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Immunofluorometric Quantification of Human Kallikrein 5 Expression in Ovarian Cancer Cytosols and Its Association with Unfavorable Patient Prognosis

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Key Words
Serine proteases · Kallikreins · Cancer biomarkers · Prognostic markers · Ovarian cancer · Human kallikrein 5

Abstract
Human kallikrein 5 (hK5; encoded by the *KLK5* gene) is a secreted serine protease expressed in hormonally regulated tissues, including the breast and ovary. We have previously reported regulation of the *KLK5* gene by estrogens and progestins and its clinical value as a marker of poor prognosis in breast and ovarian cancers. We thus hypothesized that hK5 may represent a potential biomarker for ovarian carcinomas, at the protein level. Using a newly developed ELISA, hK5 levels were quantified (nanograms per milligram of total protein) in 22 low malignant potential (LMP) and 132 epithelial ovarian tumors and correlated with various clinicopathological variables and outcome [progression-free survival (PFS), overall survival (OS)]. hK5 concentration in LMP tumors ranged from 0 to 2.3 ng/mg (mean = 0.24) and from 0 to 220 ng/mg (mean = 3.35) in ovarian tumor cytosols ($p = 0.002$). Using a cutoff value of 0.15 ng/mg, 60% of ovarian tumors were categorized as hK5 positive. We found a strong correlation between patients with hK5-positive tumors and disease stages III/IV and grade 3 tumors (all $p < 0.05$). Univariate survival analysis revealed that hK5-positive patients had a significantly shorter PFS and OS ($p < 0.05$). Kaplan-Meier survival curves further confirmed an increased risk of relapse and death in women with hK5-positive tumors ($p = 0.015$ and $p = 0.019$, respectively). Multivariate analysis indicated that the prognostic value of hK5 was not independent from other parameters in the entire group of patients. When stratified by tumor grade (G1/2 vs. G3) and debulking success (optimal vs. suboptimal), univariate and multivariate analyses demonstrated that hK5 was an independent indicator of poor prognosis for patients with grade 3 tumors and with optimal debulking ($p < 0.05$). In patients with disease stage I/II versus III/IV, hK5 positivity was independently associated with a shorter PFS ($p = 0.046$) and marginally decreased OS ($p = 0.08$), in multivariate analysis. Lastly, we observed a fairly weak, positive, but statistically significant correlation between the expression levels of tissue hK5 and tissue CA125 ($r_s = 0.297$; $p < 0.001$). Our findings provide evidence for an association between hK5 and more aggressive forms of epithelial ovarian cancer.

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Introduction

The most common and lethal form of ovarian cancer is epithelial in origin, derived from the single layer of cells that covers the ovaries [1, 2]. The American Cancer Society estimates that in 2003, 25,400 new cases of epithelial ovarian cancer will be diagnosed and approximately 14,300 women will die from this disease, despite therapeutic advances over the last decade [3]. The relatively high case-fatality ratio is usually attributed to late clinical manifestation of epithelial ovarian tumors, which generally lack early warning symptoms. As a consequence, over two thirds of patients are diagnosed at advanced International Federation of Gynecologists and Obstetricians (FIGO) stages III/IV, when the 5-year survival rate is 15–20%, compared to 80–90% at FIGO stages I/II [4]. Thus, early detection remains the most important factor in improving long-term survival of ovarian cancer patients. But until reliable screening and diagnostic strategies are available, the identification of new prognostic and predictive biomarkers will contribute to the optimal management of ovarian cancer patients, predict disease outcome and determine effective, individualized therapeutic strategies.

Proteolytic enzymes have emerged as important prognostic factors in ovarian cancer. One requirement for invasion and metastasis of ovarian cancer cells is the expression of proteases of several catalytic types, which degrade components of the basement membrane and extracellular matrix [5–9]. As expected, numerous clinical reports indicate that aberrant protease expression is often associated with a poor prognosis in ovarian cancer patients [9–13]. The prognostic relevance of proteases has also proved important in the implementation of new treatment modalities specifically targeted at metastasis formation, including the use of protease inhibitors as anticancer agents [14–17].

Human tissue kallikreins are a group of serine proteases encoded by 15 structurally similar hormonally regulated genes that colocalize to chromosome 19q13.4 [18]. Accumulating evidence indicates that at least 11 of 15 kallikreins are aberrantly expressed in ovarian cancer at the mRNA and/or protein level and many possess prognostic value (table 1) [19–21]. Furthermore, several kallikrein proteins, including hK6, hK10 and hK11, represent prospective serological screening and/or diagnostic ovarian cancer biomarkers [22–25].

Human kallikrein gene 5 *[KLK5,* previously known as *KLK-L2* and *human stratum corneum tryptic enzyme (HSCTE)],* a recently identified estrogen/progestin-regulated serine protease gene, is expressed in many endocrine tissues, including the testis, prostate, breast and ovary [26, 27]. Preliminary evidence indicates that *KLK5* is differentially regulated in a variety of hormone-dependent malignancies, including ovarian [28], breast [29], prostate [30] and testicular [31] cancers. Using RT-PCR, we subsequently discovered that *KLK5* is an indicator of poor prognosis in women with ovarian [28] and breast [29] cancer.

In order to study kallikrein 5 expression at the protein level, we developed a highly specific and sensitive immunofluorometric ELISA [32]. Using this method, we observed high levels of human kallikrein 5 (hK5) in various normal adult tissues, including the skin, breast and salivary gland [32]. Quantification of hK5 in normal, benign and cancerous ovarian tissues indicated that this protease is significantly elevated in 50% of ovarian tumor tissues compared to both normal and benign tissues. Elevated serum hK5 levels were also observed in 69% of women with ovarian cancer. Taken together, these lines of evidence support a clinical role for hK5 as a screening and/or diagnostic ovarian cancer biomarker, in addition to its prognostic value at the mRNA level. The aim of the present study was to investigate the expression of the hK5 protein in epithelial ovarian cancer tissues in relation to other clinicopathological variables and to evaluate its prognostic significance.

Table 1. Kallikrein expression (mRNA and protein) in ovarian cancer tissues

Kallikrein	Prognosis	Reference
mRNA[1]		
KLK4	unfavorable	76, 77
KLK5	unfavorable	28
KLK7	unfavorable	78
KLK8	favorable	79
KLK9	favorable	80
KLK14	favorable	81
KLK15	unfavorable	82
Protein[2]		
hK5	unfavorable	this study
hK6	unfavorable	83
hK10	unfavorable	84
hK11	favorable	85
hK13	favorable	our unpublished data

[1] RT-PCR methodology.
[2] ELISA methodology.

Materials and Methods

Ovarian Cancer Patients and Specimens

One hundred and thirty-two patients with primary epithelial ovarian cancer and 22 with low malignant potential (LMP) tumors were examined in this study, ranging in age from 20 to 85 years, with a median age of 57. 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. 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 132 patients, among which 73 (55%) had relapsed and 54 (41%) had died.

Clinical and pathological information documented at the time of surgery included tumor stage, grade, histotype, residual tumor size, debulking success and volume of ascites fluid. The staging of tumors was in accordance with the FIGO criteria [33], grading was established according to Day et al. [34] and the classification of histotypes was based on both the WHO and FIGO recommendations [35].

Patients with disease at clinical stages I–IV and grades 1–3 were represented in this study. Of the 132 ovarian tumors, the majority (95; 72%) were of the serous papillary histotype, followed by mucinous (12; 9%), undifferentiated (11; 8%), endometrioid (5; 4%) and clear cell types (4; 3%), or they were unclassified (5; 4%). The 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.

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 m*M* Tris, pH 8.0, 150 m*M* NaCl, 5 m*M* EDTA, 10 g/l of NP-40 surfactant, 1 m*M* phenylmethyl sulfonyl fluoride, 1 g/l of aprotinin, 1 g/l of leupeptin). The resulting suspensions were incubated on ice for 30 min, with repeated shaking and vortexing every 10 min. The mixtures were then centrifuged at 14,000 rpm at 4°C for 30 min, and the supernatant (cytosolic extract) was collected and stored at –80°C until further analysis. The protein concentration of the extracts was determined using the bicinchoninic acid method, with albumin as standard (Pierce Chemical Co., Rockford, Ill., USA).

Measurement of hK5 in Ovarian Cytosolic Extracts

The concentration of hK5 in the cytosolic extracts was quantified using a highly sensitive and specific noncompetitive 'sandwich-type' immunoassay, previously described and evaluated [32]. Briefly, 96-well polystyrene microtiter plates were coated with a mouse anti-hK5 monoclonal antibody (produced in-house). hK5 calibrators (recombinant hK5 in 60 g/l bovine serum albumin) or cytosolic extracts (100 µl) were then applied to each well in duplicate, incubated for 2 h with gentle shaking and washed. Rabbit anti-hK5 polyclonal antiserum was subsequently applied, incubated and washed. Finally, alkaline-phosphatase-conjugated goat antirabbit IgG (Jackson Immunoresearch, West Grove, Pa., USA) was added, incubated 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 [36]. The detection range of this assay is 0.1–50 µg/l; hK5 concentrations in micrograms per liter were converted to nanograms of hK5 per milligram of total protein to adjust for the amount of tumor tissue extracted.

Statistical Analysis

To analyze data, patients were divided into different groups according to clinical and pathological parameters, and statistical analyses were then performed with SPSS software (SPSS Inc., Richmond, Calif., USA). Since the distribution of hK5 concentration in ovarian tumor extracts was not Gaussian, the nonparametric Mann-Whitney U test was used to determine differences between two groups. Relationships between hK5 and CA125 were assessed by Spearman correlation coefficient. These tests treated hK5-specific activity in the tumor extract (nanograms hK5 per milligram total protein) as a continuous variable.

The hK5 status of ovarian tumor extracts was categorized as either hK5 positive or hK5 negative, based on the ability of hK5 values to predict the progression-free survival (PFS) of the study population. The relationship between this dichotomous variable and various clinicopathological variables was analyzed with the χ^2 test and Fisher's exact test, as appropriate.

For survival analysis, two different endpoints – cancer relapse (either local recurrence or distant metastasis) and death – were used to calculate 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 or metastatic disease. OS was defined as the time interval between the date of surgery and the date of death. The impact of hK5 on patient survival (PFS and OS) was assessed with the hazard ratio (HR; relative risk of relapse or death in the hK5-positive group) calculated with the Cox univariate and multivariate proportional hazard regression model [37]. The multivariate models were adjusted for hK15 expression in tumors and other clinical and pathological variables that may affect survival, including age, stage of disease, tumor grade, CA125 and age.

Kaplan-Meier PFS and OS curves [38] were constructed to demonstrate survival differences between the hK5-positive and hK5-negative patients. The differences between the survival curves were tested for statistical significance using the log rank test [39].

For further analysis, patients were stratified based on disease stage, by tumor grade and by debulking success. Survival rates (PFS and OS) between hK5-positive and hK5-negative patients were then compared in each subgroup.

Results

Distribution of hK5 Concentration in LMP and Ovarian Tumor Tissue Extracts

hK5 concentration in LMP tumors from 22 patients ranged from 0 to 2.3 ng/mg of total protein, with a mean of 0.24 ng/mg total protein. hK5 levels in ovarian tumor cytosols from 132 patients ranged from 0 to 220 ng/mg of total protein, with a mean of 3.35 ng/mg total protein and a median of 0.43 ng/mg total protein (table 2). hK5 levels were significantly elevated in ovarian cancer extracts compared to LMP tumors ($p = 0.002$; fig. 1), as calculated

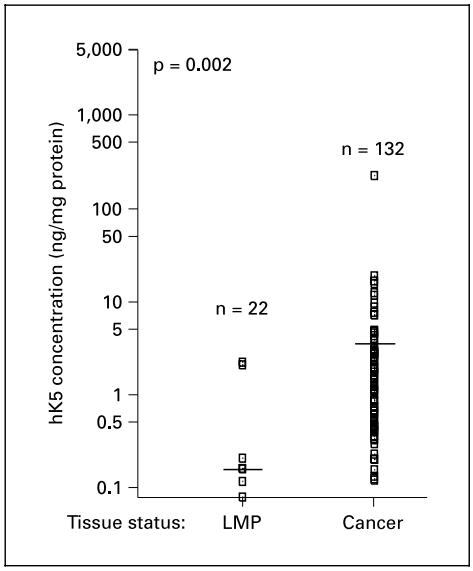

Fig. 1. Comparison of hK5 concentration in LMP and ovarian tumor extracts. Horizontal bars indicate the mean values of hK5 concentration. The Mann-Whitney test indicated that hK5 levels are significantly elevated in cancerous ovarian tumors compared to those of LMP (p = 0.002). n = Number of patients. Levels of hK5 in normal ovarian tissue were reported elsewhere.

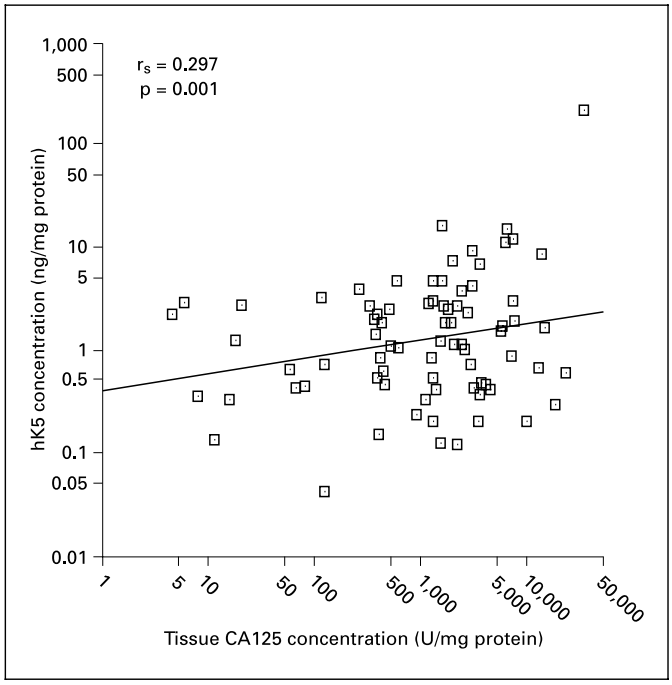

Fig. 2. Correlation between tissue CA125 and hK5 in ovarian tumor extracts. r_s = Spearman correlation coefficient.

Table 2. Distribution of hK5 concentrations (ng/mg protein) in cancer and LMP ovarian tissues

	Mean ± SE_a	Range	Percentiles					p value[1]
			10	25	50 (median)	75	90	
LMP tissues (n = 22)	0.24 ± 0.14	0.00–2.30	0.00	0.00	0.00	0.13	1.57	0.002
Cancer tissues (n = 132)	3.35 ± 1.67	0.00–220	0.00	0.00	0.43	1.97	4.78	

SE = Standard error.
[1] Calculated by the Mann-Whitney test.

by the Mann-Whitney test. Also, a weakly positive correlation was found between the expression levels of hK5 and CA125 in the ovarian tumor extracts (r_s = 0.297; p = 0.001; fig. 2).

Relationships between hK5 Status and Other Clinicopathological Variables

An optimal cutoff value of 0.15 ng/mg total protein was identified by χ^2 analysis, based on the ability of the hK5 value to predict the PFS of the study population. Based on this cutoff (40th percentile) 60% of the ovarian tumors were categorized as being hK5 positive. The distributions of various clinicopathological variables between hK5-positive and hK5-negative patients are summarized in table 3. The relationships between hK5 and these variables were examined with either the χ^2 or Fisher's exact test. Patients with hK5-positive ovarian tumors were more likely to have late-stage (stage III/IV) and -grade (G3) disease and undifferentiated or serous tumors (all $p < 0.05$). Notably, none of the 12 mucinous

Table 3. Relationship between hK5 status and other variables in 132 ovarian cancer patients

Variable	Patients	Number of patients (%)		p value
		hK5-negative	hK5-positive	
Stage				
I/II	34	22 (64.7)	12 (35.3)	0.001[a]
III	98	31 (31.6)	67 (68.4)	
Grade				
G1/G2	53	30 (56.6)	23 (43.4)	0.002[a]
G3	78	23 (29.5)	55 (70.5)	
x	1			
Histotype				
Serous	95	33 (34.7)	62 (65.3)	<0.001[b]
Mucinous	12	12 (100.0)	0 (0.0)	
Endometrioid	5	4 (80.0)	1 (20.0)	
Clear cell	4	3 (75.0)	1 (25.0)	
Undifferentiated	11	1 (9.1)	10 (90.9)	
x	5			
Residual tumor				
0	69	33 (47.8)	36 (52.2)	0.25[b]
≤2 cm	38	12 (31.6)	26 (68.4)	
>2 cm	21	8 (38.1)	13 (61.9)	
x	4			
Debulking success				
SD	59	20 (33.9)	39 (66.1)	0.15[a]
OD	69	33 (47.8)	36 (52.2)	
x	4			
Ascites fluid				
0	41	19 (46.3)	22 (53.7)	0.22[b]
≤500 ml	43	19 (44.2)	24 (55.8)	
>500 ml	44	13 (29.5)	31 (70.5)	
x	4			

Status: equal to 40th percentile (0.14 ng/mg protein). x = Status unknown. OD = optimal debulking (0–1 cm); SO = suboptimal debulking (>1 cm).

[a] Fisher's exact test.

[b] χ^2 test.

tumors were positive for hK5 while 10 of 11 undifferentiated tumors were positive. No relationship was observed between hK5 status and residual tumor size, debulking success or volume of ascites fluid.

Univariate and Multivariate Survival Analysis

The strength of association between hK5-positive tumors and survival outcome is presented in table 4. Univariate analysis indicated that hK5-positive patients had a significantly shorter PFS (HR of 1.87, p = 0.019) and OS (HR of 2.064, p = 0.023). Kaplan-Meier survival curves (fig. 3) further demonstrate that women with hK5-positive tumors have shorter PFS and OS (p = 0.015 and p = 0.019, respectively) compared with those who are hK5 negative. However, these unfavorable effects of hK5 positivity on PFS and OS were lost when hK5 was treated as a continuous variable in the univariate analysis as well as in the multivariate analysis, when survival outcomes were adjusted for all other variables.

Univariate and Multivariate Survival Analysis in Subgroups of Patients

We further examined the associations between hK5 status and survival outcomes in subgroups of patients stratified by disease stage (stage I/II vs. stage III/IV), tumor grade (G1/2 vs. G3) and debulking success (optimal vs. suboptimal; table 5). Among patients with grade 3 tumors, hK5 positivity was significantly associated with a 2- to 3-fold greater risk of relapse and death in both univariate and multivariate analyses (all $p < 0.05$). Similarly, there was a tendency for an increased risk of relapse and death in hK5-positive patients with disease stage I/II, but this did not reach statistical significance in the univariate analysis. However, in multivariate analysis, hK5-positive

Table 4. Univariate and multivariate analysis of hK5 with regard to PFS and OS

Variable	PFS			OS		
	HR	95% CI	p value	HR	95% CI	p value
Univariate analysis hK5 (n = 128)						
Negative	1.00			1.00		
Positive	1.87	1.11–3.15	0.019	2.064	1.10–3.85	0.023
As continuous variable	0.99	0.93–1.04	0.71	0.99	0.94–1.04	0.62
Multivariate analysis hK5 (n = 118)						
Negative	1.00			1.00		0.11
Positive	1.59	0.87–2.90	0.13	1.81	0.86–3.77	

HR estimated from Cox proportional hazard regression model. CI = Confidence interval of the estimated HR. Multivariate models were adjusted for stage of disease, debulking success, tumor grade, CA125 and age.

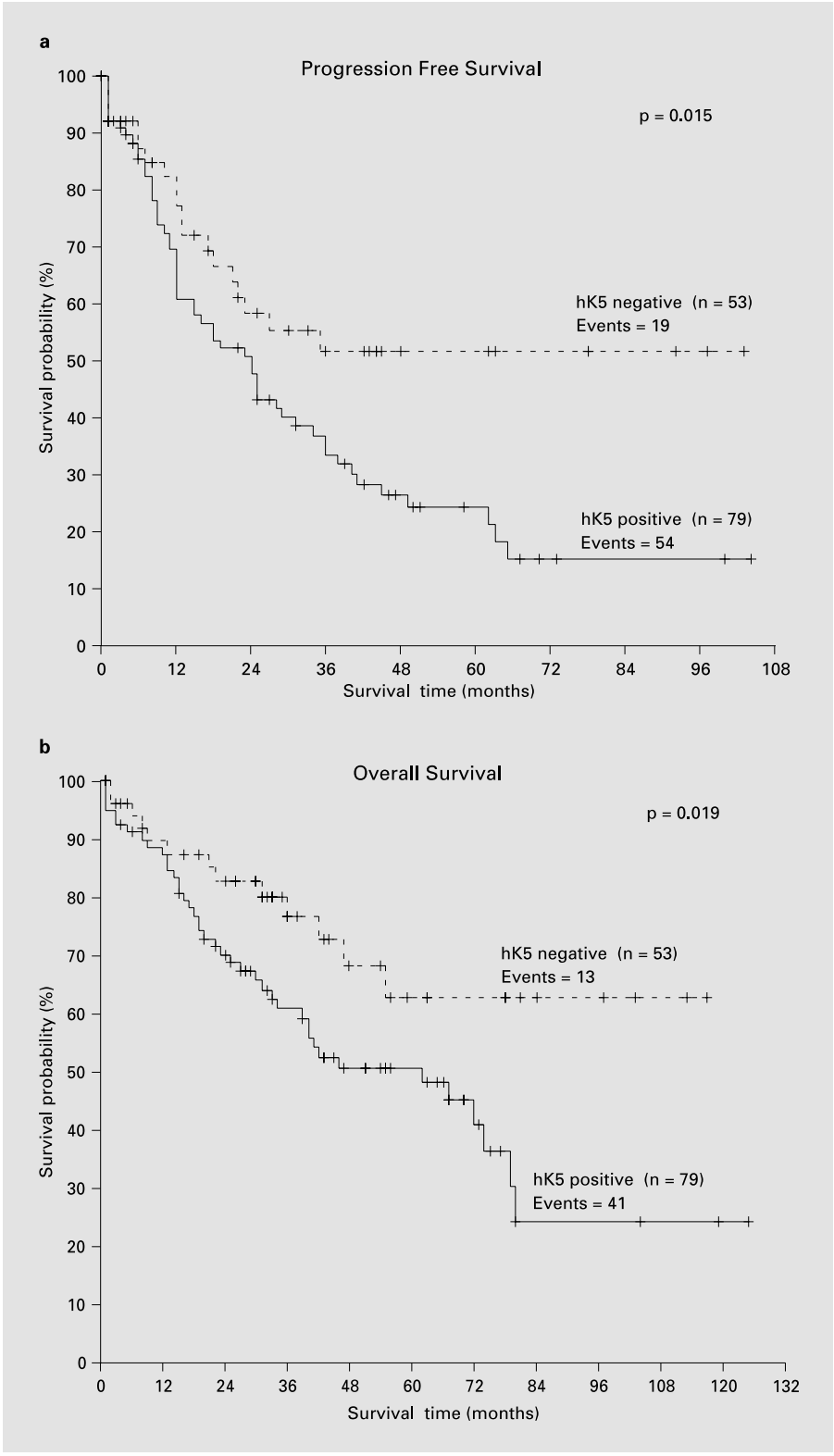

Fig. 3. Kaplan-Meier survival curves for PFS (**a**) and OS (**b**) in patients with hK5-positive and negative ovarian tumors. n = Number of samples.

Table 5. Cox proportional hazard regression analysis for subgroups of patients

Variable	PFS			OS		
	HR	95% CI	p value	HR	95% CI	p value
Tumor grade I–II						
hK5 unadjusted	1.02	0.46–2.24	0.96	0.95	0.34–2.65	0.93
hK5 adjusted[1]	1.44	0.58–3.56	0.42	1.49	0.46–4.85	0.49
Tumor grade III						
hK5 unadjusted	2.83	1.26–6.33	0.011	3.04	1.18–7.79	0.021
hK5 adjusted[1]	1.94	1.03–3.66	0.040	2.92	1.00–8.53	0.044
Stage I–II						
hK5 unadjusted	7.51	0.82–68.1	0.073	7.48	0.59–31.6	0.43
hK5 adjusted[2]	10.98	1.04–116.06	0.046	8.49	0.74–96.5	0.08
Stage III						
hK5 unadjusted	1.21	0.71–2.08	0.47	1.27	0.68–2.39	0.44
hK5 adjusted[2]	1.31	0.71–2.41	0.37	1.55	0.74–3.24	0.24
Optimal debulking success						
hK5 unadjusted	2.57	0.95–6.94	0.061	8.07	1.04–62.7	0.045
hK5 adjusted[3]	3.97	1.07–14.75	0.039	7.15	1.44–35.72	0.017
Suboptimal debulking success						
hK5 unadjusted	1.33	0.71–2.49	0.36	1.26	0.64–2.48	0.51
hK5 adjusted[3]	1.42	0.69–2.92	0.33	1.11	0.51–2.45	0.79

HR estimated from Cox proportional hazard regression model. CI = Confidence interval of the estimated HR.

[1] Multivariate models were adjusted for stage of disease, debulking success, histological type, CA125 and age.

[2] Multivariate models were adjusted for tumor grade, debulking success, histological type, CA125 and age.

[3] Multivariate models were adjusted for stage of disease, tumor grade, histological type, CA125 and age.

tumors were significantly associated with a shorter PFS (HR of 10.98, p = 0.046) and marginally related with a decreased OS (HR of 8.49, p = 0.08). Furthermore, among patients with optimal debulking success, univariate analysis indicated a marginally significant relationship between hK5 positivity and PFS and OS (p = 0.061 and p = 0.045, respectively). Multivariate analysis, however, demonstrated that the presence of hK5 was independently associated with a 3-fold risk of relapse (p = 0.039) and a 7-fold risk of death (p = 0.017) in optimally debulked patients.

Discussion

Tumor biomarkers assist in evaluating cancer risk, screening, diagnosis, clinical staging, estimating tumor volume, monitoring, assessing prognosis, evaluating success of treatment, detecting disease recurrence and predicting a likely response to therapy [40]. With respect to ovarian cancer, CA125 represents the most extensively studied biomarker for screening, diagnosis and monitoring [41]. Conventional prognostic markers in ovarian cancer have included FIGO stage, tumor grade, residual tumor after surgery, presence and absence of ascites, histology and patient age [42, 43]. The past decade has witnessed the identification of additional tumor-associated prognostic markers, ranging from DNA ploidy and oncogenes to cell cycle regulatory proteins and inhibitors, enzymes, growth factors, extracellular matrix components and proteases [44–50].

In more recent years, novel bioinformatic, genomic and proteomic technologies, such as serial analysis of gene expression, cDNA and protein arrays, two-dimensional gel electrophoresis and mass spectrometry, have been used to identify genes/proteins differentially expressed in ovarian cancer, often elucidating novel diagnostic and

prognostic information [21, 51–59]. Furthermore, the application of artificial neural networks in clinical medicine, including ovarian cancer, have allowed for the combination of independent data from a panel of prognostic factors to produce a more informative predictive index [60–63].

In the present study, we immunofluorometrically quantified and evaluated the expression of a serine protease, hK5, in LMP and epithelial ovarian tumors in relation to other established prognostic indicators and patient survival. As expected, our results are in agreement, for the most part, with our previous report documenting the prognostic value of *KLK5*, at the mRNA level, in ovarian cancer [28]. Both studies demonstrate a strong association between elevated hK5 and late-stage and high-grade ovarian carcinomas ($p < 0.05$), and its correlation with increased risks of relapse and death, as evidenced by univariate analysis and Kaplan-Meier survival curves. Also, in both cases, the prognostic value of hK5 was not independent from other clinicopathological parameters, at the mRNA or protein levels. Even so, both *KLK5* and hK5 were identified as independent indicator of poor prognosis among patients with optimal debulking success.

A few discrepancies between hK5 mRNA and protein expression levels in relation to other parameters in ovarian cancer patients were observed. For one, hK5-positive tumors were more frequently of the serous or undifferentiated histotype, a relationship not observed at the mRNA level. Second, while hK5 positivity was shown to be an independent indicator of unfavorable outcome for patients with grade 3 tumors, an equal risk of relapse and death among *KLK5*-positive and *KLK5*-negative patients with grade 3 tumors was observed in our previous study. Conversely, *KLK5* positivity was significantly associated with a prolonged PFS and marginally related with a longer OS in patients with tumors of grade 1/2. Thirdly, the current study demonstrates that hK5 has prognostic value in patients with stage I/II disease. Yet, no association between *KLK5* expression and patients stratified by stage (I/II vs. III/IV) has previously been reported. These apparent inconsistencies may be due to ethnic variations between the Italian and German ovarian cancer patients studied in the initial and present investigations, respectively. It is conceivable that these observations may stem from the limitations in the techniques used to measure *KLK5* and hK5 expression levels. Until the function of hK5 in normal and cancerous ovarian tissues is delineated, it will be difficult to explain these disparities.

In comparison to our initial mRNA study, we have also been able to demonstrate that the hK5 protein is significantly elevated in cancerous vs. LMP ovarian cytosolic extracts, in support of our initial findings of higher hK5 levels in ovarian tumors compared to normal tissues [32]. We speculate that hK5 elevation in ovarian cancer tissues may account for its elevation in the serum of ovarian cancer patients [32]. Moreover, we have observed a fairly weak, positive correlation between the expression levels of tissue hK5 and tissue CA125.

Previously, we have shown that *KLK5*, at the mRNA level, is an independent marker of poor prognosis in women with breast carcinomas [64], in agreement with its prognostic value in ovarian cancer. Although the underlying biological mechanism of hK5 involvement in the progression of breast and ovarian cancers is currently unknown, it is plausible that it may play a role in hormonal carcinogenesis [65]. First, we have shown that hK5 is encoded by an estrogen/progestin-regulated gene [27, 32]. Second, epidemiological and experimental evidence suggests that steroid hormones, such as estrogens and progestins, are implicated in the etiology of both breast and ovarian carcinomas [66–69]. Third, it has been documented that estrogen and progesterone receptors are present in about 71 and 44% of breast [70] and 30–63 and 25% of ovarian tumors, respectively [71] and that estrogens and progestins, acting through their respective receptors, can stimulate breast [72, 73] and ovarian [74] cancer cell proliferation. As such, it is likely that these hormone-receptor complexes regulate *KLK5* gene expression during breast and ovarian carcinogenesis. The identification of downstream estrogen- and progesterone-regulated genes, such as *KLK5*, is important in our understanding of the mechanism by which estrogens and progestins are implicated in hormone-related malignancies.

By virtue of the fact that hK5 is a serine protease, overexpressed in advanced forms of breast and ovarian cancer and a marker of poor prognosis, we further speculate that it is involved in metastasis, by degradation of the basement membrane and extracellular matrix. It has also been documented that inhibitors can prevent extracellular matrix degradation and thus tumor cell invasion; for instance, plasminogen activator inhibitor 1 is thought to hinder the proteolytic effect of plasminogen activator, which is also an indicator of poor prognosis in ovarian cancer [11, 75]. We have recently identified α_1-antitrypsin and α_2-macroglobulin as potential hK5 inhibitors in serum and ascites fluid [our unpubl. data]. Collectively, our findings may also have therapeutic applications.

In conclusion, we provide further evidence to support the potential clinical utility of hK5 as an indicator of unfavorable outcome in ovarian cancer patients. hK5 may be

particularly useful in subgroups of patients with advanced stage disease, grade 3 tumors and optimal debulking. The biological basis and significance of our findings are unclear and warrant further basic and clinical studies.

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