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Cancer Diagnostics:
Discovery and Clinical
Applications

Human Kallikrein Gene 5 (*KLK5*) Expression by Quantitative PCR: An Independent Indicator of Poor Prognosis in Breast Cancer

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Background: ***KLK5* is a newly discovered human kallikrein gene. Many kallikrein genes have been found to be differentially expressed in various malignancies, and prostate-specific antigen (PSA; encoded by the *KLK3* gene) is the best tumor marker for prostate cancer. Like the genes that encode PSA and other kallikreins, the *KLK5* gene was found to be regulated by steroid hormones in the BT-474 breast cancer cell line.**

Methods: **We studied *KLK5* expression in 179 patients with different stages and grades of epithelial breast carcinoma by quantitative reverse transcription-PCR (RT-PCR), using LightCycler® technology. An optimal cutoff point equal to the detection limit (65th percentile) was used. *KLK5* values were then compared with other established prognostic factors in terms of disease-free (DFS) and overall survival (OS).**

Results: **High *KLK5* expression was found more frequently in pre-/perimenopausal ($P = 0.026$), node-positive ($P = 0.029$), and estrogen receptor-negative ($P = 0.038$) patients. In univariate analysis, *KLK5* overexpression was a significant predictor of reduced DFS ($P < 0.001$) and OS ($P < 0.001$). Cox multivariate analysis indicated that *KLK5* was an independent prognostic factor for DFS and OS. *KLK5* remained an independent prognostic variable in the subgroups of patients with large tumors (>2 cm) and positive nodes. Hazard ratios derived from Cox analysis and related to DFS and OS were 2.48 ($P = 0.005$) and 2.37 ($P = 0.009$), respectively, for the node-positive group and 3.03 ($P = 0.002$) and 2.94 ($P = 0.002$), respectively, for patients with tumor sizes >2 cm. *KLK5* expression was also associated with statistically significantly shorter DFS ($P = 0.006$) and OS ($P = 0.004$) in the subgroup of patients with grade I and II tumors.**

Conclusions: ***KLK5* expression as assessed by quantitative RT-PCR is an independent and unfavorable prognostic marker for breast carcinoma.**

Breast cancer is the most common malignancy among females in North America. In the United States alone, ~200 000 new cases are diagnosed every year, and ~40 000 women die annually from the disease *(1)*. Because these carcinomas display a high variability in their biological and clinical behavior, major efforts have been directed at finding specific factors that could reflect the characteristics of each tumor. Among the different biochemical markers that can be used for this purpose, serine proteases have attracted particular interest because of their potential role in the degradation of extracellular matrix *(2, 3)* and the stimulation of cell growth and angiogenesis *(4, 5)*. Thus, it is not surprising that clinical reports have already shown that overexpression of certain serine proteases correlates positively with poor prognosis in different malignancies *(6–9)*.

Kallikreins are serine proteases with diverse physiologic functions. Accumulating evidence indicates that many members of the expanded human tissue kallikrein gene family are associated with malignancy *(10)*. Prostate-specific antigen (encoded by the *KLK3* gene) is the best tumor marker for prostate cancer *(11)*. Human glandular kallikrein protein is an emerging tumor marker for

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Table 1. Relationships between *KLK5* status[a] and other variables.

Variable	No. of patients (%) Total	*KLK5*-negative	*KLK5*-positive	*P*
Age, years				
<45	35	20 (57.1)	15 (42.9)	
45–55	42	25 (59.5)	17 (40.5)	0.23[b]
>55	102	72 (70.6)	30 (29.4)	
Menopausal status				
Pre-/perimenopausal	61	33 (54.1)	28 (45.9)	0.026[c]
Postmenopausal	118	36 (71.2)	36 (28.8)	
Tumor size, cm				
<2	85	60 (70.6)	25 (29.4)	0.21[c]
≥2	94	57 (60.6)	37 (39.4)	
Nodal status				
Negative	77	57 (74.0)	20 (26.0)	0.029[c]
Positive	90	53 (58.9)	37 (41.1)	
Unknown	12			
Stage[d]				
I	47	37 (78.7)	10 (21.3)	
II	102	63 (61.8)	39 (38.2)	0.077[b]
III–IV	18	10 (55.6)	8 (44.4)	
Unknown	12			
Grade[e]				
I	73	48 (65.8)	25 (34.2)	
II	61	44 (72.1)	17 (27.9)	0.21[c]
III	45	25 (55.6)	20 (44.4)	
Histology				
Ductal	112	71 (63.4)	41 (36.6)	
Lobular	29	20 (69.0)	9 (31.0)	0.77[b]
Other	38	26 (68.4)	12 (31.6)	
ER status				
Negative	71	40 (56.3)	31 (43.7)	0.038[c]
Positive	106	76 (71.7)	30 (28.3)	
Unknown	2			
PR status				
Negative	82	50 (61.0)	32 (39.0)	0.27[c]
Positive	95	66 (69.5)	29 (30.5)	
Unknown	2			
Adjuvant treatment				
None	43	29 (67.4)	14 (32.6)	
Tamoxifen	83	61 (73.5)	22 (26.5)	0.025[b]
Chemotherapy ± tamoxifen	53	27 (50.9)	26 (49.1)	

[a] The cutoff point was the 65th percentile value.
[b] χ^2 test.
[c] Fisher exact test.
[d] TNM system.
[e] Bloom–Scarff–Richardson grading system.

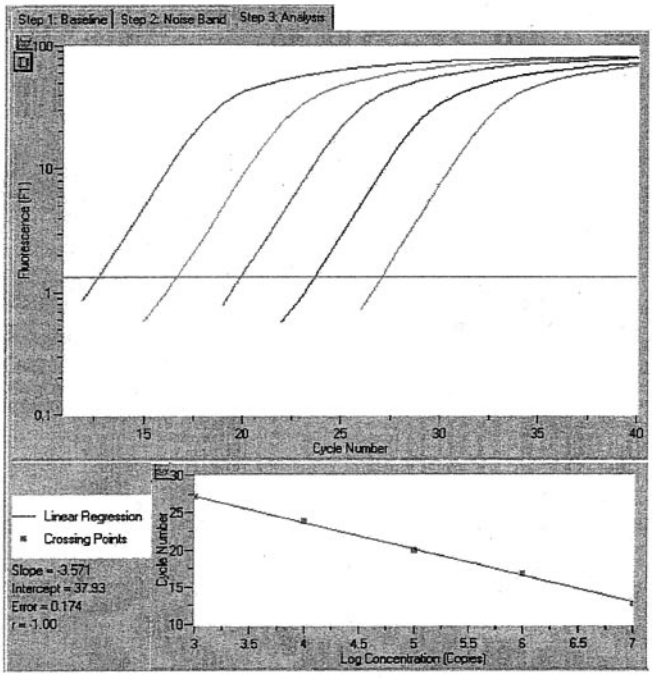

Fig. 1. Quantification of *KLK5* gene expression by real-time PCR.
(*Top*), logarithmic plot of fluorescence signal above the background noise (*horizontal line*) during amplification. Serial dilutions of a total RNA preparation from breast tissue were prepared, and an arbitrary copy number was assigned to each sample according to the dilution factor. (*Bottom*), crossing points (cycle number) plotted against the log of copy number to obtain a calibration curve. For details, see text.

prostate cancer *(12–14)*. *KLK10* (also known as the normal epithelial cell-specific 1 gene; *NES1*) appears to be a novel tumor suppressor that is down-regulated during breast cancer progression *(15)*. *KLK6* (zyme/protease M/neurosin) is expressed in primary breast and ovarian cancers *(16)*, and preliminary studies indicate that it may have utility as a serum biomarker for ovarian carcinoma *(17)*. Two additional kallikrein genes, *KLK8* (also known as neuropsin or *TADG-14*) *(18)* and the gene that encodes stratum corneum chymotryptic enzyme *(19, 20)* are up-regulated in ovarian cancer. Human kallikrein gene 5 [designated *KLK5*[5] according to the human gene nomenclature committee *(21)*, and also known as kallikrein-like gene-2 (*KLK-L2*) *(22)* or human stratum corneum tryptic enzyme (*HSCTE*) *(23)*] is a newly identified member of the human kallikrein gene family that maps to chromosome 19q13.3-q13.4, close to other kallikrein genes *(24)*. *KLK5* is expressed mainly in testis, breast, brain, and epidermis *(22, 23)*. The KLK5 protein has the conserved catalytic triad of serine proteases *(22)*, and the enzyme has proteolytic activity *(23)*. KLK5 was also found to be regulated by steroid hormones in the BT-474 breast cancer cell line *(22)*. On the basis of these new findings, we hypothesized that *KLK5* may be differentially expressed in breast cancer tissues and may have prognostic/predictive value as a breast cancer biomarker.

Materials and Methods

STUDY POPULATION

Included in this study were tumor specimens from 179 consecutive patients undergoing surgical treatment for

[5] Nonstandard abbreviations: *KLK5*, human kallikrein gene 5; ER, estrogen receptor; PR, progesterone receptor; DFS, disease-free survival; OS, overall survival; and EMSP, enamel matrix serine proteinase.

Table 2. Univariate and multivariate analysis of *KLK5* expression and DFS and OS.

	DFS			OS		
Variable	**HR[a]**	**95% CI[b]**	***P***	**HR[a]**	**95% CI[b]**	***P***
Univariate analysis						
KLK5						
Negative (n = 115)	1.00			1.00		
Positive (n = 61)	2.83	1.63–4.89	<0.001	2.89	1.61–5.18	<0.001
As a continuous variable	1.001	1.00–1.003	0.031	1.002	1.00–1.004	0.042
Nodal status	5.78	2.82–11.8	<0.001	7.03	2.97–16.6	<0.001
Grading (ordinal)	1.61	1.16–2.21	0.004	1.82	1.28–2.60	<0.001
Tumor size	1.42	1.27–1.58	<0.001	1.36	1.23–1.50	<0.001
ER status	0.58	0.34–0.96	0.036	0.41	0.23–1.50	0.002
PR status	0.55	0.32–0.92	0.023	0.39	0.22–0.71	0.002
Histologic type[c]	0.62	0.36–1.09	0.10	0.57	0.31–1.06	0.074
Age	0.98	0.96–1.01	0.14	0.99	0.96–1.01	0.42
Multivariate analysis						
KLK5						
Negative (n = 109)	1.00			1.00		
Positive (n = 56)	2.78	1.49–5.21	0.001	2.97	1.49–5.90	0.002
As a continuous variable	1.00	0.99–1.001	0.90	1.00	0.99–1.01	0.90
Nodal status	6.05	2.63–13.9	<0.001	7.83	2.96–20.7	<0.001
Grading (ordinal)	0.91	0.51–1.61	0.74	0.82	0.38–1.73	0.61
Tumor size	1.34	1.17–1.55	<0.001	1.32	1.14–1.52	<0.001
ER status	0.67	0.29–1.54	0.35	0.55	0.22–1.39	0.21
PR status	0.77	0.34–1.74	0.79	0.67	0.27–1.69	0.41
Histologic type[c]	0.72	0.38–1.39	0.33	0.48	0.12–1.96	0.31
Age	0.99	0.97–1.02	0.79	1.01	0.98–1.04	0.62

[a] HR, hazard ratio estimated from Cox proportional hazard regression model.
[b] CI, confidence interval of the estimated hazard ratio.
[c] Lobular and others vs ductal.

primary breast carcinoma at the Department of Gynecologic Oncology at the University of Turin (Turin, Italy). The selection criterion for specimens was confirmation of the diagnosis by histopathology. Tumor tissues were frozen in liquid nitrogen immediately after surgery.

This study was approved by the Institutional Review Board of the University of Turin. The patients were 29–83 years of age (median, 58 years), and tumors were 0.1–15 cm in size (median, 2.15 cm). Follow-up information (median follow-up period, 75 months) was available for 176 patients, among whom 55 (31%) had relapsed and 46 (26%) had died. The histologic type and steroid hormone receptor status (assessed by an Abbott ELISA) of each tumor and the number of positive axillary nodes were established at the time of surgery, as shown in Table 1. Of the 179 patients, 112 (63%) had ductal carcinoma, 29 (16%) had lobular carcinoma, and 38 (21%) had other histologic types. Patients from clinical stages I–III were included in the study, with staging determined according to the TNM classification. Tumors were graded according to the Bloom–Richardson grading system *(25)*. Forty-three patients (24%) received no adjuvant treatment, 83 (46%) received tamoxifen, and 53 (30%) received chemotherapy with or without tamoxifen. Estrogen (ER) and progesterone receptor (PR) status was established as described by the European Organization for Research and Treatment of Cancer *(26)*.

TOTAL RNA EXTRACTION AND cDNA SYNTHESIS

Tumor tissues were minced with a scalpel, on dry ice, and transferred immediately to 2-mL polypropylene tubes. The tissues were then homogenized, and total RNA was extracted with Trizol™ reagent (Invitrogen), according to the manufacturer's instructions. The concentration and purity of mRNA were determined spectrophotometrically. Two micrograms of total RNA was reverse-transcribed into first-strand cDNA by use of the Superscript™ preamplification system with an oligo(dT) primer (Invitrogen). The final volume was 20 μL.

QUANTITATIVE REAL-TIME PCR AND CONTINUAL MONITORING OF PCR PRODUCTS

On the basis of the published genomic sequence of *KLK5* (GenBank accession no. AF135028), two gene-specific primers were designed: L2-3 (5′-CAA GAC CCC CCT GGA TGT GG-3′) and 5L2 (5′-AGT TTT CAG AGT CCG TCT CGG-3′). These primers spanned more than two exons to avoid contamination by genomic DNA.

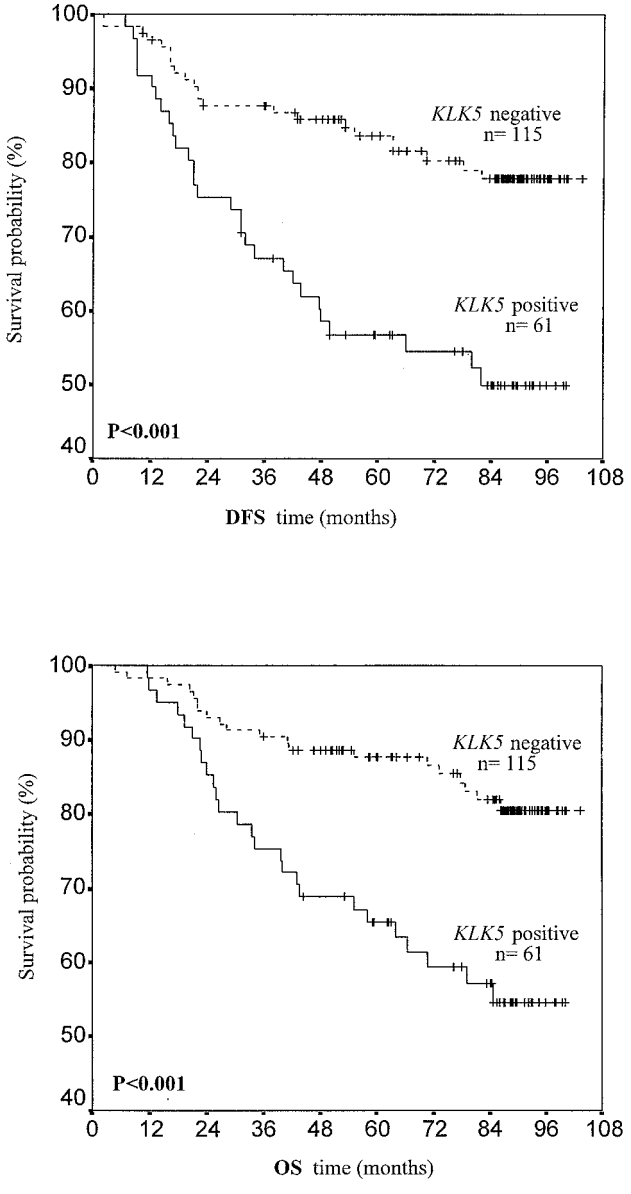

Fig. 2. DFS (*top*) and OS (*bottom*) for patients with *KLK5*-positive and -negative tumors.
For details, see text.

The PCR was monitored in real time with the LightCycler™ system (Roche Molecular Systems) and SYBR Green I dye, which binds preferentially to double-stranded DNA *(27)*. The reaction is characterized by the time during cycling when amplification of the PCR products is first detected, rather than the amount of PCR product accumulated after a fixed number of cycles. The higher the starting quantity of the template, the earlier a significant increase in fluorescence is observed *(28)*. The threshold cycle is defined as the fractional cycle number at which fluorescence passes a fixed threshold above baseline *(29)*. For each sample, the amounts of the target and an endogenous control (*β-actin*, a housekeeping gene) were determined from a calibration curve (see below). The amount of the target molecule was then divided by the amount of the endogenous reference to obtain a normalized target value

CALIBRATION CURVE CONSTRUCTION

Separate calibration curves for *β-actin* and *KLK5* were constructed from serial dilutions of healthy human breast tissue total cDNA (Clontech) and were included in each assay (Fig. 1). Calibrators were defined to contain arbitrary units of *KLK5* and *β-actin* RNA, and all calculated concentrations are relative to these concentrations.

PCR AMPLIFICATION

PCR was performed with the LightCycler system. For each assay, a master mixture containing 1 μL of cDNA, 2 μL of LC DNA Master SYBR Green 1 mixture, 50 ng of the primers, and 1.2 μL of 25 mM $MgCl_2$ was prepared on ice. After the reaction mixture was loaded into the glass capillary tube, the cycling conditions were as follows: initial denaturation at 94 °C for 10 min, followed by 45 cycles of denaturation at 94 °C for 0 s, annealing at 60 °C for 5 s, and extension at 72 °C for 16 s. The temperature transition rate was set at 20 °C/s. The amount of fluorescent product was measured in single-acquisition mode at 86 °C after each cycle.

MELTING CURVE

To distinguish specific from nonspecific products and primer-dimers, a melting curve was obtained after amplification by maintaining the temperature at 70 °C for 30 s, followed by a gradual increase in temperature to 98 °C at a rate of 0.2 °C/s, with the signal acquisition mode set at step-acquisition mode, as described previously *(30)*. To verify the melting curve results, representative samples of the PCR products were assayed on 1.5% agarose gels, purified, and cloned into the pCR 2.1-TOPO vector (Invitrogen) according to the manufacturer's instructions. The inserts were sequenced from both directions with vector-specific primers in an automated DNA sequencer.

STATISTICAL ANALYSIS

Patients were subdivided into groups based on different clinical or pathologic variables, and statistical analyses were performed using SAS software (SAS Institute). An optimal cutoff point equal to the 65th percentile was defined using χ^2 analysis based on the ability of *KLK5* to predict the disease-free survival (DFS) for the population studied. According to this cutoff, *KLK5* expression was classified as positive or negative, and associations between *KLK5* status and other qualitative variables were analyzed by the χ^2 or the Fisher exact test, where appropriate. Differences in *KLK5* values between groups of patients were analyzed with the nonparametric Mann–Whitney *U*-test or Kruskal–Wallis tests. In this analysis, *KLK5* was the continuous variable. The cutoff value for tumor size was 2 cm. Lymph node status was either

Table 3. Cox proportional hazard regression analysis for subgroups of breast cancer patients.

	DFS			OS		
Variable	HR[a]	95% CI[b]	*P*	HR[a]	95% CI[b]	*P*
Node negative						
KLK5 unadjusted	3.85	0.86–17.2	0.077	4.11	0.68–24.6	0.12
KLK5 adjusted[c]	6.61	0.95–45.7	0.056	4.80	0.55–41.9	0.16
Node positive						
KLK5 unadjusted	2.48	1.32–4.67	0.005	2.37	1.23–4.57	0.009
KLK5 adjusted[c]	2.77	1.37–5.61	0.004	2.83	1.33–6.02	0.007
Tumor size ≤2 cm						
KLK5 unadjusted	1.94	0.73–5.10	0.18	1.93	0.61–6.10	0.26
KLK5 adjusted[d]	2.08	0.68–6.10	0.19	1.16	0.22–5.99	0.85
Tumor size >2 cm						
KLK5 unadjusted	3.03	1.52–6.02	0.002	2.94	1.47–5.89	0.002
KLK5 adjusted[d]	2.31	1.08–4.95	0.031	2.45	1.12–5.35	0.024
Grade I–II						
KLK5 unadjusted	2.57	1.27–5.21	0.009	2.84	1.31–6.16	0.008
KLK5 adjusted[e]	3.03	1.37–6.68	0.006	3.63	1.49–8.85	0.004
Grade III						
KLK5 unadjusted	2.66	1.09–6.46	0.030	2.38	0.97–5.86	0.057
KLK5 adjusted[e]	3.55	0.99–12.7	0.051	3.84	0.93–15.8	0.062

[a] HR, hazard ratio estimated from Cox proportional hazard regression model.
[b] CI, confidence interval of the estimated hazard ratio.
[c] Multivariate models were adjusted for tumor size, grade, ER, PR, histologic type, and age.
[d] Multivariate models were adjusted for tumor grade, nodal status, ER, PR, histologic type, and age.
[e] Multivariate models were adjusted for tumor size, nodal status, ER, PR, histologic type, and age.

positive (any positive number of nodes) or negative. Age was categorized into three groups: <45 years, 45–55 years, and >55 years. Survival analyses were performed by constructing Kaplan–Meier DFS and overall survival (OS) curves *(31)*, and differences between curves were evaluated by the log-rank test as well as by estimating the relative risks for relapse and death from the Cox proportional-hazards regression model *(32)*. The Cox model was used for both univariate and multivariate analyses. Only patients for whom the status of all variables was known were included in the multivariate regression models, which incorporated *KLK5* and all other variables by which the patients were characterized. In the multivariate analysis of *KLK5*, the Cox regression models were adjusted for patient age, nodal status, tumor size, grade, histologic type, and ER and PR status.

During this study, the PCR operators were blinded to the clinical data, and the biostatistician was also blinded until all PCRs had been completed and entered into the database.

Results

KLK5 expression and relation to other variables

KLK5 mRNA concentrations ranged from 0.00 to 6953 arbitrary units in breast cancer tissues, with a mean ± SE of 278 ± 74. A cutoff point equal to the detection limit (65th percentile) was used. Of 179 breast tumors examined, 62 (35%) were positive for *KLK5* expression, and the remaining 117 (65%) were negative. Table 1 depicts the distribution of *KLK5* expression in breast tissues in relation to other established prognostic factors, such as menopausal status, tumor size, nodal status, tumor stage and grade, histologic type, receptor status, and adjuvant therapy. High *KLK5* expression was found more frequently in pre-/perimenopausal ($P = 0.026$), node-positive ($P = 0.029$), and ER-negative ($P = 0.038$) patients. Significant associations between *KLK5* status and tumor size, stage, grade, histologic type, or PR status were not observed.

survival analysis

Of the 179 patients included in this study, follow-up information was available for 176 patients, among whom 55 (31%) had relapsed and 46 (26%) had died. The strength of the association between each clinicopathologic variable and DFS and OS is shown in the univariate analysis of Table 2. *KLK5* expression was a significant predictor of DFS and OS (hazard ratios, 2.8 and 1.6, respectively; $P < 0.001$ for both). Kaplan–Meier survival curves (Fig. 2) also demonstrated that patients with *KLK5*-positive tumors had substantially shorter DFS and OS ($P < 0.001$ for both) compared with those who were *KLK5* negative.

In the multivariate analysis, Cox models were adjusted for nodal status, tumor grade, tumor size, ER and PR status, histologic type, and age. In this analysis, nodal status, tumor size, and *KLK5* expression were the strongest independent indicators for DFS and OS (Table 2). TNM stage was not included in the multivariate models because it is a function of tumor size and nodal status, variables that were included in the multivariate models.

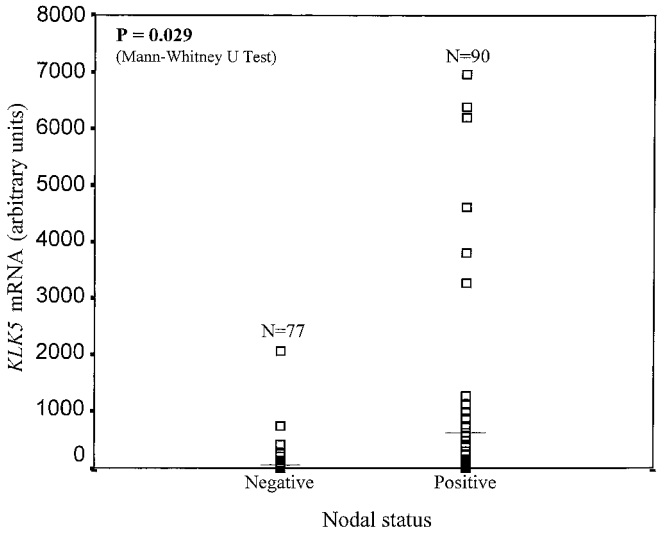

Fig. 3. Relationship between *KLK5* expression and nodal status.
P was determined by the Mann–Whitney *U*-test. *Horizontal lines* represent the mean *KLK5* mRNA concentrations.

Table 3 shows a Cox proportional-hazard regression analysis for *KLK5* expression in breast cancer patients stratified for nodal status, tumor size, and tumor grade. *KLK5* was a significant prognostic factor in the subgroup of patients who were node positive (Fig. 3 and Table 3), those with a tumor size >2 cm, or those with grade I and II cancer (Table 3). Hazard ratios derived from the Cox regression analysis and related to DFS and OS were 2.48 ($P = 0.005$) and 2.37 ($P = 0.009$), respectively, for the node-positive group and 3.03 ($P = 0.002$) and 2.94 ($P = 0.002$), respectively, for patients in whom the tumor size was >2 cm. After adjustment for other known prognostic variables, *KLK5* retained its independent prognostic value in all of these subgroups of patients.

These results were also demonstrated by the Kaplan–Meier curves, whereby patients with *KLK5*-positive tumors were found to have a less favorable progression-free survival and OS than patients with *KLK5*-negative tumors in the subgroup of node-positive patients (Fig. 4). Furthermore, *KLK5* expression was associated with substantially

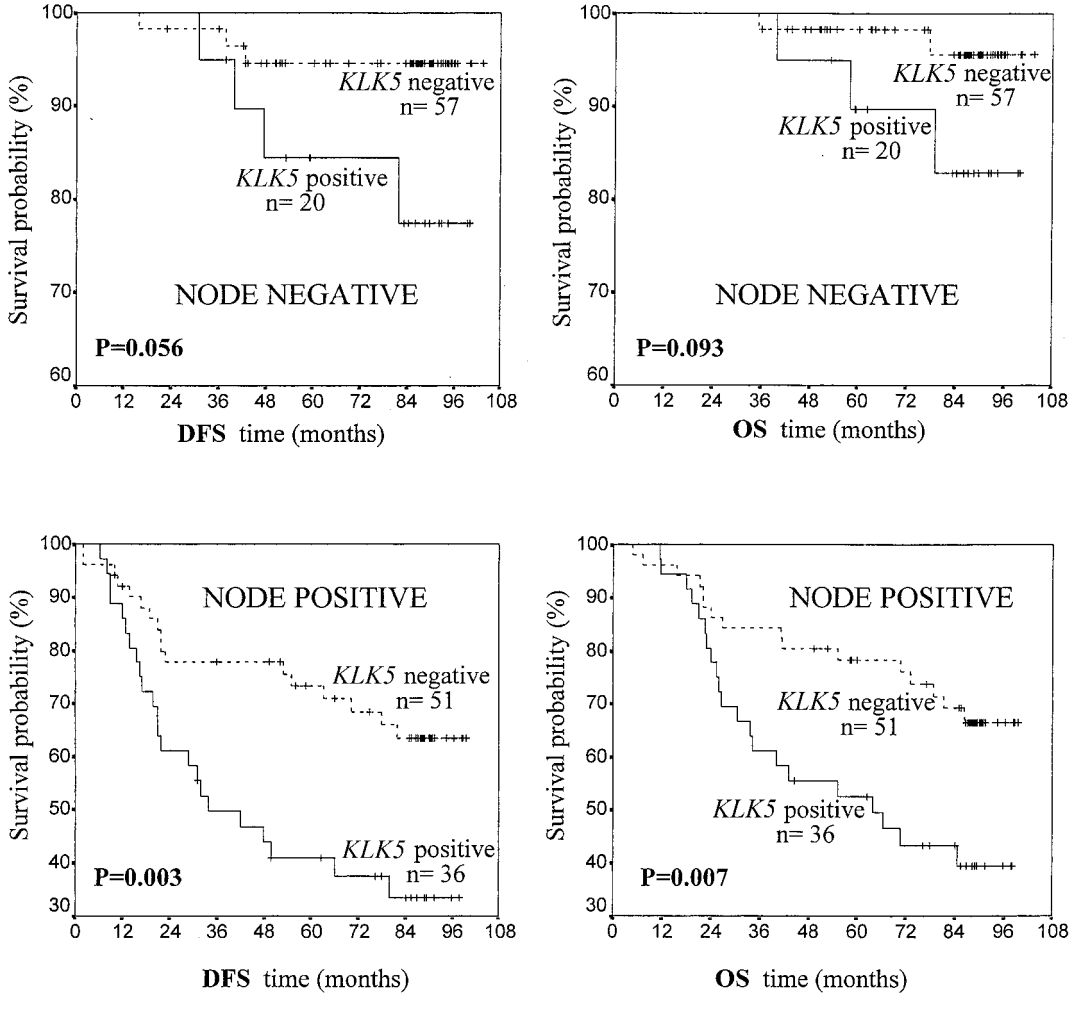

Fig. 4. Kaplan–Meier survival curves for patients with *KLK5*-positive and -negative breast cancers, stratified by nodal status.

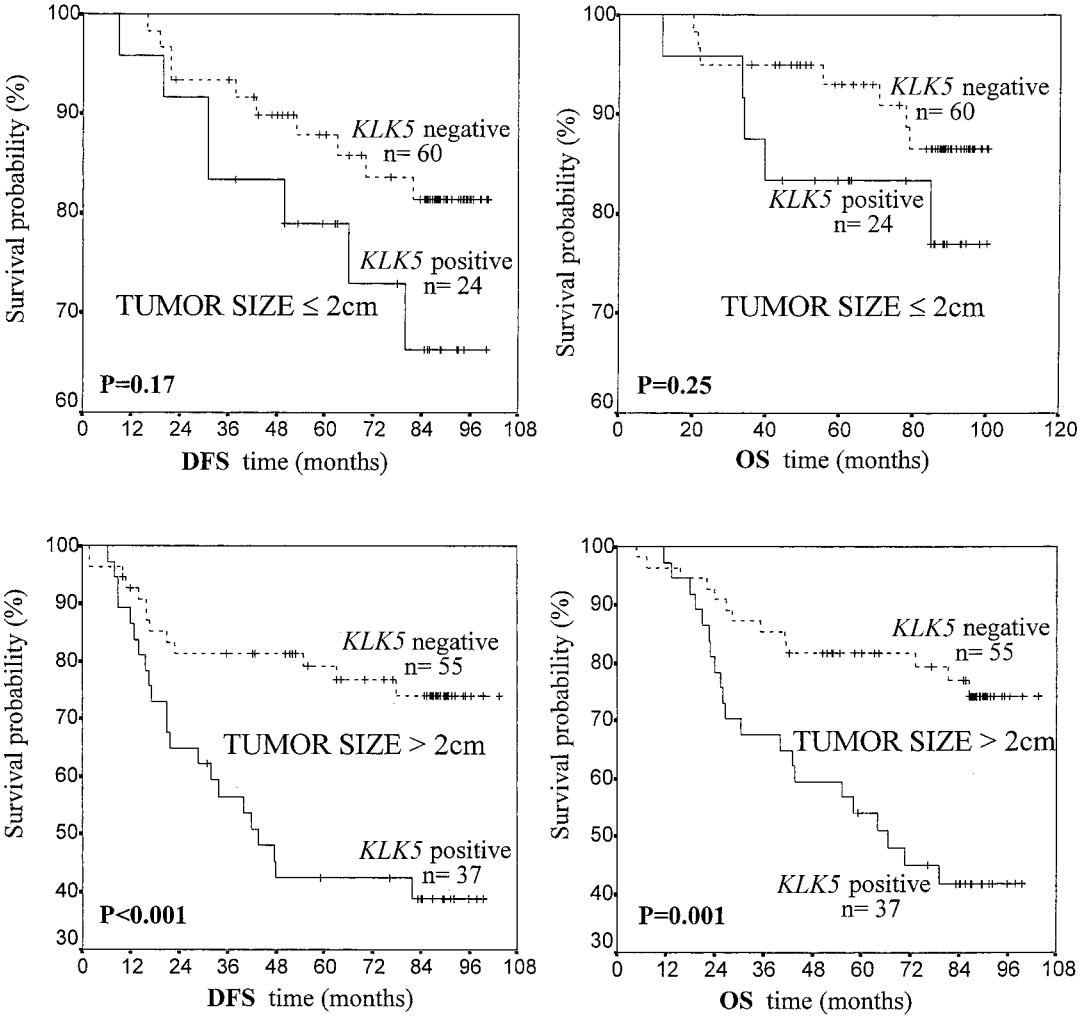

Fig. 5. Kaplan–Meier survival curves for patients with *KLK5*-positive and -negative breast cancers, stratified by tumor size.

shorter DFS and OS in patients with a tumor size ≥2 cm ($P = 0.001$ for both DFS and OS; Fig. 5). Fig. 6 shows that *KLK5* expression was associated with statistically significantly shorter DFS ($P = 0.006$) and OS ($P = 0.004$) in the subgroup of patients with grade I and II tumors and, more weakly, in those with grade III tumors.

Discussion

The selection of therapies for breast cancer is based on grouping patients according to the presence or absence of certain clinical characteristics. The identification of new prognostic/predictive markers will contribute to more optimal patient subgrouping and individualization of treatment *(33)*. The classic prognostic markers for breast cancer, including lymph node status, tumor size, and stage, have prognostic importance *(34)*. Many other potential prognostic/predictive markers has been identified, including steroid receptors, p53, c-erbB2, BCL-2, carcinoembryonic antigen, CA15.3, CA27.29, cathepsin D, and polyadenylate polymerase *(33–37)*. However, only hormone receptor status is recommended by the American Society of Clinical Oncology *(38)* and the College of American Pathologists Consensus Statement *(34)* for routine use. None of the remaining biomarkers has sufficient prognostic/predictive value by itself. Some markers may have applications in particular cases, e.g., HER-2 evaluation is useful for selection of patients for Herceptin therapy *(33)*. Furthermore, there is now growing interest in neural networks, which show the promise of combining weak but independent information from various biomarkers to produce a prognostic/predictive index that is more informative than each biomarker alone *(39)*. In this report, we show that *KLK5* expression has independent prognostic value in breast cancer.

Tumor formation and progression are complex processes involving many genes *(40, 41)*. Like other serine proteases, *KLK5* is a potential candidate that might be involved in stimulating cellular growth, angiogenesis, or degradation of extracellular matrix. Our finding that *KLK5* is a marker of poor prognosis in breast cancer is not

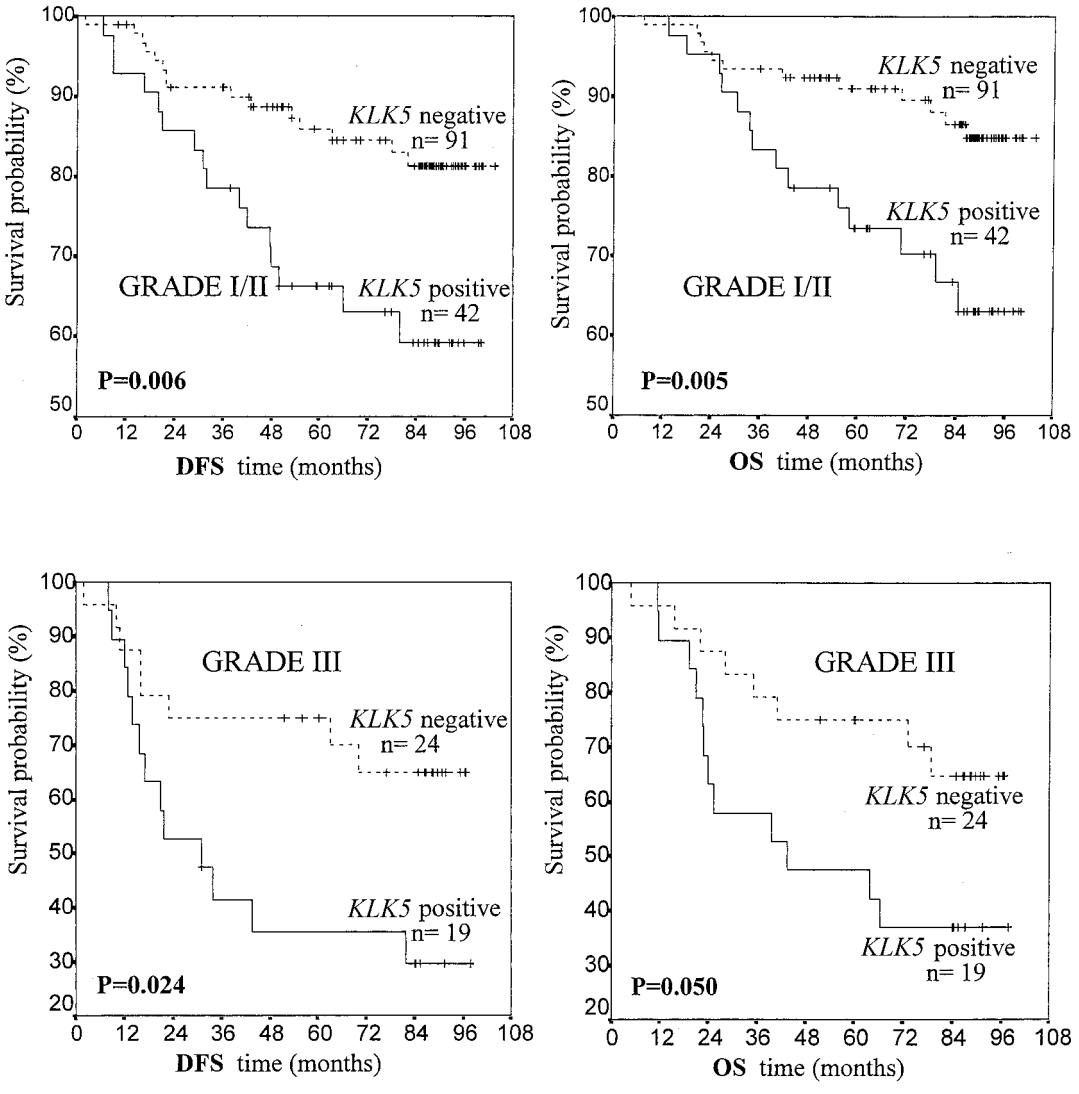

Fig. 6. Kaplan–Meier survival curves for patients with *KLK5*-positive and -negative breast cancers, stratified by tumor grade.

surprising. Protease-mediated degradation of extracellular matrix promotes tumor invasiveness and metastasis, and many other serine proteases, such as plasminogen activator *(9)*, were found to correlate with poor prognosis. Phylogenetic analysis and protein homology analysis indicate that hK5 is most structurally similar to enamel matrix serine proteinase (EMSP; 68% amino acid homology) *(22)*. The function of EMSP is to degrade the enamel matrix proteins during enamel maturation *(42)*. The homology between hK5 and EMSP is intriguing in view of the high propensity of breast cancer to metastasize to bone. Metastatic breast cancer cells alter the normal balance of bone remodeling, which involves interactions among osteoblasts, osteoclasts, and constituents of the bone matrix *(43)*. Although some of the components of bone remodeling that are involved in tumor metastasis have been identified, the process remains ill defined. If the homology between hK5 and EMSP extends to their function, then hK5 should be investigated as a potential contributor to bone manifestations of metastatic breast cancer.

A large and compelling body of evidence implicates estrogens in the pathogenesis of breast cancer *(40)*. Animal studies have demonstrated that estrogens can induce and promote mammary tumors in rodents *(44)*, and the most widely accepted risk factors for breast cancer are related to cumulative estrogen exposure *(45)*. However, the exact role of estrogen in breast cancer remains poorly defined. Recent experimental data suggest that progestins are breast mitogens and, as such, are likely to increase beast cancer risk *(46)*. We have previously demonstrated that *KLK5* is up-regulated by both estrogens and progestins *(22)*. Together with the evidence we provide in this study, that overexpression of *KLK5* is a marker of poor prognosis, we hypothesize that *KLK5* might be involved in the pathway through which

estrogens and progestins promote breast cancer development and progression.

An interesting observation is that the chromosomal region 19q13.3-q13.4 harbors many steroid hormone-regulated genes *(10)* and that *KLK6* (which encodes KLK6, also known as protease M or zyme), the gene most adjacent to *KLK5*, is differentially expressed in breast cancer *(16, 47)*. This observation points to the possibility that this group of genes is involved in a cascade of activation events in cancer.

In conclusion, we studied the quantitative expression of *KLK5* in breast tumors and found that higher *KLK5* expression is associated with decreased DFS and OS in both univariate and multivariate analyses. Larger studies will be necessary to confirm these data and to further establish the clinical value of this biomarker.

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