multivariate analysis[‡]						
hK8 (*n* = 121)						
Negative	1.00			1.00		
Positive	0.48	0.24-0.95	0.037	0.47	0.21-1.08	0.076
As continuous variable	0.99	0.98-1.00	0.066	0.98	0.96-1.00	0.11
Stage of disease (ordinal)	1.92	1.32-2.79	0.001	2.92	1.83-4.83	<0.001
Grading (ordinal)	1.12	0.78-1.61	0.53	1.17	0.77-1.78	0.44
CA125	1.01	0.93-1.09	0.81	1.03	0.93-1.13	0.59
Age (y)	1.01	0.99-1.04	0.17	1.026	1.00-1.05	0.053

*HR estimated from Cox proportional hazard regression model.
[†]95% confidence interval of the estimated HR.
[‡]Multivariate models were adjusted for stage of disease, tumor grade, CA125 and age.

As expected, disease staging was found to be strongly associated with decreased PFS and OS in both univariate and multivariate analyses ($P \leq 0.001$). However, tumor CA125 was not a significant predictor of PFS or OS in univariate and multivariate analyses (all $P > 0.2$).

Discussion

During the last decade, numerous studies have been published that attempt to refine our understanding of determinants of prognosis in epithelial ovarian cancer. Through the use of traditional approaches and more recent microarray and proteomics-based expression profiling technologies, a number of tumor-associated prognostic biomarkers with biological relevance in ovarian tumorigenesis or tumor progression have been discovered. Proteolytic enzymes of several catalytic classes (e.g., serine, cysteine, and metallo) have emerged as important prognosticators in ovarian cancer

Fig. 3. Kaplan-Meier survival curves for disease-free survival (*A*) and overall survival (*B*) in patients with hK8-positive and hK8-negative ovarian tumors. *n*, number of samples.

(46). Among these enzymes are many members of human tissue kallikrein family of secreted serine proteases, including *KLK8*/hK8, a promising biomarker for ovarian cancer diagnosis, prognosis, and monitoring (24, 38, 39).

In the present study, we quantified hK8 protein expression levels in epithelial ovarian tumor extracts and correlated these values with traditional prognostic indicators and patient survival. We found that women with hK8-positive ovarian tumors most frequently had early-stage disease, lower-grade tumors, no residual tumor, and optimal debulking success. We also showed that patients with hK8-positive tumors have a longer PFS and OS as evidenced by multivariate Cox proportional hazards regression analysis and Kaplan-Meier survival curves. Overall, our results indicate that hK8 is an independent predictor of favorable prognosis in ovarian cancer.

Our present findings are in general agreement with previous studies on the expression and prognostic value of *KLK8*/hK8 in ovarian carcinoma (20, 24, 37–39). In our earlier study, we measured *KLK8* mRNA levels in ovarian tumor cytosolic extracts by quantitative reverse transcription-PCR and established that patients with higher tumor *KLK8* mRNA levels had lower-grade tumors, smaller residual tumors after surgery, and a longer PFS and OS than patients with lower tumor *KLK8* levels (38). We also provided evidence that *KLK8* mRNA expression is an independent predictor of increased PFS in multivariate analysis. A recent study by Shigemasa et al. (39) also reported an association between higher hK8 protein levels in ovarian tumors, as detected by immunohistochemistry, with early-stage disease and longer OS in univariate analysis but not in multivariate analysis. Furthermore, when patients were stratified into subgroups according to clinical stage, tumor histotype, and grade, hK8 expression also correlated with a longer OS in patients with nonserous and low-grade tumors, suggesting that hK8 may be of clinical interest in these patient subgroups. Furthermore, we have also observed that higher hK8 ascites levels are present in women with lower-stage ovarian carcinoma (24). Lastly, studies have shown that *KLK8* mRNA is also highly overexpressed in ovarian tumors of low malignant potential compared with normal ovarian tissues (37, 39) and serous ovarian tumors (20). These findings further validate the association of *KLK8* expression with favorable prognosis because patients with low malignant potential tumors have a relatively better outcome than those with serous ovarian carcinomas. Collectively, these studies confirm that high *KLK8*/hK8 expression in ovarian cancer indicates a favorable course of disease.

In addition to hK8, extensive correlative clinical data have linked the overexpression of 11 other kallikreins to ovarian cancer patient prognosis. Although most reports link high kallikrein expression with poor patient prognosis (e.g., kallikreins 4, 5, 6, 7, 10, and 15), several studies also recognize some kallikreins as favorable prognostic indicators (e.g., kallikreins 9, 11, 13, and 14; ref. 15). Besides hK family members, the expression of other serine proteases (e.g., TADG-15; ref. 47) and matrix metalloproteases (48) also forecast a favorable outcome in ovarian and other malignancies. Although these clinical findings seem to be contradictory, they may be explained by the dual role that hKs, and proteases in general, have during tumor progression (15, 48). For instance, evidence suggests that hKs can promote and inhibit cancer cell growth, angiogenesis,
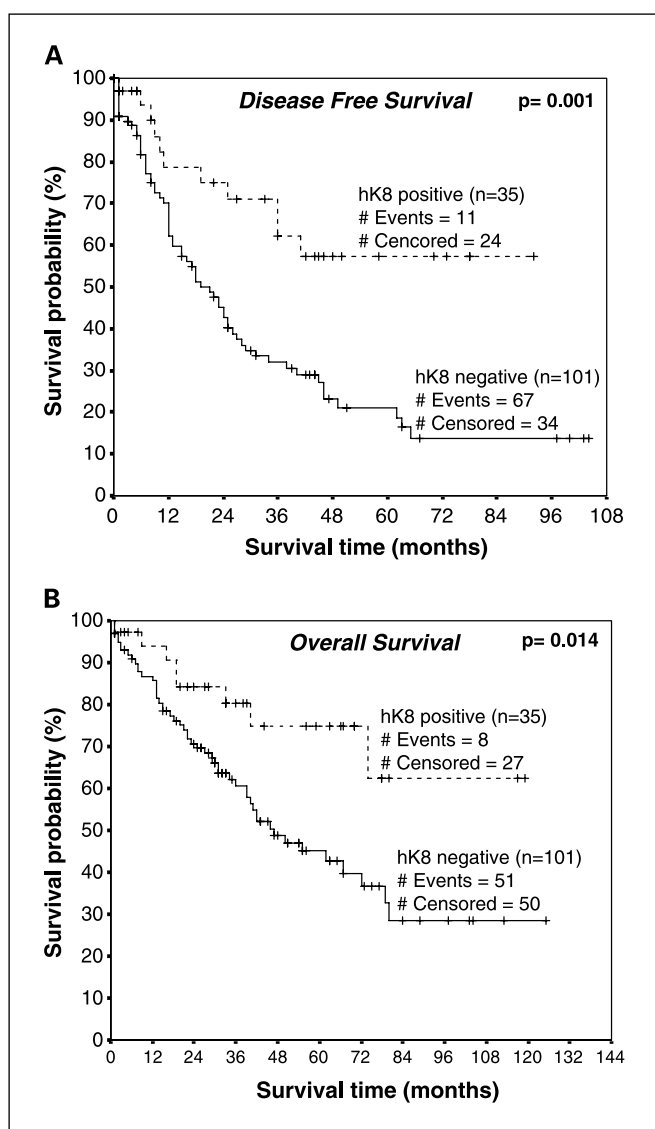
invasion, and metastasis by proteolytic processing of growth factor–binding proteins, activation of growth factors and other proteases, release of angiogenic or antiangiogenic factors, and degradation of extracellular matrix components, depending on the microenvironment (i.e., factors present in different tissues and steroid hormone balances; ref. 15). Although the function of hK8 is currently unknown, the fact that it has been immunohistochemically localized near the invasive front of ovarian tumors (37) and that its mouse orthologue can cleave the extracellular matrix protein fibronectin (49) suggests that hK8 may have a role in pericellular proteolysis during tumor progression.

In addition to proteases, a plethora of other prognostic indicators for ovarian cancer have been identified and evaluated with variable success. In fact, it has become evident that measurements of single biomarkers may not provide sufficient prognostic information to be clinically useful. Instead, panels of biomarkers will need to be simultaneously measured to produce more informative prognostic indices for ovarian cancer. However, no such multiparametric model will improve the ability to significantly predict outcome in ovarian cancer if the individual factors do not carry independent prognostic value. In this respect, it might be interesting to examine the combined prognostic value of hK8 with other kallikreins and other favorable prognosticators in ovarian cancer such as TADG-15 (47), bikunin (7), and the progesterone receptor (50). This may be particularly useful given the contrasting reports on the prognostic value of CA125 in this malignancy (51).

In conclusion, we provide further evidence to support the potential clinical utility of tissue hK8 as an indicator of favorable outcome in ovarian cancer patients. In the future, it may be worthwhile to evaluate the prognostic value of serum hK8 levels as well as to include hK8 in a panel of with other independent prognostic factors. Because *KLK8* mRNA is also expressed in prostate, breast, and colon cancer (37) and highly overexpressed in cervical cancer (52), the clinical usefulness of this protease in other malignancies should be examined as well. hK8 may also represent a therapeutic target for the inhibition of tumor progression once its biological pathways are delineated. Further clinical and basic studies are warranted to determine the biological basis and significance of our findings.

References

1. Jemal A, Murray T, Ward E, et al. Cancer statistics. CA Cancer J Clin 2005;55:10–30.

2. Schink JC. Current initial therapy of stage III and IV ovarian cancer: challenges for managed care. Semin Oncol 1999;26:2–7.

3. Cannistra SA. Cancer of the ovary. N Engl J Med 2004;351:2519–29.

4. Bandera CA, Ye B, Mok SC. New technologies for the identification of markers for early detection of ovarian cancer. Curr Opin Obstet Gynecol 2003;15:51–5.

5. Dogan E, Saygili U, Tuna B, et al. p53 and mdm2 as prognostic indicators in patients with epithelial ovarian cancer: a multivariate analysis. Gynecol Oncol 2005; 97:46–52.

6. Konecny G, Untch M, Pihan A, et al. Association of urokinase-type plasminogen activator and its inhibitor with disease progression and prognosis in ovarian cancer. Clin Cancer Res 2001;7:1743–9.

7. Matsuzaki H, Kobayashi H, Yagyu T, et al. Plasma bikunin as a favorable prognostic factor in ovarian cancer. J Clin Oncol 2005;23:1463–72.

8. Nielsen JS, Jakobsen E, Holund B, et al. Prognostic significance of p53, Her-2, and EGFR overexpression in borderline and epithelial ovarian cancer. Int J Gynecol Cancer 2004;14:1086–96.

9. Spentzos D, Levine DA, Ramoni MF, et al. Gene expression signature with independent prognostic significance in epithelial ovarian cancer. J Clin Oncol 2004;22:4700–10.

10. Diamandis EP, Yousef GM, Clements J, et al. New nomenclature for the human tissue kallikrein gene family. Clin Chem 2000;46:1855–8.

11. Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr Rev 2001;22:184–204.

12. Borgono CA, Michael IP, Diamandis EP. Human tissue kallikreins: physiologic roles and applications in cancer. Mol Cancer Res 2004;2:257–80.

13. Diamandis EP. Prostate-specific antigen—its usefulness in clinical medicine. Trends Endocrinol Metab 1998;9:310–6.

14. Diamandis EP, Yousef GM. Human tissue kallikreins: a family of new cancer biomarkers. Clin Chem 2002; 48:1198–205.

15. Borgono CA, Diamandis EP. The emerging roles of human tissue kallikreins in cancer. Nat Rev Cancer 2004;4:876–90.

16. Shvartsman HS, Lu KH, Lee J, et al. Overexpression of kallikrein 10 in epithelial ovarian carcinomas. Gynecol Oncol 2003;90:44–50.

17. Welsh JB, Sapinoso LM, Kern SG, et al. Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. Proc Natl Acad Sci U S A 2003;100:3410–5.

18. Adib TR, Henderson S, Perrett C, et al. Predicting biomarkers for ovarian cancer using gene-expression microarrays. Br J Cancer 2004;90:686–92.

19. Hibbs K, Skubitz KM, Pambuccian SE, et al. Differential gene expression in ovarian carcinoma: identification of potential biomarkers. Am J Pathol 2004;165: 397–414.

20. Gilks CB, Vanderhyden BC, Zhu S, et al. Distinction between serous tumors of low malignant potential and serous carcinomas based on global mRNA expression profiling. Gynecol Oncol 2005;96:684–94.

21. Santin AD, Zhan F, Bellone S, et al. Gene expression profiles in primary ovarian serous papillary tumors and normal ovarian epithelium: identification of candidate molecular markers for ovarian cancer diagnosis and therapy. Int J Cancer 2004;112:14–25.

22. Yousef GM, Polymeris ME, Grass L, et al. Human kallikrein 5: a potential novel serum biomarker for breast and ovarian cancer. Cancer Res 2003;63: 3958–65.

23. 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.

24. Kishi T, Grass L, Soosaipillai A, et al. Human kallikrein 8, a novel biomarker for ovarian carcinoma. Cancer Res 2003;63:2771–4.

25. Luo LY, Katsaros D, Scorilas A, et al. The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis. Cancer Res 2003;63:807–11.

26. Diamandis EP, Okui A, Mitsui S, et al. Human Kallikrein 11: A new biomarker of prostate and ovarian carcinoma. Cancer Res 2002;62:295–300.

27. Borgono CA, Grass L, Soosaipillai A, et al. Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. Cancer Res 2003;63:9032–41.

28. Yoshida S, Taniguchi M, Hirata A, et al. Sequence analysis and expression of human neuropsin cDNA and gene. Gene 1998;213:9–16.

29. Chen ZL, Yoshida S, Kato K, et al. Expression and activity-dependent changes of a novel limbic-serine protease gene in the hippocampus. J Neurosci 1995;15:5088–97.

30. Okabe A, Momota Y, Yoshida S, et al. Kindling induces neuropsin mRNA in the mouse brain. Brain Res 1996;728:116–20.

31. Momota Y, Yoshida S, Ito J, et al. Blockade of neuropsin, a serine protease, ameliorates kindling epilepsy. Eur J Neurosci 1998;10:760–4.

32. Komai S, Matsuyama T, Matsumoto K, et al. Neuropsin regulates an early phase of schaffer-collateral long-term potentiation in the murine hippocampus. Eur J Neurosci 2000;12:1479–86.

33. Mitsui S, Tsuruoka N, Yamashiro K, et al. A novel form of human neuropsin, a brain-related serine protease, is generated by alternative splicing and is expressed preferentially in human adult brain. Eur J Biochem 1999;260:627–34.

34. Shimizu-Okabe C, Yousef GM, Diamandis EP, et al. Expression of the kallikrein gene family in normal and Alzheimer's disease brain. Neuroreport 2001;12: 2747–51.

35. Kishi T, Grass L, Soosaipillai A, et al. Human kallikrein 8: immunoassay development and identification in tissue extracts and biological fluids. Clin Chem 2003; 49:87–96.

36. Yousef GM, Polymeris ME, Yacoub GM, et al. Parallel overexpression of seven kallikrein genes in ovarian cancer. Cancer Res 2003;63:2223–7.

37. Underwood LJ, Tanimoto H, Wang Y, et al. Cloning of tumor-associated differentially expressed gene-14, a novel serine protease overexpressed by ovarian carcinoma. Cancer Res 1999;59:4435–9.

38. Magklara A, Scorilas A, Katsaros D, et al. The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer. Clin Cancer Res 2001;7:806–11.

39. Shigemasa K, Tian X, Gu L, et al. Human kallikrein 8 (hK8/TADG-14) expression is associated with an early clinical stage and favorable prognosis in ovarian cancer. Oncol Rep 2004;11:1153–9.

40. Pettersson F. Annual report on the treatment in gynecological cancer. Stockholm: International Federation of Gynecology and Obstetrics; 1994. p. 83–102.

41. Day TG, Jr., Gallager HS, Rutledge FN. Epithelial carcinoma of the ovary: prognostic importance of histologic grade. J Natl Cancer Inst Monogr 1975;42:15–21.

42. Serov SF, Sorbin LH. Histological typing of ovarian tumors. World Health Organization; 1973.

43. Cox DR. Regression models and life tables. J R Stat Soc B 1972;34:187–202.

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

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

46. Duffy MJ. Proteases as prognostic markers in cancer. Clin Cancer Res 1996;2:613–8.

47. Tanimoto H, Shigemasa K, Tian X, et al. Trans-membrane serine protease TADG-15 (ST14/Matriptase/MT-SP1): expression and prognostic value in ovarian cancer. Br J Cancer 2005;92:278–83.

48. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002;2:161–74.

49. Shimizu C, Yoshida S, Shibata M, et al. Characterization of recombinant and brain neuropsin, a plasticity-related serine protease. J Biol Chem 1998;273:11189–96.

50. Lee P, Rosen DG, Zhu C, et al. Expression of progesterone receptor is a favorable prognostic marker in ovarian cancer. Gynecol Oncol 2005;96:671–7.

51. Meyer T, Rustin GJ. Role of tumor markers in monitoring epithelial ovarian cancer. Br J Cancer 2000;82:1535–8.

52. Cane S, Bignotti E, Bellone S, et al. The novel serine protease tumor-associated differentially expressed gene-14 (KLK8/Neuropsin/Ovasin) is highly overexpressed in cervical cancer. Am J Obstet Gynecol 2004;190:60–6.