

Cellular Proteolysis and Oncology

Expression analysis of the human kallikrein 7 (KLK7) in breast tumors: a new potential biomarker for prognosis of breast carcinoma

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Summary

Kallikreins are a subgroup of serine proteases that are involved in the post-translational processing of polypeptide precursors. Growing evidence suggests that many kallikreins are implicated in carcinogenesis. Human kallikrein gene 7 (KLK7; HSCCE) is a new member of the human kallikrein gene family. KLK7 is expressed in normal breast tissue and is up-regulated in breast cancer cells by estrogens and glucocorticoids. In the present study, expression of the KLK7 gene in 92 breast cancer tissues was analyzed by reverse transcription-PCR (RT-PCR) and direct sequencing of several samples. The results were correlated

with other clinicopathological variables and patient outcome. KLK7 gene expression was significantly lower in breast cancer patients of low stage (I/II) ($p = 0.011$) and patients with negative progesterone receptors ($p = 0.022$). Survival analysis showed that breast cancer patients with KLK7 positive tumors have relatively shorter disease-free survival (DFS) and overall survival (OS) than patients with KLK7 negative tumors. These data suggest that KLK7 gene expression may be used as a marker of unfavorable prognosis for breast cancer patients.

Keywords

KLK7, hK7, HSCCE, breast cancer, kallikrein, KLK, prognosis, tumor markers

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Introduction

The human kallikrein gene family is a subfamily of serine proteases, located at the chromosomal locus 19q13.3-q13.4 (1). Until recently, this family was known to include only three members: the pancreatic/renal kallikrein gene (KLK1), the human granular kallikrein 2 gene (KLK2), and prostate-specific antigen (KLK3). In the past few years, another 12 kallikrein-like genes were discovered (2). Some of the newly

identified kallikrein-like genes have been found to be either underexpressed or overexpressed in certain carcinomas (2). Hence it is possible that a few members of this expanded kallikrein gene family may serve as valuable cancer biomarkers for disease diagnosis or monitoring.

The kallikrein cascade is implicated in tumorigenesis, firstly by induction of kallikrein gene expression as the initial step of the cascade, and then sequentially by the formation of kinins (3). During injury, cellular activities of kinins mediate

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venoconstriction, arteriolar dilation, increased capillary permeability and interaction with sensory nerve terminals. During tumorigenesis, kinins stimulate proliferation of tumor cells by their mitogenic action and by promoting the process of diapedesis-enhanced metastases of cancer cells. A molecular response to infection, tissue injury and proliferation of tumor cells is the secretion of chemotactic molecules that attract neutrophils to sites of inflammation (4). Neutrophils function as one arm of the defense reaction, essentially to promote cellular healing that contrasts with the pro-inflammatory actions of cytoplasmic and surface-bound enzymes. When neutrophils are drawn into tumors, kallikreins initiate a cascade of molecular events that play a crucial role in the specific inflammatory process (5).

The human kallikrein gene 7 (KLK7), previously known as stratum corneum chymotryptic enzyme (HSCCE), was first identified as a chymotrypsin-like proteinase that may be involved in the desquamation of plantar stratum corneum (6). The enzyme has been shown to be secreted into the stratum corneum extracellular space and to catalyze the degradation of desmosomes during remodeling of the deeper layers of the skin. The tissue levels of KLK7 have been reported to be highly up-regulated in ovarian carcinomas (7).

The cloning and genomic organization of the KLK7 gene, have been recently reported (8, 9). This gene maps to the same chromosomal locus as other serine proteases of the kallikrein gene family. More precisely, the KLK7 gene lies between the KLK6 (centromere) and KLK8 (telomere) genes. Previous reports indicated that KLK7 is primarily expressed in the skin and, to a lesser extent, in other tissues (6, 10). Yousef et al. showed that the gene is also expressed at relatively high amounts in a number of tissues, including kidney, the central nervous system, as well as in mammary and salivary glands (9). Recent data further indicate that in addition to its involvement in certain skin diseases (10), the hK7 enzyme may also be involved in carcinogenesis (7). Kyriakopoulou, et al. demonstrated overexpression of this serine protease in a subset of ovarian carcinomas (11). Previously, we used the steroid hormone receptor-positive breast carcinoma cell line BT-474 as a model system to examine whether the KLK7 gene is under steroid hormonal regulation (9). The KLK7 gene was found to be up-regulated primarily by estrogens and glucocorticoids. The above data prompted us to examine the expression of KLK7 gene in breast cancer.

Materials and methods

Study group

Tumor specimens from 90 patients who underwent surgery for primary breast cancer at the Oncologic Hospital of Athens "Saint Savas" were evaluated in this study. Additionally, for 15 of the samples, tissue from the same breast but distal to the site of malignant transformation (as determined by a pathologist)

was available and examined in order to verify KLK7 gene expression in normal breast tissue. Informed consent was obtained from all patients. A database containing updated information concerning each patient, together with receptor status, nodal status, size of primary tumor, number of positive nodes, age and menopausal status and/or differentiation grade of tumor, was available for statistical analysis. Patient ages ranged from 29 to 82 years, with a median of 57.5 years. Follow up information (median follow-up period, 99 months) was available for all patients, among whom 32 (34.8%) had relapsed and 29 (31.5%) had died. All patients had a histologically confirmed diagnosis of primary breast cancer and received no treatment before surgery. Clinical staging was performed according to the Postsurgical International Union against cancer Tumor-Node-Metastasis (TNM) classification system (12). Histological grade of the tumors was determined according to criteria reported by Bloom and Richardson (13). The post-operative treatment modality was known for all patients; 5 patients received no further treatment after tumor resection, 27 were given adjuvant chemotherapy, 52 were treated with endocrine therapy and 8 were given both chemotherapy and endocrine therapy. Postoperative loco-regional radiotherapy was administered to 60 patients.

Reverse transcriptase-polymerase chain reaction

Total RNA was extracted from the breast tissue using Trizol reagent (Gibco, BRL) following the manufacturer's instructions. RNA concentration was determined spectrophotometrically. Two micrograms of total RNA were reverse-transcribed into first-strand cDNA using the SuperscriptTM pre-amplification system (Gibco BRL) according to the manufacturer's instructions. The final volume was 20 µl. Based on the information obtained from our previous report (9), two gene-specific primers were designed (see Table 1), and PCR was carried out in a reaction mixture containing 1 µl of cDNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 mM dNTPs (deoxynucleoside triphosphates), 150 ng of primers, and 2.5 units of AmpliTaq Gold DNA polymerase (Roche Molecular Systems, Branchburg, NJ, USA) on a thermal cycler (MJ, Research, USA). The cycling conditions were a denaturation step at 94°C for 9 min, followed by 40 cycles of 94°C for 30s, 68°C for 1 min and a final extension step at 72°C for 10 min. Equal amounts of PCR products were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining. Actin was used as internal control for the integrity of the mRNA. The cycling conditions were a denaturation step at 94°C for 15 min, followed by 30 cycles of 94°C for 30s, 62°C for 30 sec and a final extension step at 72°C for 10 min.

Table 1: Primers used for reverse transcription polymerase chain reaction (RT-PCR) analysis.

Gene		Primer Sequence	Product size (bp)
KLK7	Forward	5'-GAATGAGTACACCGTGCACC-3'	360
	Reverse	5'-TGCCAGCGCACAGCATGGAA-3'	
Actin	Forward	5'-ATCTCGCACCACACCTTCTA -3'	832
	Reverse	5'-CGTCATACTCCTGCTTGCTG -3'	

Table 2: Distribution of numerical variables in the study

Variable	No. of patients	Mean \pm SE	Median	Range
Age (years)	92	56.71 \pm 1.27	57.5	29-82
Tumor size (cm)	92	2.48 \pm 0.13	2.20	0.80-7.00
Lymph nodes ^a	89	3.46 \pm 0.64	1	0-34
DFS time (months)	92	66.59 \pm 3.16	77.18	2-103
OS time (months)	92	73.27 \pm 2.65	84.96	7-103

^a Number of lymph nodes positive for malignancy

Cloning and sequencing of the PCR products

To verify the identity of the PCR products, they were cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The inserts were sequenced using vector-specific primers, with an automated DNA sequencer.

Steroid hormone receptor analysis

Steroid hormone receptors were quantified as described elsewhere (14, 15). The results of the dual ligand binding assay, in which dextran-coated charcoal was used to separate bound ligand from free ligand, were interpreted by Scatchard analysis (16). Tumors with ER (estrogen receptor) and PR (progesterone receptor) concentrations of ≤ 10 fmol/mg protein were considered receptor negative, whereas those with ER and PR concentrations of 10-300 fmol/mg were characterized as positive (17, 18).

Statistical analysis

KLK7 expression at the mRNA level was classified as positive or negative, and associations between KLK7 status and other qualitative variables were analyzed using the χ^2 or the Fisher exact test, where appropriate. A Cox proportional hazard regression model (19) was developed to evaluate the association (i.e. the hazard ratio and its confidence interval) between the potential prognostic marker and disease-free (DFS) or overall survival (OS). This analysis was conducted at both univariate and multivariate levels. The multivariate model was adjusted for KLK7 expression in tumors, lymph node status, tumor size, grade, histologic type, ER and PR status as well as adjuvant treatment (hormonal alone vs chemo \pm hormonal treatment)

status. Survival analyses were performed by constructing Kaplan-Meier DFS and OS curves (20) for KLK7-positive and KLK7-negative patients, and the log-rank test was used to examine the differences between them. DFS 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.

Results

KLK7 gene expression and relation to other variables

Of the 92 breast cancer patients examined (Table 2), 42 patients (45.65%) were classified as positive and 50 patients (54.34%) as negative for KLK7 gene expression (Table 3). The cut-off value, used for classifying KLK7 gene expression as positive or negative, was the presence of a DNA band of 360 bp or the non-existence of this band for equally loaded samples (Fig. 1).

Table 3 presents the associations between KLK7 gene expression and other clinical or pathological variables, including age of patients, menopausal status, tumor size, nodal status, clinical stage, histological grade, histotype, estrogen receptor (ER) and progesterone receptor (PR). KLK7 gene expression was marginally related to the cohort of women aged 45 to 55 years. KLK7 gene expression was positively associated to stage of the disease ($p=0.011$) and negatively related to PR status ($p=0.022$). On the other hand, no significant associations were found between KLK7 gene expression and different histological types, tumor size, nodal status, tumor grade, ER status or menopausal status (Table 3).

Table 3: Associations between KLK7 status and other clinicopathological variables

Variable	Total	No. of patients (%)		p value
		KLK7-negative	KLK7-positive	
Age (years)				
<45	20	13 (65.0)	7 (35.0)	0.047 ^a
45-55	22	7 (31.8)	15 (68.2)	
>55	50	30 (60.0)	20 (40.0)	
Menopausal status				
Pre/peri	32	16 (50.0)	16 (50.0)	0.66 ^b
Post	60	34 (56.7)	26 (43.3)	
Tumor size (cm)				
<2	41	26 (63.4)	15 (36.6)	0.14 ^b
≥2	51	24 (47.1)	27 (52.9)	
Nodal status				
Negative	41	26 (63.4)	15 (36.6)	0.13 ^b
Positive	48	22 (45.8)	26 (54.2)	
x	3			
Stage ^c				
I/II	77	46 (59.7)	31 (40.3)	0.011 ^b
III	12	2 (16.7)	10 (83.3)	
x	3			
Grade ^d				
I/II	69	40 (58.0)	29 (42.0)	0.23 ^b
III	23	10 (43.5)	13 (56.5)	
Histology				
Ductal	56	30 (53.6)	26 (46.4)	0.78 ^a
Lobular	18	9 (50.0)	9 (50.0)	
Other	18	11 (61.1)	7 (38.9)	
ER status				
Negative	34	14 (41.2)	20 (58.8)	0.082 ^b
Positive	57	35 (61.4)	22 (38.6)	
x	1			
PR status				
Negative	40	16 (40.0)	24 (60.0)	0.022 ^b
Positive	51	33 (64.7)	18 (35.3)	
x	1			

^a χ^2 test

^b Fisher's Exact Test

^c TNM system

^d Bloom-Scarff-Richardson grading system

x. Status unknown

Survival analysis

The strength of the associations between KLK7 gene expression and relapse free survival or death in univariate and multivariate analysis are shown in Table 4. In Cox univariate analysis, KLK7 expression showed a reverse relation with DFS and OS

($p = 0.001$ and $p < 0.001$, respectively), while in multivariate analysis this relation also remained statistically significant with DFS and OS ($p = 0.011$ and $p = 0.005$, respectively). Kaplan-Meier survival curves (Fig. 2) demonstrated that patients with KLK7-negative tumors have relatively longer DFS and OS

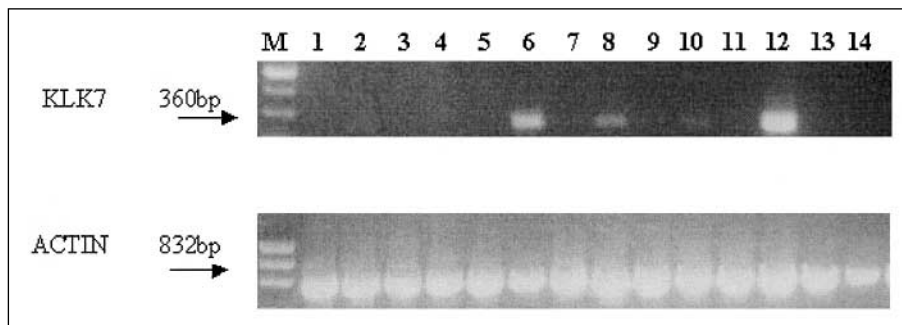


Figure 1: Expression of the KLK7 gene in breast tissue. M, DNA molecular weight marker; 2,4,6,8,10,12,14, randomly selected breast tumor samples; 1,3, 5, 7,9,11, 13: normal counterparts of corresponding tumor samples;

Table 4: Associations between KLK7 and disease-free and overall survival

Variable	Disease-free survival			Overall survival		
	HR ^a	95% CI ^b	p value	HR ^a	95% CI ^b	p value
Univariate analysis						
KLK7						
negative	1.00			1.00		
positive	3.41	1.61-7.23	0.001	4.01	1.77-9.07	<0.001
Nodal status	4.69	1.91-11.49	<0.001	4.71	1.78-12.42	<0.001
Grading (ordinal)	1.39	0.91-2.13	0.004	1.46	1.01-2.13	0.045
Tumor size	1.54	1.21-1.96	<0.001	1.38	1.07-1.75	0.011
ER status	0.52	0.26-1.05	0.068	0.43	0.21-0.91	0.027
PR status	0.51	0.25-1.03	0.059	0.45	0.21-0.94	0.034
Histologic type ^c	0.84	0.58-1.21	0.11	0.85	0.58-1.25	0.42
Adjuvant treatment ^d	0.34	0.16-0.74	0.007	0.29	0.12-0.66	0.003
Multivariate analysis ^f						
KLK7						
negative	1.00			1.00		
positive	2.92	1.28-6.63	0.011	3.71	1.49-9.23	0.005
Nodal status	4.02	1.48-10.91	0.006	4.52	1.52-13.4	0.006
Grading (ordinal)	0.90	0.46-1.76	0.76	0.91	0.41-1.96	0.81
Tumor size	1.32	0.99-1.77	0.042	1.19	0.87-1.62	0.25
ER status	0.81	0.32-2.04	0.65	0.64	0.24-1.73	0.38
PR status	0.70	0.29-1.66	0.41	0.70	0.28-1.75	0.45
Histologic type ^c	0.65	0.34-1.27	0.21	0.67	0.31-1.44	0.31
Adjuvant treatment ^d	0.58	0.23-1.45	0.24	0.45	0.17-1.20	0.11

^a Hazard ratio (HR) estimated from Cox proportional hazard regression model
^b Confidence interval of the estimated HR
^c Lobular and others vs. ductal
^d Hormonal alone vs chemo±hormonal treatment
^f Multivariate models were adjusted for lymph node status; tumor size; grade; histologic type; ER and PR status; adjuvant treatment status.

($p < 0.001$) compared to patients with KLK7-positive tumors, showing that KLK7 gene expression may serve as an unfavorable prognostic marker for DFS and OS of breast cancer patients.

Discussion

Proteases play essential roles in the process of tumor invasion and metastasis. Kallikreins are a group of serine proteases that are found in diverse tissues and biological fluids. So far, 15 members of the kallikrein gene family have been identified. According to the approved official kallikrein gene nomenclature (21), the KLK7 gene encodes the serine protease human stratum corneum chymotryptic enzyme (SCCE or hK7) and is known to contribute to the cell shedding process by catalyzing the degradation of intercellular cohesive structures at the skin surface (22). The presence of hK7 on the surface of tumor cells suggests

that it may also contribute to the process of tumor cell shedding, resulting in early metastasis of carcinoma.

Using immunohistochemical methods, Sondell, et al. (23) examined the expression of hK7 in two diseases of human oral mucosa producing a pathological keratinization of the epithelium at sites which are normally non-keratinised, and suggested that hK7 expression may be a marker of terminal differentiation of squamous epithelia. Tanimoto, et al. (26) reported that the expression of SCCE, known until then to be expressed in skin (8, 24, 25), is increased in ovarian cancer. Later it was shown (9) that KLK7 is also expressed in kidney, spinal cord, cerebellum, breast, brain and salivary glands. In ovarian cancer, other proteases and inhibitors, like hepsin and anti-leukoprotease (ALP) (26, 27), are also overexpressed. ALP has been shown to be a natural inhibitor of SCCE, isolated from human stratum corneum (27). Expression of the serine protease inhibi-

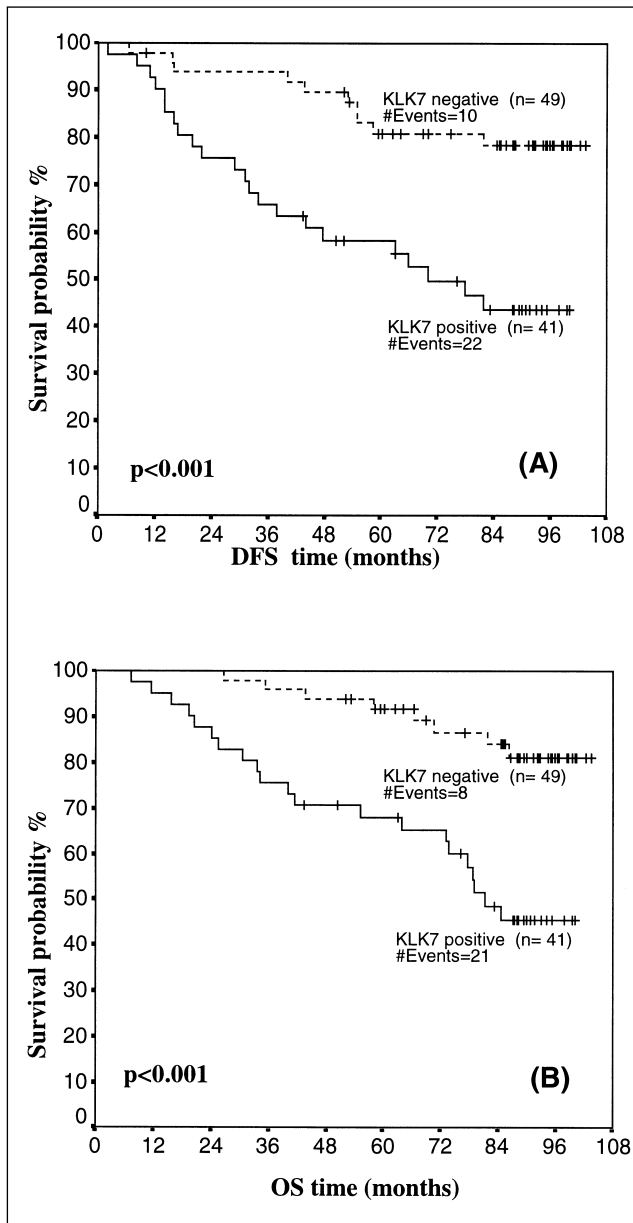


Figure 2: Kaplan-Meier survival curves presenting the associations between KLK7 gene expression and DFS (A) or OS (B), n = number of patients examined.

tor ALP and hK7 was shown to be coordinated in ovarian tumors (28).

In the present study we report, for first time, that the KLK7 gene is differentially expressed at the mRNA level of breast cancer tissues and that this can serve as an independent marker of unfavorable prognosis for disease-free survival (DFS) and overall survival (OS) both in univariate and in multivariate analysis. Also, KLK7 gene expression in multivariate analysis was found to be the most significant independent prognostic marker for the overall survival (OS) of patients, but was not associated to other clinicopathological features such as histological type, tumor size, nodal status, tumor grade, menopausal status, ER and PR status, (Table 4). Analysis of other kallikreins in breast and other cancers has shown the following results: The KLK5 gene (29) has been predicted to be phylogenetically linked to KLK7 (4) and is also an independent indicator of poor prognosis in breast cancer; KLK10 (NES1) was shown to be over-expressed in aggressive ovarian tissues (30, 31) and down-regulated in breast cancer; KLK13 was reported to be an independent indicator of favorable prognosis in breast (32) and KLK9 an independent indicator of favorable prognosis of ovarian cancer (33).

Although it is evident from Table 4 that patients receiving hormonal treatment alone seem to have a longer survival time than patients receiving chemotherapy treatment, this, nevertheless, may be due to selection of patients for hormonal treatment according to low tumor grade and stage of the disease. KLK7 gene expression was found to be positively associated with disease stage.

The expression of the KLK7 gene was previously found to be up-regulated by estrogens and glucocorticoids in breast cancer cells (9). In the present study, positive KLK7 gene expression was negatively correlated to the stage of differentiation and progesterone receptor status.

In summary, we provided evidence that higher KLK7 gene expression is associated with the unfavorable outcome of patients with breast cancer. Since the KLK7 gene encodes for a potentially secreted enzyme, analysis of this protein (hK7) in serum may serve as a new biomarker for breast cancer.

References

1. Yousef G, Chang A, Scorilas A, et al.. Genomic organization of the human kallikrein locus on chromosome 19q13.3-q13.4. *Biochem Bioph Res Co* 2000; 276: 125-33.
2. Diamandis EP, Yousef GM, Luo LY, et al.. The new kallikrein gene family-implications in carcinogenesis. *Trends Endocrinol Metab* 2000; 11: 54-60.
3. Bhoola K, Ramsaroop R, Plendl J, et al.. Kallikrein and kinin receptor in inflammation and cancer. *Biol Chem* 2001; 382: 77-89.
4. Bhoola K, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens and kininases. *Pharmacol Rev* 1992; 44: 1-80.
5. Bhoola K. Translocation of the neutrophil kinin moiety and changes in the regulation of kinin receptors in inflammation. *Immunopharmacology* 1996; 33: 247-56.
6. Lundstrom A, Egelrud T. Stratum corneum chymotryptic enzyme: a proteinase which may be generally present in the stratum corneum and with a possible involvement in desquamation. *Acta Derm Venereol* 1991; 71: 471-4.
7. Tanimoto H, Underwood LJ, Shigemasa K, et al.. The stratum corneum chymotryptic enzyme that mediates shedding and desquamation of skin cells is highly overexpressed in ovarian tumor cells. *Cancer* 1999; 86: 2074-82.
8. Hansson L, Stromqvist M, Backman A, et al.. Cloning, expression and characterization of stratum corneum chymotryptic enzyme. A

- skin-specific human serine proteinase. *J Biol Chem* 1994; 269: 19420-6.
9. Yousef GM, Scorilas A, Magklara A, et al.. The KLK7 (PRSS6) gene, encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family-genomic characterization, mapping, tissue expression and hormonal regulation. *Gene* 2000; 254: 119-28.
10. Ekholm E, Egelrud T. Stratum corneum chymotryptic enzyme in psoriasis. *Arch Dermatol Res* 1999; 29: 195-200.
11. Kyriakopoulou LG, Yousef GM, Scorilas A, et al.. Prognostic value of quantitatively assessed KLK7 expression in ovarian cancer. *Clin Biochem* 2003; 36: 135-43.
12. Scott-Conor CE, Christie DW. Cancer staging using the American Joint Committee on Cancer TNM System. *J Am Coll Surg* 1995; 181(2): 182-8.
13. Doussal VL, Tubiana-Hulin M, Friedman S, et al.. Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson SBR: an improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. *Cancer* 1989; 64: 1914-22.
14. Dressler LG, Seamer LC, Owens MA, et al.. Dna flow cytometry and prognostic factors in 1131 frozen breast cancer specimens. *Cancer(Phila.)* 1988; 61: 420-7.
15. Scorilas A, Talieri M, Ardavanis A, et al.. Polyadenylate polymerase enzymatic activity in mammary tumor cytosols: A new independent prognostic marker in primary breast cancer. *Cancer Res* 2000; 60: 5427-33.
16. Scatchard G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 1949; 51: 660-72.
17. Alexiera-Figusch J, Van Putten WLS, Blankenstein MA, et al.. The prognostic value and relationships of patient characteristics, estrogen and progesterin receptors and site of relapse in primary breast cancer. *Cancer (Phila.)* 1988; 61: 758-68.
18. Reiher A, Kolb R, Reiner G, et al.. Prognostic significance of steroid hormone receptor and histopathological characterization of human breast cancer. *J Cancer Res Clin Oncol* 1987; 113: 285-90.
19. Cox DR. Regression models and life tables R. *Stat Soc. B*, 1972; 34: 187-202.
20. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; 53: 457-81.
21. Diamandis EP, Yousef GM, Clements J, et al.. New nomenclature for the human tissue kallikrein gene family. *Clin Chem* 2000; 46: 1855-8.
22. Sondell B, Thornell LE, Egelrud T. Evidence that stratum corneum chymotryptic enzyme is transported to the stratum corneum extracellular space via lamellar bodies. *J Invest Dermatol* 1995; 104: 819-23.
23. Sondell B, Dysberg P, Anneroth GK, et al.. Association between expression of stratum corneum chymotryptic enzyme and pathological keratinization in human oral mucosa. *Acta Derm Venereol* 1996; 76: 177-81.
24. Egelrud T. Purification and preliminary characterization of stratum corneum chymotryptic enzyme: a proteinase that may be involved in desquamation. *J Invest Dermatol* 1993; 101: 200-4.
25. Sondell B, Thornell LE, Stigbrand T, et al.. Immunolocalization of stratum corneum chymotryptic enzyme in human skin and oral epithelium with monoclonal antibodies: evidence of a proteinase specifically expressed in keratinizing squamous epithelia. *J Histochem Cytochem* 1994; 42: 459-65.
26. Tanimoto H, Yan Y, Clarke J, et al.. Hepsin: a cell surface serine protease identified in hepatoma cells is overexpressed in ovarian cancer. *Cancer Res* 1997; 57: 2884-7.
27. Shigemasa K, Tanimoto H, Underwood LJ, et al.. Expression of the protease inhibitor antileukoprotease and the serine protease stratum corneum chymotryptic enzyme (SCCE) is coordinated in ovarian tumors. *Int J Gynecol Cancer* 2001; 11: 454-61.
28. Franzke CW, Baici A, Bartels J, et al.. Antileukoprotease inhibits stratum corneum chymotryptic enzyme. *J Biol Chem* 1996; 271: 21886-90.
29. Yousef GM, Scorilas A, Kyriakopoulou LG, et al.. Human kallikrein gene 5 (KLK5) expression by quantitative PCR: an independent indicator of poor prognosis in breast cancer. *Clin Chem* 2002; 48: 1241-50.
30. Luo Y, Katsaros D, Scorilas A, et al.. Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. *Clin Cancer Res* 2001; 7: 2372-9.
31. Luo LY, Bunting P, Scorilas A, et al.. Human kallikrein 10: A novel tumor marker for ovarian carcinoma? *Clin Chim Acta* 2001; 306: 111-8.
32. Chang A, Yousef G, Scorilas A, et al.. Human Kallikrein gene 13 (KLK13) expression by quantitative RT-PCR: An independent indicator of favorable prognosis in breast cancer. *Br J Cancer* 2002; 86:1457-64.
33. Yousef GM, Kyriakopoulou L, 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* 2001; 61: 7811-8.
34. Yousef GM, Scorilas A, Nakamura T, et al.. The prognostic value of the human kallikrein gene 9 (KLK9) in breast cancer. *Breast Cancer Res Treat* 2003; 78: 149-58.
35. 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(6): 1035-43.
36. 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* 2003; 119: 346-55.
37. 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.
38. Yousef GM, Borgono CA, Scorilas A, et al.. Quantitative analysis of human kallikrein gene 14 expression in breast tumours indicates association with poor prognosis. *Br J Cancer* 2002; 87: 1287-93.
39. 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* 2002; 87: 763-71.