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ORIGINAL REPORT

Human Kallikrein 13 Protein in Ovarian Cancer Cytosols: A New Favorable Prognostic Marker

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ABSTRACT

Purpose
Human kallikrein 13 (hK13; encoded by the *KLK13* gene) is a secreted serine protease expressed in endocrine tissues, including the prostate, testis, breast, and ovary. We have previously reported steroid hormone regulation of the *KLK13* gene and its clinical value as a marker of favorable prognosis in breast cancer at the mRNA level. We hypothesized that hK13 may represent a potential biomarker for ovarian carcinomas.

Patients and Methods
Using a newly developed enzyme-linked immunosorbent assay (ELISA), hK13 levels were quantified in 131 ovarian tumor extracts and correlated with various clinicopathological variables and outcome (progression-free survival [PFS], overall survival [OS]), over a median follow-up period of 42 months.

Results
hK13 concentration in ovarian tumor cytosols ranged from 0 to 18.4 ng/mg of total protein. An optimal cutoff value of 0.13 ng/mg (67th percentile) was selected, based on the ability of hK13 values to predict the PFS of the study population, to categorize tumors as hK13-positive or negative. Women with hK13-positive tumors most often had early stage (stage I/II) disease, no residual tumor after surgery and optimal debulking success ($P < .05$). Univariate and multivariate Cox regression analyses revealed that patients with hK13-positive tumors had a significantly longer PFS and OS than hK13-negative patients ($P < .05$). Kaplan-Meier survival curves further confirmed a reduced risk of relapse and death in women with hK13-positive tumors ($P = .007$ and $P = .002$, respectively).

Conclusion
These results indicate that hK13 is an independent marker of favorable prognosis in ovarian cancer.

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INTRODUCTION

Epithelial ovarian cancer is the most lethal gynecologic malignancy, constituting approximately 90% of all ovarian cancer cases [1,2]. The high mortality rate is usually ascribed to late diagnosis, since epithelial ovarian tumors commonly lack early warning symptoms. Furthermore, ovarian carcinomas often lack definite precursor lesions, are quite heterogeneous, and the molecular pathways underlying their progression are still elusive. Thus, many attempts have been made to predict the biology of ovarian tumors in order to determine prognosis and develop individualized treatment strategies. The International Federation of Gynecology and Obstetrics (FIGO) stage at diagnosis represents the major prognostic factor in ovarian cancer. FIGO stage I patients have a 5-year survival of 80% to 90%, compared with only 15% to 20% for women with stage III and IV disease [3]. Other well established conventional prognostic markers include tumor grade, patient age, residual tumor after surgery, presence and absence of ascites, and histology [4,5].

In addition to these clinicopathologic parameters, numerous tumor markers with prognostic potential have also been identified, including DNA ploidy, oncogenes, cell cycle regulatory proteins and inhibitors, enzymes, growth factors, extracellular matrix components, and proteases [6-12]. More re-

cently, cDNA microarray analyses and bioinformatic approaches, such as serial analysis of gene expression, have also been used to identify genes differentially expressed in ovarian cancer in order to subcategorize tumors on the basis of molecular profile, often unveiling important biologic, diagnostic, and prognostic information [13-17].

Among the newly identified prognostic factors are human tissue kallikreins, a group of serine proteases encoded by 15 structurally similar, hormonally-regulated genes, clustered in tandem on chromosome 19q13.4 [18]. Accumulating evidence indicates that at least 11 kallikrein family members are differentially expressed in ovarian cancer at both the mRNA and protein levels, and several demonstrate clinical utility as prognostic biomarkers [19,20]. Moreover, the serum levels of kallikrein proteins, hK6, hK10, and hK11, are elevated in a proportion of ovarian cancer patients and as such, they may represent putative serological screening and/or diagnostic biomarkers for ovarian cancer [21-25].

Human kallikrein gene 13 (*KLK13*, previously known as *KLK-L4*), recently discovered by the positional candidate cloning approach, is a novel androgen/progestin-regulated serine protease gene, predominantly expressed in endocrine tissues, including the prostate, testis, and breast [26]. Preliminary evidence indicates that *KLK13* is implicated in hormone-dependent malignancies and has prognostic utility. For one, *KLK13* is downregulated at the mRNA level in breast cancer tissues and cell lines [26]. A subsequent and extensive quantitative reverse transcriptase polymerase chain reaction study demonstrated that *KLK13* expression in breast tumor tissues is an indicator of favorable prognosis, since patients with *KLK13*-positive tumors exhibit a longer progression-free survival (PFS) and overall survival (OS) [27]. Furthermore, five testis-specific splice variants of the *KLK13* gene were identified and found to be downregulated in testicular cancer tissues compared to the matched normal counterparts [28].

In order to study hK13 expression at the protein level, we recently developed a highly specific and sensitive immunofluorometric assay [29]. Using this method, we observed high hK13 expression in esophageal, tonsil, and salivary gland tissues, as well as in endocrine-dependent tissues and their corresponding biologic fluids, namely, the prostate, testis, breast, and seminal plasma and breast milk, respectively [29]. By immuohistochemical analysis, we demonstrated that hK13 is predominantly localized in the glandular epithelia of a variety of normal tissues, including the epithelium of the prostate and surface epithelium of the breast and ovary [30]. Quantification of hK13 in normal, benign, and cancerous ovarian tissues indicated that hK13 is elevated in 50% of ovarian tumor tissues compared to the relatively low levels observed in both normal and benign tissues [29], suggesting that hK13 may have prognostic utility as an ovarian cancer biomarker. Given the above, the aim of the present study was to investigate the expression of hK13 in ovarian cancer tissues and to evaluate its prognostic significance.

PATIENTS AND METHODS

Ovarian Cancer Patients and Specimens

One hundred and thirty-one 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 years (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 131 patients, among which 74 (56%) had relapsed and 54 (41%) had died.

Histological examination, performed during intrasurgery frozen section analysis, allowed representative portions of each tumor containing more than 80% tumor cells to be selected for storage until analysis. Clinical and pathologic information documented at the time of surgery included tumor stage, grade, histotype, residual tumor size, debulking success, and volume of ascites fluid (Table 2). The staging of tumors was in accordance with the FIGO criteria [31], grading was established according to Day et al [32], and the classification of histotypes was based on both the WHO and FIGO recommendations [33].

Patients with disease at clinical stages I to IV and grades 1 to 3 were represented in this study. Of the 134 ovarian tumors, the majority (93 [71%]) were of the serous papillary histotype, followed by mucinous (12 [9%]), undifferentiated (11 [8%]), endometrioid (6 [5%]), clear-cell (4 [3%]), or were unclassified (5 [4%]). The residual size of tumors 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

			Percentiles (median)						
Variable	Mean ± SE	Range	10	25	40	50	60	75	90
hK13, ng/mg	0.61 ± 0.19	0.00-18.4	0.00	0.00	0.00	0.00	0.0043	0.25	1.2
CA-125, KU/mg	2.7 ± 0.4	0.00-32.7	0.02	0.14	0.46	1.05	1.55	2.94	7.2
Age, years	58 ± 1.1	20-85	41	51	55	57	62	68	76

Abbreviation: hK13, human kallikrein 13.

Table 2. Relationship Between hK13 Status and Other Variables in 131 Ovarian Cancer Patients

Variable	No. of Patients	hK13-Negative		hK13-Positive		*P*
		No. of Patients	%	No. of Patients	%	
Stage						
I/II	32	15	46.9	17	53.1	.009*
III/IV	99	73	73.7	26	26.3	
Grade						
G1/G2	53	32	60.4	21	39.6	.19*
G3	78	56	71.8	22	28.2	
Histotype						
Serous	93	66	71.0	27	29.0	
Mucinous	12	5	41.7	7	58.3	
Endometrioid	6	5	83.3	1	16.7	.089†
Clear-cell	4	1	25.0	3	75.0	
Undifferentiated	11	7	63.6	4	36.4	
Status unknown	5					
Residual tumor, cm						
0	68	39	57.4	29	42.6	
≤ 2	37	30	81.1	7	18.9	.026†
> 2	22	17	77.3	5	22.7	
Status unknown	4					
Debulking success						
SD	59	47	79.7	12	20.3	.008*
OD	68	39	57.4	29	42.6	
Status unknown	4					
Acites fluid, ml						
0	41	25	61.0	16	39.0	
≤ 500	43	28	65.1	15	34.9	.27†
> 500	43	33	76.7	10	23.3	
Status unknown	4					

NOTE. Cutoff used was equal to the 67th percentile (0.13 ng/mg protein).
Abbreviations: hK13, human kallikrein 13; SD, suboptimal debulking (> 1 cm); OD, optimal debulking (0-1 cm).
*Fisher's exact test.
†χ^2 test.

Preparation of Cytosolic Extracts

Tumor specimens were snap-frozen in liquid nitrogen immediately after surgery and stored at −80ºC until extraction. Frozen tissues (20 to 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 of NP-40 surfactant, 1 mmol/L phenylmethylsulfonyl fluoride, 1 g/L of aprotinin, 1 g/L of 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 4ºC for 30 minutes and the supernatant (cytosolic extract) was collected and stored at −80ºC 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 hK13 in Ovarian Cytosolic Extracts

The concentration of hK13 in cytosolic extracts was quantified using a highly sensitive and specific noncompetitive "sandwich-type" immunoassay, previously described and evaluated [29]. Briefly, microtiter plates were coated directly with a mouse anti-hK13 monoclonal antibody (code 2-17; 500 ng/well). After a 1 hour incubation, the plates were washed six times with washing buffer (9g/L NaCl and 0.5 g/L Tween 20 in 10 mmol/L Tris buffer, pH 7.40). Then, either recombinant hK13 calibrators (50 μL/well and 50 μL of a general diluent [60 g/L BSA, 50 mmol/L Tris, pH 7.80, 0.5 g/L sodium azide]) or cytosolic extracts (50 μL/well and 50 μL of the general diluent) were applied to each well in duplicate, incubated for 2 hours with gentle shaking and washed. Rabbit anti-hK13 polyclonal antiserum (diluted 1,000-fold in assay buffer containing the components of the general diluent plus 25 mL/L normal mouse serum, 100 mL/L normal goat serum, and 10 g/L bovine IgG) was subsequently applied, incubated for 1 hour and washed. Finally, alkaline phosphatase-conjugated goat antirabbit IgG (Jackson Immunoresearch, West Grove, PA), diluted 2,000-fold in assay buffer, was added, incubated for 45 minutes and washed as before. Signal detection and data reduction were performed automatically by the CyberFluor 615 Immunoanalyzer, which uses time resolved fluorometry, as described elsewhere [34]. The detection range of this assay is 0.1 to 20 ng/mL. hK13 measurements in ng/mL were converted to ng of hK13/mg of total protein, by dividing the ng of hK13/mL of cytosolic extracts with the mg of total protein/mL of cytosolic extracts, to adjust for the amount of tumor tissue extracted.

Statistical Analysis

The relationship between hK13 status and various clinicopathologic variables was analyzed with the χ^2 test and the Fisher's exact test, as appropriate. The hK13 status of ovarian tumor extracts was categorized as either hK13-positive or hK13-negative.

For survival analysis, two different end points—cancer relapse (either local recurrence or distant metastasis) and death—

were used to calculate PFS and OS, respectively. PFS was defined as the time interval between the date of surgery and the date of identification of recurrent metastatic disease. OS was defined as the time interval between the date of surgery and the date of death. The impact of hK13 on patient survival (PFS and OS) was assessed with the hazard ratio (relative risk of relapse or death in the hK13-positive group) calculated with the Cox univariate and multivariate proportional hazard regression model [35]. Only patients for whom the status of all variables was known were included in the multivariate regression models. The multivariate models were adjusted for hK13 expression in tumors and other clinical and pathologic variables that may affect survival, including stage of disease, tumor grade, cytosolic extract CA-125 values, and age. Kaplan-Meier PFS and OS curves [36] were also constructed in order to demonstrate survival differences between the hK13-positive and hK13-negative patients. The differences between the survival curves were tested for statistical significance using the log-rank test [37].

RESULTS

Distribution of hK13 Concentration in Ovarian Tumor Tissues

hK13 concentration in ovarian tumor cytosols from 131 patients ranged from 0 to 18.4 ng/mg of total protein, with a mean of 0.61 ng/mg total protein and a median of 0 ng/mg total protein (Table 1). An optimal cutoff value of 0.13 ng/mg total protein was identified by χ^2 analysis, based on the ability of hK13 to predict the PFS of the study population. Based on this cutoff (67th percentile), 33% of the ovarian tumors were categorized as hK13-positive.

Relationships Between hK13 Status and Other Clinincopathologic Variables

The distributions of various clinicopathologic variables between hK13-postitive and hK13-negative patients are summarized in Table 2. The relationships between hK13 and these variables were examined with either the χ^2 or Fisher's exact test. Patients with hK13-positive ovarian tumors were more likely to have early stage (stage I/II) disease, no residual tumor, and optimal debulking success ($P < .05$). Although marginally significant, hK13-positive tumors were mainly of the clear-cell and mucinous histotypes ($P = .089$). No relationship was observed between hK13 status and tumor grade or volume of ascites fluid.

Univariate and Multivariate Survival Analysis

The strength of association between hK13-positive tumors and survival outcome is presented in Table 3. In univariate Cox regression analysis, hK13-positive patients had a lower risk of relapse (hazard ratio [HR], 0.46; $P = .009$) and death (HR, 0.33; $P = .004$). Since the hK13-positive and negative tumors were classified according the above optimal cutoff, we also evaluated the prognostic significance of hK13 as a continuous variable. hK13 concentrations were transformed logarithmically using half of the lowest positive observation (0.015 ng/mg) for the undetectable hK13 samples. The continuous variable log (hK13) was found to have statistically significant prognostic value for overall survival (HR, 0.63; $P = .028$).

Table 3. Univariate and Multivariate Analysis of hK13 Status With Regard to Progression-Free and Overall Survival

Variable	Progression-Free Survival			Overall Survival		
	HR	95% CI	*P*	HR*	95% CI†	*P*
Univariate analysis						
hK13, n = 131						
Negative	1.00			1.00		
Positive	0.46	0.25 to 0.82	.009	0.33	0.15 to 0.69	.004
Log hK13, continuous variable	0.81	0.59 to 1.11	.19	0.63	0.42 to 0.95	.028
Stage of disease ordinal	2.14	1.50 to 3.04	< .001	3.03	1.95 to 4.67	< .001
Grading, ordinal	1.42	1.04 to 1.94	.027	1.55	1.08 to 2.18	.017
CA-125‡	0.97	0.91 to 1.04	.42	0.98	0.91 to 1.05	.59
Age	1.02	0.99 to 1.03	.084	1.025	1.00 to 1.05	.029
Multivariate analysis§						
hK13, n = 120						
Negative	1.00			1.00		
Positive	0.53	0.29 to 0.97	.042	0.34	0.15 to 0.76	.010
Log hK13, continuous variable	0.86	0.62 to 1.20	.38	0.66	0.42 to 1.03	.068
Stage of disease, ordinal	2.02	1.36 to 2.98	< .001	3.37	1.99 to 5.71	< .001
Grading, ordinal	1.13	0.81 to 1.57	.48	1.21	0.81 to 1.80	.36
CA-125	1.00	0.93 to 1.08	.71	1.03	0.93 to 1.13	.56
Age	1.00	0.98 to 1.02	.45	1.02	0.99 to 1.04	.19

Abbreviations: hK13, human kallikrein 13; HR, hazard ratio.
*HR estimated from Cox proportional hazard regression model.
†Confidence interval of the estimated HR.
‡Tissue CA-125 in KU/mg total protein.
§Multivariate models were adjusted for stage of disease, tumor grade, CA-125, and age.

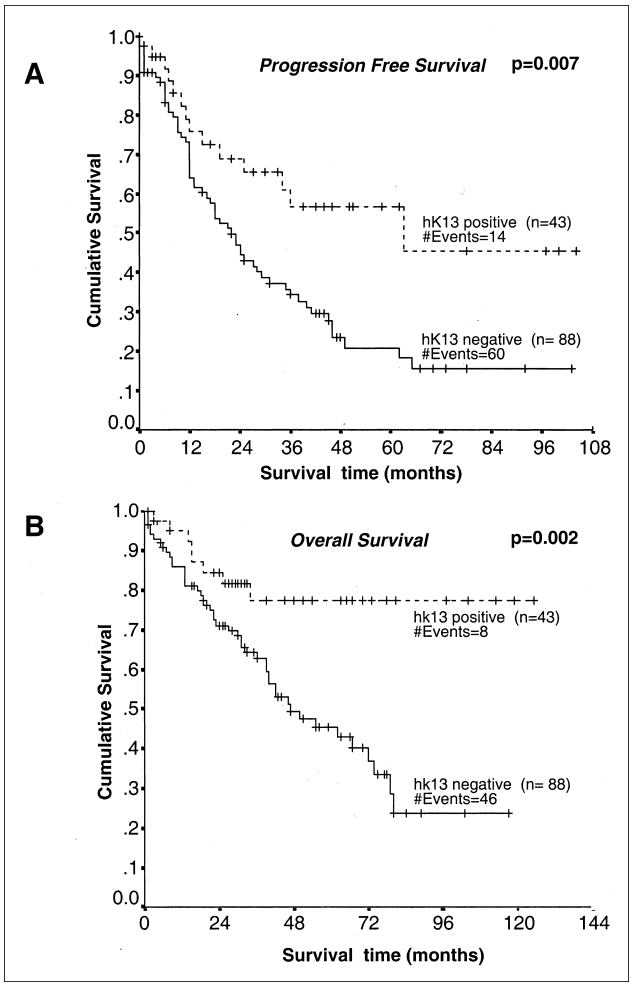

Fig 1. Kaplan-Meier survival curves for (A) progression-free survival and B) overall survival in patients with hK13-positive and negative ovarian tumors.

In multivariate Cox regression analysis, hK13 positivity was found to be significantly associated with a longer PFS and OS (HR, 0.53 and 0.34; $P = .042$ and $P = .01$, respectively). This regression model suggests that there is approximately a 47% to 66% reduction in either the risk of relapse or death in patients with hK13-positive tumors, compared to those who are hK13-negative. Kaplan-Meier survival curves (Fig 1) further demonstrate that women with hK13-positive ovarian tumors have substantially longer PFS and OS ($P < .01$), compared to those with hK13-negative tumors. As expected, disease staging was found to be strongly associated with decreased PFS and OS, in both univariate and multivariate analyses ($P < .001$).

DISCUSSION

During the last few years, a plethora of studies have been published which attempt to refine our understanding of determinants of prognosis in ovarian cancer by analyzing tumor-associated markers thought to be of biologic relevance in the carcinogenic process. Proteases, of several catalytic types (serine, cysteine, metallo), are among these prognostic factors [38]. In the present study, we have evaluated the expression of a serine protease, hK13, in epithelial ovarian tumors in relation to other established prognostic indicators and patient survival. hK13-positive ovarian tumors were most frequently found in patients with early stage disease, no residual tumor, and optimal debulking success. We have also demonstrated that hK13 is an independent predictor of favorable prognosis in ovarian cancer, as evidenced by multivariate Cox proportional hazards regression analysis and Kaplan-Meier survival curves.

Typically, the expression of proteases in cancer tissues correlates with poor patient prognosis in different malignancies [39-44], including ovarian cancer [45-49], likely as a result of the well established roles of proteolytic enzymes in extracellular matrix degradation, which facilitates invasion and metastasis [50,51]. However, in recent years, with the identification of non-extracellular matrix substrates for secreted proteases, new roles for proteolytic enzymes have emerged in the regulation of cellular functions during tumor development, including cell proliferation, differentiation, survival, genomic (in)stability, and angiogenesis [52-54]. In fact, it has been documented that certain proteases, including matrix metalloproteinase-19 [55], and serine proteases such as testisin [56], prostasin [57,58], and human kallikreins 3, 4, 5, 10, 12, 13, and 14 [18], are downregulated in hormone-dependent cancers, and several may function as tumor suppressors [59-61]. These findings may help to explain why certain proteases, including hK13, are associated with a favorable prognosis in cancer patients [62-65]. The human kallikreins 6, 8, 10, and 11 were also analyzed in a fraction of the same samples. hK13 was not found to have statistically significant correlation with the other examined kallikreins (data not shown).

Accumulating evidence suggests that at least 10 of the 15 human kallikrein family members have prognostic value in ovarian cancer (listed in Table 4), exclusive of hK13 [19]. Among these, kallikreins 8, 9, 11, and 14 are comparable to hK13 since they are most often expressed in early stage ovarian tumors and correlate with a favorable patient prognosis [66-69]. The remainder, kallikreins 4, 5, 6, 7, 10, and 15, are expressed in advanced ovarian tumors and are markers of poor prognosis [70-76]. Since the vast majority of kallikreins are coexpressed, and likely coordinately regulated in ovarian cancer, we speculate that they may represent an enzymatic pathway involved in ovarian carcinogenesis by, as yet, unknown mechanisms [19].

In contrast to earlier studies, which reported high kallikrein 4, 6, and 10 expression in serous epithelial ovarian tumors [71,73,75], hK13-positive tumors were more frequently of the nonserous (ie, clear-cell and mucinous) histotypes (Table 4). Similar findings were obtained with kal-

Table 4. Kallikrein Expression (mRNA and protein) in Ovarian Cancer Tissues

Kallikrein	Prognosis	Reference
mRNA*		
KLK4	Unfavorable	[70,71]
KLK5	Unfavorable	[72]
KLK7	Unfavorable	[74]
KLK8	Favorable	[66]
KLK9	Favorable	[67]
KLK14	Favorable	[69]
KLK15	Unfavorable	[76]
Protein†		
hK5	Unfavorable	Our unpublished data
hK6	Unfavorable	[73]
hK10	Unfavorable	[75]
hK11	Favorable	[68]
hK13	Favorable	Present study

*Reverse transcriptase polymerase chain reaction methodology
†Enzyme-linked immunosorbent assay methodology

likreins 5 and 11, which are also associated with nonserous tumors (ie, undifferentiated and mucinous, respectively; unpublished data) [68]. This data suggests that hK13, together with hK5 and hK11, may be clinically useful as determinants of prognosis in the subgroup of ovarian cancer patients with nonserous epithelial tumors.

Previously, we have shown that *KLK13*, at the mRNA level, is an independent marker of favorable prognosis in women with breast carcinomas [27], in agreement with our present findings in ovarian cancer. Although the underlying biologic mechanism of hK13 involvement in the progression of breast and ovarian cancers is currently unknown, it is plausible that this effect is related to steroid hormones. First, we have shown that hK13 is encoded by an androgen-regulated gene [26]. Second, epidemiologic and experimental evidence suggests that steroid hormones, such as androgens, are implicated in the etiology of both breast and ovarian carcinomas [77-79]. Third, it has been documented that the androgen receptor (AR) is present in over 80% of breast [80] and in 84% of ovarian tumors [81] and that androgens, acting through the AR, can stimulate and inhibit breast [78] and increase ovarian [79] cancer cell proliferation. As such, it is likely that AR complexes regulate *KLK13* gene expression during breast and ovarian carcinogenesis. We further speculate that hK13, under androgenic stimulation, may inhibit breast and ovarian cancer metastasis in early stage carcinomas, by initiating or terminating events through the activation of favorable proteins (ie, inhibitors) or cleavage of unfavorable ones (ie, growth factors). Identification of downstream AR-regulated genes, such as *KLK13*, is also important in our understanding of the mechanism by which androgens are implicated in hormone-related malignancies. These findings may have therapeutic applications.

Quantification of hK13 in normal, benign, and cancerous ovarian tissues indicated that hK13 is elevated in 50% of ovarian tumor tissues compared to the relatively low levels observed in both normal and benign tissues. It seems that hK13 is not expressed much in the normal ovarian tissue, but its expression increases in the first stages of tumorigenesis, and then it is progressively suppressed as the malignancy advances. Because there is no information available on the pathologic role of hK13 in ovarian tissue, it would be difficult to formulate a hypothesis that could explain the way of regulation of gene expression and the mechanism by which *KLK13* confers a favorable prognostic outcome in ovarian cancer.

CA-125, a traditional serologic ovarian cancer biomarker, has clinical value for disease diagnosis and it is used as an aid for the early detection of relapse and for assessing response to treatment. CA-125, quantified in ovarian cancer cytosols, was used as a continuous variable in the present study and it was not found to have statistically significant prognostic value. The prognostic significance of CA-125 in cytosolic extracts, in combination with other biomarkers and clinocopathologic features, is still under investigation.

In conclusion, this is the first report to describe the prognostic utility of hK13 as an independent indicator of favorable prognosis in ovarian cancer patients. By virtue of its predominance in nonserous ovarian tumors, hK13 may also be applied clinically in the corresponding subgroup of patients. The biologic basis and significance of our findings are unclear and warrant further basic and clinical studies.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

REFERENCES

1. Jemal A, Murray T, Samuels A, et al: Cancer statistics, 2003. CA Cancer J Clin 53:5-26, 2003
2. Auersperg N, Edelson MI, Mok SC, et al: The biology of ovarian cancer. Semin Oncol 25:281-304, 1998
3. Schink JC: Current initial therapy of stage III and IV ovarian cancer: Challenges for managed care. Semin Oncol 26:2-7, 1999
4. Trope C: Prognostic factors in ovarian cancer. Cancer Treat Res 95:287-352, 1998
5. Eisenhauer EA, Gore M, Neijt JP: Ovarian cancer: Should we be managing patients with good and bad prognostic factors in the same manner? Ann Oncol 10:9-15, 1999 (suppl 1)
6. Ozalp S, Yalcin OT, Gulbas Z, et al: Effect of cellular DNA content on the prognosis of epithelial ovarian cancers. Gynecol Obstet Invest 52:93-97, 2001
7. Milde-Langosch K, Hagen M, Bamberger AM, et al: Expression and Prognostic Value of

the Cell-cycle Regulatory Proteins, Rb, p16MTS1, p21WAF1, p27KIP1, Cyclin E, and Cyclin D2, in Ovarian Cancer. Int J Gynecol Pathol 22:168-174, 2003

8. Masciullo V, Ferrandina G, Pucci B, et al: P27Kip1 expression is associated with clinical outcome in advanced epithelial ovarian cancer: Multivariate analysis. Clin Cancer Res 6:4816-4822, 2000

9. Gotlieb WH, Goldberg I, Weisz B, et al: Topoisomerase II immunostaining as a prognostic marker for survival in ovarian cancer. Gynecol Oncol 82:99-104, 2001

10. Shen GH, Ghazizadeh M, Kawanami O, et al: Prognostic significance of vascular endothelial growth factor expression in human ovarian carcinoma. Br J Cancer 83:196-203, 2000

11. Hata K, Fujiwaki R, Nakayama K, et al: Expression of the Endostatin gene in epithelial ovarian cancer. Clin Cancer Res 7:2405-2409, 2001

12. Schmalfeldt B, Prechtel D, Harting K, et al: Increased expression of matrix metalloproteinases (MMP)-2, MMP-9, and the urokinase-type plasminogen activator is associated with progression from benign to advanced ovarian cancer. Clin Cancer Res 7:2396-2404, 2001

13. Schummer M, Ng WV, Bumgarner RE, et al: Comparative hybridization of an array of 21, 500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. Gene 238: 375-385, 1999

14. Welsh JB, Zarrinkar PP, Sapinoso LM, et al: Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. Proc Natl Acad Sci U S A 98:1176-1181, 2001

15. Haviv I, Campbell IG: DNA microarrays for assessing ovarian cancer gene expression. Mol Cell Endocrinol 191:121-126, 2002

16. Sawiris GP, Sherman-Baust CA, Becker KG, et al: Development of a highly specialized cDNA array for the study and diagnosis of epithelial ovarian cancer. Cancer Res 62:2923-2928, 2002

17. Hough CD, Sherman-Baust CA, Pizer ES, et al: Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. Cancer Res 60:6281-6287, 2000

18. Yousef GM, Diamandis EP: The new human tissue kallikrein gene family: Structure, function, and association to disease. Endocr Rev 22:184-204, 2001

19. Yousef GM, Diamandis EP: Kallikreins, steroid hormones and ovarian cancer: Is there a link? Minerva Endocrinol 27:157-166, 2002

20. Diamandis EP, Yousef GM: Human tissue kallikreins: A family of new cancer biomarkers. Clin Chem 48:1198-1205, 2002

21. Diamandis EP, Yousef GM, Soosaipillai AR, et al: Human kallikrein 6 (zyme/protease M/neurosin): a new serum biomarker of ovarian carcinoma. Clin Biochem 33:579-583, 2000

22. 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 21:1035-1043, 2003

23. Luo LY, Bunting P, Scorilas A, et al: Human kallikrein 10: a novel tumor marker for ovarian carcinoma? Clin Chim Acta 306:111-118, 2001

24. 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 63:807-811, 2003

25. Diamandis EP, Okui A, Mitsui S, et al: Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. Cancer Res 62:295-300, 2002

26. Yousef GM, Chang A, Diamandis EP: Identification and characterization of KLK-L4, a new kallikrein-like gene that appears to be down-regulated in breast cancer tissues. J Biol Chem 275:11891-11898, 2000

27. Chang A, Yousef GM, Scorilas A, et al: Human kallikrein gene 13 (KLK13) expression by quantitative RT-PCR: An independent indicator of favourable prognosis in breast cancer. Br J Cancer 86:1457-1464, 2002

28. Chang A, Yousef GM, Jung K, et al: Identification and molecular characterization of five novel kallikrein gene 13 (KLK13; KLK-L4) splice variants: Differential expression in the human testis and testicular cancer. Anticancer Res 21: 3147-3152, 2001

29. Kapadia C, Chang A, Sotiropoulou G, et al: Human kallikrein 13: production and purification of recombinant protein and monoclonal and polyclonal antibodies, and development of a sensitive and specific immunofluorometric assay. Clin Chem 49:77-86, 2003

30. Petraki CD, Karavana VN, Diamandis EP: Human Kallikrein 13 Expression in Normal Tissues. An immunohistochemical study. J Histochem Cytochem 51:493-501, 2003

31. Pettersson F: Annual report on the treatment in gynecological cancer. Stockholm, International Federation of Gynecology and Obstetrics, 1994

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

33. Serov SF, Sorbin LH: Histological typing of ovarian tumors. World Health Organization, 1973

34. Christopoulos TK, Diamandis EP: Enzymatically amplified time-resolved fluorescence immunoassay with terbium chelates. Anal Chem 64:342-346, 1992

35. Cox DR: Regression models and life tables. J R Stat Soc B 34:181-220, 1972

36. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

37. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163-170, 1966

38. Duffy MJ: Proteases as prognostic markers in cancer. Clin Cancer Res 2:613-618, 1996

39. Duffy MJ, Maguire TM, McDermott EW, et al: Urokinase plasminogen activator: A prognostic marker in multiple types of cancer. J Surg Oncol 71:130-135, 1999

40. Vihinen P, Kahari VM: Matrix metalloproteinases in cancer: Prognostic markers and therapeutic targets. Int J Cancer 99:157-166, 2002

41. Kos J, Lah TT: Cysteine proteinases and their endogenous inhibitors: Target proteins for prognosis, diagnosis and therapy in cancer (review). Oncol Rep 5:1349-1361, 1998

42. Harbeck N, Alt U, Berger U, et al: Prognostic impact of proteolytic factors (urokinase-type plasminogen activator, plasminogen activator inhibitor 1, and cathepsins B, D, and L) in primary breast cancer reflects effects of adjuvant systemic therapy. Clin Cancer Res 7:2757-2764, 2001

43. Seetoo DQ, Crowe PJ, Russell PJ, et al: Quantitative expression of protein markers of plasminogen activation system in prognosis of colorectal cancer. J Surg Oncol 82:184-193, 2003

44. Foekens JA, Ries C, Look MP, et al: The prognostic value of polymorphonuclear leukocyte elastase in patients with primary breast cancer. Cancer Res 63:337-341, 2003

45. Ghosh S, Wu Y, Stack MS: Ovarian cancer-associated proteinases. Cancer Treat Res 107: 331-351, 2002

46. 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 7:1743-1749, 2001

47. Lengyel E, Schmalfeldt B, Konik E, et al: Expression of latent matrix metalloproteinase 9 (MMP-9) predicts survival in advanced ovarian cancer. Gynecol Oncol 82:291-298, 2001

48. Wu X, Li H, Kang L, et al: Activated matrix metalloproteinase-2–a potential marker of prognosis for epithelial ovarian cancer. Gynecol Oncol 84:126-134, 2002

49. Sui L, Dong Y, Ohno M, et al: Survivin expression and its correlation with cell proliferation and prognosis in epithelial ovarian tumors. Int J Oncol 21:315-320, 2002

50. Duffy MJ: The role of proteolytic enzymes in cancer invasion and metastasis. Clin Exp Metastasis 10:145-155, 1991

51. Del Rosso M, Fibbi G, Pucci M, et al: Multiple pathways of cell invasion are regulated by multiple families of serine proteases. Clin Exp Metastasis 19:193-207, 2002

52. Noel A, Gilles C, Bajou K, et al: Emerging roles for proteinases in cancer. Invasion Metastasis 17:221-239, 1997

53. DeClerck YA, Imren S, Montgomery AM, et al: Proteases and protease inhibitors in tumor progression. Adv Exp Med Biol 425:89-97, 1997

54. Aimes RT, Zijlstra A, Hooper JD, et al: Endothelial cell serine proteases expressed during vascular morphogenesis and angiogenesis. Thromb Haemost 89:561-572, 2003

55. Impola U, Toriseva M, Suomela S, et al: Matrix metalloproteinase-19 is expressed by proliferating epithelium but disappears with neoplastic dedifferentiation. Int J Cancer 103:709-716, 2003

56. Hooper JD, Nicol DL, Dickinson JL, et al: Testisin, a new human serine proteinase expressed by premeiotic testicular germ cells and lost in testicular germ cell tumors. Cancer Res 59:3199-3205, 1999

57. Chen LM, Chai KX: Prostasin serine protease inhibits breast cancer invasiveness and is transcriptionally regulated by promoter DNA methylation. Int J Cancer 97:323-329, 2002

58. Takahashi S, Suzuki S, Inaguma S, et al: Down-regulated expression of prostasin in high-

grade or hormone-refractory human prostate cancers. Prostate 54:187-193, 2003

59. Chen LM, Hodge GB, Guarda LA, et al: Down-regulation of prostasin serine protease: A potential invasion suppressor in prostate cancer. Prostate 48:93-103, 2001

60. Balbay MD, Pettaway CA, Kuniyasu H, et al: Highly metastatic human prostate cancer growing within the prostate of athymic mice overexpresses vascular endothelial growth factor. Clin Cancer Res 5:783-789, 1999

61. Goyal J, Smith KM, Cowan JM, et al: The role for NES1 serine protease as a novel tumor suppressor. Cancer Res 58:4782-4786, 1998

62. Scorilas A, Diamandis EP, Levesque MA, et al: Immunoenzymatically determined pepsinogen C concentration in breast tumor cytosols: An independent favorable prognostic factor in node-positive patients. Clin Cancer Res 5:1778-1785, 1999

63. Scorilas A, Karameris A, Arnogiannaki N, et al: Overexpression of matrix-metalloproteinase-9 in human breast cancer: A potential favourable indicator in node-negative patients. Br J Cancer 84:1488-1496, 2001

64. Rojo JV, Merino AM, Gonzalez LO, et al: Expression and clinical significance of pepsinogen C in epithelial ovarian carcinomas. Eur J Obstet Gynecol Reprod Biol 104:58-63, 2002

65. Hsia JY, Chen CY, Chen JT, et al: Prognostic significance of caspase-3 expression in primary resected esophageal squamous cell carcinoma. Eur J Surg Oncol 29:44-48, 2003

66. 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 7:806-811, 2001

67. Yousef GM, Kyriakopoulou LG, Scorilas A, et al: Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: A new independent and favorable prognostic marker. Cancer Res 61:7811-7818, 2001

68. Borgono CA, Fracchioli S, Yousef GM, et al: Favorable prognostic value of tissue human kallikrein 11 (hK11) in patients with ovarian carcinoma. Int J Cancer 106:605-610, 2003

69. Yousef GM, Fracchioli S, Scorilas A, et al: Steroid hormone regulation and prognostic value of the human kallikrein gene 14 in ovarian cancer. Am J Clin Pathol 119:346-355, 2003

70. Obiezu CV, Scorilas A, Katsaros D, et al: Higher human kallikrein gene 4 (KLK4) expression indicates poor prognosis of ovarian cancer patients. Clin Cancer Res 7:2380-2386, 2001

71. Dong Y, Kaushal A, Bui L, et al: Human kallikrein 4 (KLK4) is highly expressed in serous ovarian carcinomas. Clin Cancer Res 7:2363-2371, 2001

72. Kim H, Scorilas A, Katsaros D, et al: Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. Br J Cancer 84:643-650, 2001

73. Hoffman BR, Katsaros D, Scorilas A, et al: Immunofluorometric quantitation and histochemical localisation of kallikrein 6 protein in ovarian cancer tissue: A new independent unfavourable prognostic biomarker. Br J Cancer 87:763-771, 2002

74. Kyriakopoulou LG, Yousef GM, Scorilas A, et al: Prognostic value of quantitatively assessed KLK7 expression in ovarian cancer. Clin Biochem 36:135-143, 2003

75. Luo LY, Katsaros D, Scorilas A, et al: Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. Clin Cancer Res 7:2372-2379, 2001

76. Yousef GM, Scorilas A, Katsaros D, et al: Prognostic value of the human kallikrein gene 15 (KLK15) expression in ovarian cancer. J Clin Oncol 21:3119-3126, 2003

77. Henderson BE, Feigelson HS: Hormonal carcinogenesis. Carcinogenesis 21:427-433, 2000

78. Liao DJ, Dickson RB: Roles of androgens in the development, growth, and carcinogenesis of the mammary gland. J Steroid Biochem Mol Biol 80:175-189, 2002

79. Risch HA: Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. J Natl Cancer Inst 90:1774-1786, 1998

80. Isola JJ: Immunohistochemical demonstration of androgen receptor in breast cancer and its relationship to other prognostic factors. J Pathol 170:31-35, 1993

81. Cardillo MR, Petrangeli E, Aliotta N, et al: Androgen receptors in ovarian tumors: Correlation with oestrogen and progesterone receptors in an immunohistochemical and semiquantitative image analysis study. J Exp Clin Cancer Res 17:231-237, 1998