Expression analysis and study of KLK4 in benign and malignant breast tumours

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Summary

The steroid hormone-regulated gene KLK4 (kallikrein 4) is a new member of the human kallikrein-related peptidase gene family. Up to date, studies report that KLK4 is differentially expressed in many tumours. The purpose of this study was the expression analysis and study of KLK4 in benign and malignant breast tumours. Total RNA was isolated from 16 benign and 45 malignant breast tissue specimens. After testing RNA quality, cDNA was prepared by reverse transcription. Highly sensitive quantitative real-time PCR method for KLK4 mRNA quantification was developed using the SYBR Green chemistry. GAPDH served as a housekeeping gene. Relative quantification analysis was performed using the comparative Cₜ method 2−ΔΔCₜ. KLK4 expression was found to vary in both patients’ cohorts; however, a statistically significant elevation of the KLK4 mRNA levels was observed in malignant compared to benign tumour patients. Low KLK4 expression levels were found in well-differentiated tumours (p=0.011) as well as in stage I (p=0.024) patients. Moreover, a statistically significant (rₛ=-0.318, p=0.035) negative correlation between the KLK4 expression and progesterone receptor staining was observed. ROC and logistic regression analysis recommended that KLK4 gene expression may be used as a new potential biomarker in breast cancer.

Keywords
KLK4, kallikreins, breast cancer, biomarker, prognosis

Introduction

Breast cancer is the most frequent type of malignancy among women. It can also arise in men, though it is less common. According to the American Cancer Society, each year alone in the United States 200,000 women and 1,600 men are affected by invasive breast carcinoma while about 50,000 women die. Current data show that breast cancer is the second leading cause of cancer-related deaths following lung cancer. Therefore, this disease accounts for numerous social, economic and clinical problems and in general, constitutes an important health issue worldwide (1). The rate of incidence is higher in the industrialized countries -where 10% of women are affected- than in the developing ones. It is also remarkable that the descendants of immigrants from the latter countries, who have moved to the former ones, gradually acquire the same rate of incidence as that of the indigenous people (2). Apart from heredity, which is associated with cancer progression (3), age is also a very important risk factor in tumorigenesis (4). Estrogen levels, fibroblast growth factors and malnutrition predispose to mammary neoplasms (5).

The diagnosis of breast cancer, at an initial stage, is vital, since studies have shown that five-year survival rates are reduced from 97%, concerning localized breast cancer, to 79% for regionally spread and 23% for metastatic breast cancer (6). Towards this, biomarkers such as steroid receptors (ER, PR), p53, cerbB₂, CEA, CA15–3, cathepsin D and polyadenylate polyme-rase, including factors involved in different cell functions (proliferation, hormonal regulation, apoptosis, angiogenesis, invasion, metastasis), have been identified as potential predictive markers (7–11). Nevertheless, patients’ survival is substantial to be improved, thus the detection and classification of new tumor markers could aid in the early divulgence of breast cancer’s biological profile.

Human kallikrein-related peptidase genes (tissue kallikreins, KLKs) belong to the serine protease family of proteolytic enzymes and map to chromosome 19q13.4. The 15 members of the human kallikrein-related peptidase gene family of proteolytic enzymes and map to chromosome 19q13.4. The 15 members of...
this family have the same exon-intron organization and are flanked by the ACP7 and two other genes – a cancer-associated (CAG) and a Siglec-9 gene (12–14). They play a key role in the normal development and progress of many functions such as digestion, cellular and humoral immunity, fertilization and embryonic development. In addition, they participate in cellular proliferation, extracellular matrix degradation, regulation of local blood flow, angiogenesis and mitogenesis, implying that they account for both carcinogenesis and metastasis. According to an increasing number of reports, KLKs have been implicated in tumorigenesis; it has been shown that these steroid-regulated genes are part of a hormonal cascade contributing to the promotion and progression of cancer. Moreover, they have an aptitude to play a dual role – promoting and inhibiting – in the pathogenesis of endocrine-related cancers. The differential expression of KLKs is present in hormone-regulated malignancies at both the mRNA and protein levels (15, 16).

Several kallikrein genes are correlated to the mammary gland, as supported by the following data. Prostate-specific antigen (PSA), encoded by the KLK3 gene, is expressed in benign or normal breast tissues as well as in more aggressive malignant tumors, while it is down-regulated in cancerous breast specimens (17, 18). It has also been reported that PSA levels are reduced in the nipple aspirate fluid of women, indicating its involvement, as a high risk factor, in the development of cancer (19). Except for KLK3, another classical gene, KLK2, was observed to be expressed in the female breast tissues at low levels (20). The KLK3 mRNA expression in breast tumors indicates an association with poor prognosis (21). Additionally, KLK6 levels were found to be absent in metastatic sites, in contrast to primary breast cancer in which these are up-regulated (22). The lower KLK7 expression in the early stages of breast cancer development and in progesterone receptor-positive tissues revealed that this gene may be useful as a new potential prognostic biomarker (23). Moreover, KLK9 and KLK15 have been proposed as independent favorable biomarkers in cancerous breast tumors (24, 25). Studies in a proportion of breast cancer patients regarding the serum levels of kallikrein proteins hK3, hK5 and hK14 have established their detection as putative diagnostic biomarkers (26, 27).

The recently identified KLK4 is located between the KLK2 and KLK5. It was previously designated as KLK-L1, prostatease, Enamel Matrix Serine Protease1 (EMSP1), Androgen Related Message1 (ARM 1) and PRSS17 (28, 29). Initially, based on Northern blot analyses, this gene was thought to be expressed exclusively in the male prostate (29); however, real-time polymerase chain reaction (RT-PCR) studies have shown that KLK4 is also detected in mammary glands, testes, uterus, adrenals, colon, brain, thyroid, salivary glands and lungs. KLK4 regulation is controlled by steroid hormones; particularly, its expression is increased by androgens in the prostate cancer-derived cell line, LNCaP; whereas in the breast cancer cells BT-474, it is elevated by both androgens and progesterones (30). In women, most androgens are changed into estrogens by fat and muscle cells. This is the main source of estrogens in a menopausal woman's body when the ovaries cease to produce them (31).

On the basis of its amino acid sequence, the KLK4 gene resembles other kallikrein members that encode PSA (38% identity) and the KLK6 gene (zyme / neurosin / protease M; 38% identity). The mRNA of KLK4 is translated into a 254-amino acid polypeptide whose first 26 NH₂-terminal amino acids comprise the signal peptide and the next four NH₂- acids are the pro-piece. The remaining 224 amino acids compose the active serine protease sequence, since hK4 is a secreted protein (32). The hK4 functions have been proposed to affect tooth maturation as well as pro-PSA and urokinase-type plasminogen activator (uPA) activation (29, 33). Contrary to other kallikreins, the sequence of KLK4 mRNA does not include the exon 1, which encodes the 26 amino acid signal peptide. The absence of exon 1 offers an advantage to hK4 regarding intracellular location and function. In particular, the mRNA transcript is translated into a 205 amino acid hK4 isoform, initially localized to the nucleus (34).

The KLK4 expression has revealed its clinical value in ovarian cancer, considering that increased KLK4 levels have been proposed as an unfavorable prognostic marker and have been correlated with a higher risk of patient relapse and death. Moreover, its expression was found to be significantly elevated in late-stage and more aggressive tumors, mainly in serous ovarian carcinoma (35). It has, also, been mentioned that hK4 is present in secretions of steroid hormone-regulated organs and may be a useful disease biomarker in prostate, ovarian and breast cancers (36).

The above data encouraged us to analyze and study the KLK4 mRNA expression of 61 breast tissue specimens determined by an ultra-sensitive quantitative real-time PCR (qRT-PCR) methodology, using the SYBR Green chemistry. GAPDH was utilized as a reference gene and the LNCaP cell line as a calibrator in qRT-PCR and as positive control in the conventional PCR.

Materials and methods

Study group

Breast tissue specimens were obtained from 45 breast cancer patients and 16 patients without malignancy who underwent surgical treatment at the Oncological Hospital “G. Gennimatas” IKA-ETAM of Athens, between October 2005 and April 2008. A database including individualized information about age, Tumor-Node-Metastasis (TNM) stage, receptor status, size and differentiation grade of tumor and was provided for statistical analysis. Age ranged from 24 to 57 years (median, 37.6 years) for non-cancerous patients and from 35 to 83 years (median, 58.1 years) for those with malignant tumors. In all cases, the diagnosis was performed with the aid of histopathology. Cancerous tissue results revealed primary breast cancer (ductal carcinoma 100%). The histological types of eight fibroadenomas, six fibrocysts, one tubular adenoma and one fibroepitheliosis were proven to be benign alterations. The determination of the grade of tumors was based on the Bloom and Richardson grading system, while TNM classification was used for the clinical stage. The steroid hormone receptors were defined according to the European Organization for Research and Treatment of Cancer (EORTC). Before surgery, patients had not received any hormonal therapy. Our study was performed with respect to the ethical standards of the 1975 Declaration of Helsinki, as revised in 1983.
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Cell lines and culture conditions
The prostate cancer cell line LNCaP was derived from the American Type Culture Collection (ATCC, Rockville, MD, USA). A quantity of 4x10^5 cells/ml was cultured in RPMI 1640 medium (PAA Laboratories, Pasching, Austria) with 10% fetal bovine serum (PAA Laboratories), 100 U/ml penicillin (PAA Laboratories), 0.1 mg/ml streptomycin (PAA Laboratories) and 2 mM L-Glutamine (PAA Laboratories) at 37°C and 5.0% CO_2 in accordance with instructions.

Total RNA extraction and evaluation of mRNA quality
Firstly, frozen tissues (40–90 mg) were pulverized with BioPulverizer (Biopic Inc.) on dry ice. Then, the resulting fine powder was treated with TRI-Regent (Ampion) – following the manufacturer’s instructions – in order to extract total RNA. The homogenized tissues were transferred into 2-ml polypropylene tubes and were stored at –80°C. RNA integrity was assessed using agarose gel electrophoresis, while its concentration and purity were evaluated spectrophotometrically at 260 and 280 nm.

Reverse transcription
The reverse transcription of 1 µg isolated total RNA was performed in a 20 µl reaction mixture with the use of the M-MuLV Reverse Transcriptase RNase H-(Finnzymes) and an oligo(dT) primer. The accrued first-strand cDNAs from each tissue or cell sample were verified through PCR amplification using specific primers for the housekeeping gene GAPDH.

Quantitative real-time polymerase chain reaction (qRT-PCR)
According to the information provided on the database and with the aid of the Primer Express software (Applied Biosystems) two pairs of gene specific primers were designed for GAPDH (endogenous reference gene) (NM 002046) and KLK4 (NM 004917). The upstream primer ATGGGGAAGGTGAAGGTCG and the downstream GGGTATTGATTGGCAACAATATC for GAPDH lead to a 107 bp product; CTGTCAGCCGCACACTGTGGT for KLK4 produced a 144 bp amplicon.

A highly sensitive qRT-PCR method was developed and performed in 96-well plates on an ABI Prism 7500 Thermal Cycler (Applied Biosystems), using the SYBR Green® Dye detection system (Applied Biosystems), GAPDH as an endogenous reference gene, LNCaP cells as a calibrator and gene specific primers. The 10 µl reaction mixture contained 0.2 µl cDNA, 50 nM primers and 2× Power SYBR® Green PCR Master Mix (Applied Biosystems) including AmpliTaq Gold® hot-start DNA polymerase. The thermal protocol consisted of 10 minutes (min) polymerase activation at 95°C, followed by 40 cycles of denaturation at 95°C for 15 seconds and primer annealing and extension at 60°C for 1 min. During the conduction of the expression analysis, each sample was amplified in triplicates, the average C_T value was calculated and a dissociation curve was generated by plotting each of the PCR products against its specific melting temperature (Tm) for verification.

The comparative C_T method 2^-△△C_T was used for performing relative quantification analysis (37). The normalization of the KLK4 mRNA expression between different specimens was implemented through GAPDH amplification using the cell line as a calibrator.

Statistical analysis
Due to the fact that the KLK4 expression levels in benign and malignant breast tissues did not follow a Gaussian distribution, the analysis of the different parameters between the two groups was performed through the non-parametric Mann-Whitney U test. Receiver operating characteristic (ROC) curve was constructed for KLK4 expression levels, by plotting sensitivity versus (1-specificity). The areas under the ROC curves (AUC) were analyzed by the Hanley and McNeil method. The ability of the variables to predict presence of breast cancer was studied using univariate and multivariate unconditional logistic regression analysis. The Spearman correlation coefficient (r_s) was used for assessing the relationship between the different variables. A p-value of <0.05 was considered statistically significant.
Results

**KLK4 mRNA expression analysis in benign and malignant breast tumors**

The expression of *KLK4* was detected in the benign as well as in the malignant mammary specimens. The real-time PCR (Fig. 1) method revealed that mRNA levels were higher in cancer tumors than in non-cancerous. The *KLK4* expression profiles ranged between 0.5 – 373 *KLK4* copies/10^3 GAPDH mRNA copies (c/Kc) in the cancerous samples and 0.5 – 17 c/Kc in the benign. According to the average (mean) and the median (50th percentile) expression of the *KLK4* gene presented an about 21– and 40-fold increased value in cancer tumors (mean ± standard error of the mean [SEM]: 64.1 ± 13.9 c/Kc, median: 20.0 c/Kc) compared to the value of the non-malignant tissues (mean ± SEM: 3.2 ± 1.1 c/Kc, median: 0.5 c/Kc) (Table 1). The distribution of *KLK4* mRNA levels in the two breast patients’ cohorts is depicted in Figure 2.

We performed ROC and logistic regression analyses in order to evaluate the discriminative potential of *KLK4* mRNA expression between non cancer and breast cancer patients. The ROC curve (Fig. 3) depicts the significant value of *KLK4* in distinguishing malignant from benign tissues (area under the curve [AUC]=0.81; 95% confidence interval [CI]=0.70–0.91; p<0.001). The univariate logistic regression analysis was developed to estimate the differential diagnostic value of the log_{10} *KLK4* mRNA expression in both patients’ cohorts. It was demonstrated that patients with increased *KLK4* levels had a statistically significant (p=0.015) higher risk to suffer from mammary neoplasms (crude odds ratio [OR]: 1.11; 95% CI= 1.02–1.21). With regards to the multivariate analysis, the logistic regression models were adjusted to log_{10} *KLK4* expression profiles, tumor size and patients’ age. Taking this finding into account, it is suggested that *KLK4* is an independent and unfavorable predictor of breast cancer patients (crude OR: 1.21; 95% CI= 1.02–1.41; p=0.029) (Table 2).

**KLK4 mRNA expression in relation to histopathological parameters of breast cancer**

The study of *KLK4* mRNA levels divulged a statistically significant (p=0.011) increase of this gene expression in women patients with advanced grade breast cancer. Grade I patients showed low expression levels of *KLK4* mRNA, whereas those with grade II displayed higher and even more elevated was indicated in grade III patients. These data are depicted in Figure 4, which exhibits the *KLK4* mRNA expression in malignant tumors of different grade. Moreover, it is clearly illustrated in Figure 5 the statistically significant (p=0.024) relationship of *KLK4* mRNA copies with TNM stage.

Additionally, the correlation between *KLK4* levels and progesterone receptors (PR) was verified through the Spearman correlation coefficient. Statistically significant negative correlation (r_s= –0.318, p=0.035) was observed between *KLK4* mRNA expression and the PR status of the breast cancer tissue specimens (Fig. 6). However, the benign samples showed statistically insignificant correlation.

**Discussion**

Taking into consideration the heterogeneous nature of mammary carcinomas, the purpose of researchers is to discover the best or a panel of biomarkers so as to predict disease progression and relapse. Classical histopathological parameters (e.g. TNM stage, histological type, grade, hormone receptor status, etc) have been proven of prognostic importance. However, predictive value has solely been attributed to the hormone receptor status of patients who are likely to respond to therapy. Hormone receptors (mainly ER and PR) are the only markers recommended for routine use by the College of American Pathologist Consensus Statement (38). Up to now, biomarkers such as e.g. p53, bcl-2, HER-2, c-myc, VEGF, uPA represent prognostic factors for breast cancer. Nevertheless, only few of them have predictive value. It is known, that HER-2 is a predictive factor, especially in patients following Herceptin therapy. Additionally, according to latest

### Table 1: Descriptive statistics of *KLK4* expression in breast tissues.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SEM</th>
<th>Range</th>
<th>Quartiles (median)</th>
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<tr>
<td><strong>Cancer (N = 45)</strong>*&lt;br&gt;KLK4 (c/Kc)&lt;br&gt;<strong>Non Cancer (N= 16)</strong>&lt;br&gt;KLK4 (c/Kc)</td>
<td>64.1 ± 13.9&lt;br&gt;3.2 ± 1.1</td>
<td>0.5 – 373.0&lt;br&gt;0.5 – 17.0</td>
<td>50</td>
</tr>
</tbody>
</table>
| **Covariant** | **Univariate analysis** | **Multivariate analysis**<br>Crude OR | 95% CI | P-value<br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br><br>
studies, the urokinase-type plasminogen activator (uPA) successfully predicts advanced breast cancer (39–43).

The circulating levels of estrogens, androgens and their precursors have been connected with breast neoplasm, while, the increased levels of precursors and metabolites of estradiol comprise another strong risk factor (44). The presence of androgen receptors (AR) fluctuates from 70–90% for primary breast carcinoma and 70–80% for breast cancer metastases (45). Previous studies have unveiled AR mutations in breast cancer (46). These data recommend that AR-mediated pathways may be of close biological and clinical relevance to breast cancer. Also, AR possibly plays the role of a ligand-activated transcriptional factor, which monitors gene expression during cancer progression. Moreover, animal studies have suggested that androgen-regulated genes may interact with other transcriptional regulators in cell growth modulation, strengthening the possibility of cross-talking with other growth pathways (47). Thus, the identification of genes regulated by androgens, such as KLK4, could contribute to breast cancer treatment and a new therapeutic strategy in metastatic diseases, where ARs are exclusively expressed. Consequently, KLK4 seems to be a potential novel biomarker for breast cancer, fact supported by the plethora of similarities it
shares with other KLKs related to breast malignancies and also
by the steroid hormone regulation and the detection of ARs in
breast tumors.

In the current study, we evaluated the mRNA expression of
the human kallikrein 4 gene, KLK4, in benign and malignant
breast tumors so as to further estimate its prognostic value and its
potential use as a diagnostic biomarker. The present study is the
first to investigate KLK4 expression in a cohort of human breast
tumors and we were based on existing data regarding the breast
cancer line BT-474, already identified as expressing KLK4 at the
mRNA level.

KLK4 mRNA levels were detected in both tissues’ cohorts.
The cancerous specimens presented a statistically significant
more frequent and higher expression (mean ± SEM: 64.1 ± 13.9
c/Kc, median: 20.0 c/Kc) compared to the benign (mean ± SEM:
3.2 ± 1.1 c/Kc, median: 0.5 c/Kc). The elevated KLK4 ex-
pression was associated with breast cancer patients and implied
a potential involvement of the enzyme in the pathogenesis of
cancer. Furthermore, it is considered possible that the resulting
augmentative synthesis of hK4, which then participates in the
metabolic biochemical reactions, could modify the breast cell
phenotype, promoting cancer progression. Additionally, kalli-
rein 4 may be one of the adhesion molecules interacting with
breast cells, stroma and surrounding normal tissues. The higher
average and median value in malignant specimens is consistent
with the fact that KLK4 is a steroid-regulated gene. Regarding
the ROC curve (p<0.01) and logistic regression analysis, KLK4
illustrated significant potential as an independent factor in the
discrimination of the two breast abnormalities. Besides, the
evaluation of the KLK4 mRNA expression revealed a statis-
tically significant negative correlation (r_{s} = -0.318, p=0.035)
with PR staining only in the cancerous samples. The absence of
PRs suggests their poor prognostic value as a favorable indicator
of hormone-related breast cancer and confirms that KLK4 may
indeed serve as a potential unfavorable biomarker. However, in
the present study, we did observe a reversible biochemical pro-
cess between gene expression and PRs during the development
and spreading of cancer cells. This phenomenon could be at-
tributed either to the assumption that PRs are recreated during
the transitional stage, something that has still remained unde-
tected, or to some other unknown interim biochemical inter-
actions which generate a differentiated biological level.

In connection with our data, a statistically significant
(p=0.011) elevation of the KLK4 mRNA expression was ob-
served in advanced grade breast cancer patients. This positive
correlation between KLK4 levels and tumor grade divulges the
prognostic value of this gene. Grade I patients showed consider-
ably lower KLK4 mRNA profiles, which appeared to be in-
creased in grade II and were even significantly higher in grade III
patients. Moreover, a statistically significant (p=0.024) associ-
ation was found between the gene expression copies and TNM
stage. KLK4 elevated levels were more frequently detected in
higher grade tumors as well as in advanced stage patients, indi-
cating its poor prognostic value, making it a putative unfavorable
biomarker for breast cancer. Respectively, the overexpression of
KLK4 correlates with an enhanced hazard for metastasis. It is
widely acceptable that KLKs are considered responsible for
extracellular function, so we estimate that KLK4 may have a
negative impact on the extracellular matrix causing the degrada-
tion of proteins. Consequently, this function of KLK4 facilitates
the penetration of cancerous cells into adjacent breast stroma
and the corresponding blood vessels, promoting angiogenesis, tumor
invasiveness and metastasis.

At this point, it is important to discuss the diverse role of
KLK4 and KLK3 (PSA) in breast cancer. It was mentioned above
that hK4 activates PSA via the degradation of the pro-PSA. In
contrast to KLK4, PSA expression is higher in benign or normal
tissues than in malignant ones (17, 18). The decreased PSA ex-
pression in the cancer tissues is provoked by a potential cell-react-
ive mechanism. With this mechanism, the cells counterbalance
the possibly stronger pro-PSA activation triggered by the aug-
mentative KLK4 levels through the higher hK4 formation. In
case of any deregulation, the higher PSA activation facilitates
invasiveness of surrounding tissues, metastasis and development
of more aggressive phenotypes. This fact is supported by the
elevated PSA expression in the more aggressive compared to the
early grade breast tumors. It could, also, be hypothesized that this
inverse behavior of the two genes illustrates a two-directional
hormonal interdependence, which can modify the molecular
structure of cells. Their interaction gradually becomes weak
upon the uncontrolled spreading of breast cancer.

In conclusion, our results reveal that KLK4 is definitely ex-
pressed in breast tumors. Elevated expression is associated with
advanced grade and more aggressive tumor behavior, as well as
with reduced progesterone receptor staining. Additional bio-
chemical and molecular studies are recommended in order to
pinpoint the exact location of hormonal action and gene ex-
pression modification. We believe that KLK4 may constitute a
new independent potential biomarker of poor prognosis in breast
cancer, though basic and clinical studies would further delineate
its predictive and clinical value.
References