

Human kallikrein 7: an unfavorable prognostic marker of ovarian cancer

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ABSTRACT

Human tissue kallikrein 7 (hk7), also known as human stratum corneum chymotryptic enzyme (HSCCE), is a member of the kallikrein family of secreted serine proteases. Previous reports indicated that seven KLK genes, including KLK7, are significantly up-regulated in ovarian cancer at the mRNA level. Nevertheless, few have studied hk7 protein expression in ovarian cancer. The aim of this study was to determine whether tissue hk7 may serve as a prognostic biomarker for ovarian cancer. Using a newly developed enzyme-linked immunosorbent assay (ELISA) with two mono-clonal antibodies, we quantified hk7 expression in 260 ovarian tumor cytosols and correlated these results with various clinicopathological variables and patient outcome (progression-free survival [PFS] and overall survival [OS]) over a median follow-up period of 52 months. hk7 concentration in ovarian tumor cytosols ranged from 0 to 32.8 ng/mg of total protein, with a median of 2.84 ng/mg. In comparison to normal and benign ovarian tissues and non-ovarian metastatic tumors, malignant ovarian tumor cytosols highly over-expressed hk7 ($p < 0.001$). The median of 2.84 ng/mg of total protein was selected as the cutoff value to categorize tumors as hk7-positive and hk7-negative. Women with hk7-positive tumors most frequently had advanced stage diseases, suboptimal debulking, larger residual tumors, and serous papillary histotype ($p < 0.001$). Furthermore, univariate analysis showed hk7-positivity to be associated with significantly shorter progression-free survival ($p = 0.01$). Kaplan-Meier survival curves confirmed an increased risk of relapse in women with hk7-positive tumors ($p = 0.009$). Our results indicated that hk7 is an independent unfavorable prognostic marker for ovarian cancer.

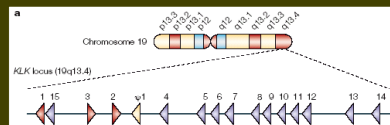
BACKGROUND

Ovarian cancer:

- The most lethal gynecologic malignancy for women in the industrialized countries.
- Independent prognostic biomarkers can facilitate disease management.

Human tissue kallikreins (hKs) are potential prognostic markers

- Secreted serine proteases
- Human tissue kallikrein 7 (hk7, human stratum corneum chymotryptic enzyme [HSCCE])



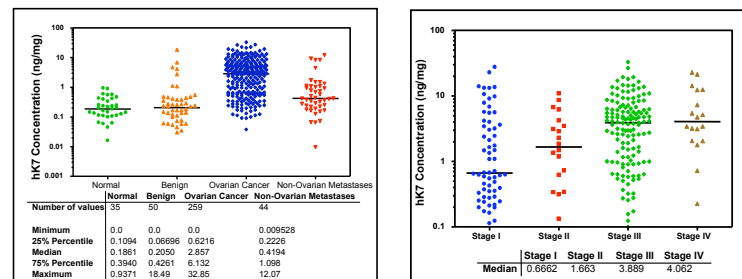
RATIONALE & HYPOTHESIS

- Previous reports indicated that seven KLK genes, including KLK7, are significantly up-regulated in ovarian cancer at the mRNA level.
- Few have studied hk7 protein expression in ovarian cancer.
- AIM: to determine whether tissue hk7 may serve as a prognostic biomarker for ovarian cancer.

EXPERIMENTAL DESIGN

- Quantify hk7 protein expression in ovarian cancer tissue cytosols.
- Correlate hk7 expression with other clinicopathological variables.
- Determine the prognostic value of hk7 using univariate and multivariate Cox regression analysis and Kaplan Meier survival curves.

hk7 PROTEIN UPREGULATION IN OVARIAN CANCER

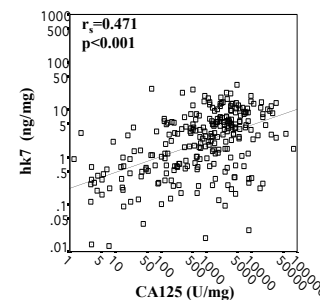
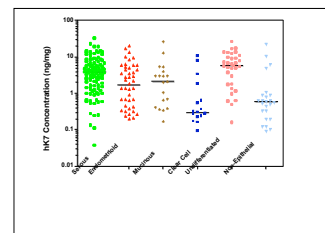


hk7 CORRELATION WITH CLINICOPATHOLOGICAL VARIABLES

Table 2 Relationship between tissue hk7 status* and other variables in ovarian cancer patients.

Variable	Patients	hk7 negative	hk7 positive	p-value
Stage				
I	65	45 (69.2)	20 (30.8)	
II	20	12 (60.0)	8 (40.0)	<0.001 [†]
III	143	59 (41.3)	84 (58.7)	
IV	18	5 (27.8)	13 (72.2)	
Grade				
G1	88	37 (63.8)	21 (36.2)	
G2	45	31 (68.9)	14 (31.1)	<0.001 [†]
G3	139	50 (36.0)	89 (64.0)	
Histotype				
Serous	110	44 (40.0)	66 (60.0)	
Endometrioid	46	26 (56.5)	20 (43.5)	
Mucinous	20	11 (55.0)	9 (45.0)	
Clear cell	18	15 (83.3)	3 (16.7)	<0.001 [†]
Undifferentiated	33	9 (27.3)	24 (72.7)	
Other sero-epithelial	23	19 (82.6)	4 (17.4)	
Debulking success [‡]				
SD	103	36 (35.0)	67 (65.0)	<0.001 [†]
OD	140	83 (59.3)	57 (40.7)	
Response to CTX [§]				
NC/PD	19	8 (42.1)	11 (57.9)	
PR	41	19 (46.3)	22 (53.7)	
CR	180	93 (51.7)	87 (48.3)	0.64 [†]
NE	20			

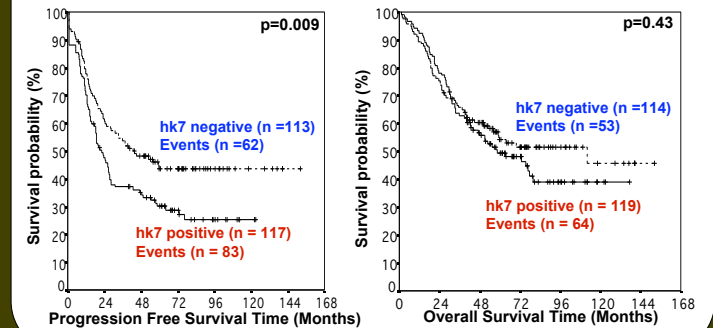
* cutoff = 2.84 ng/mg (50th percentile, median)
[†] χ^2 test
[‡] Fisher's Exact Test
[§] OD, Optimal debulking (0-1 cm); SD, Suboptimal debulking (>1 cm)
[¶] CTX, chemotherapy; NC, no change; PD, progressive disease; CR, complete response; PR, partial response; NE, not evaluated
^{||} *n*, Status unknown



MATERIALS & METHODS

- Tissue cytosol from 259 malignant, 50 benign, 44 non-ovarian metastatic tumors, and 35 normal ovarian tissues were extracted and analyzed with an enzyme-linked immunosorbent assay (ELISA) using two hk7-specific monoclonal antibodies.
- The strength of the association between hk7 expression and other clinicopathological variables such as tumor stage, grade, histotype, debulking success, and response to chemotherapy were statistically determined using the χ^2 or the Fisher's exact test.
- The prognostic value of hk7 was evaluated using univariate and multivariate Cox analyses and Kaplan-Meier survival curves.

PROGNOSTIC SIGNIFICANCE OF hk7



CONCLUSIONS

- hk7 was significantly up-regulated with an average concentration that was approximately 15 fold of normal and benign ovarian tissues, and 8 fold of non-ovarian metastatic tumors.
- Patients with hk7-positive ovarian tumors had later stage (stage III/IV) diseases, higher tumor grades, suboptimal debulking, and serous papillary histotypes.
- hk7-positive patients had shorter progression-free survival and 54% increase in risk of cancer relapse.
- hk7 is an independent molecular marker of unfavorable prognosis that can be used in conjunction with other prognostic markers in a multi-parametric approach to determining ovarian cancer prognosis.

REFERENCES

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- Kishi et al. (2004) Clin Chem 50 (4): 709-16

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