Clinical Chemistry 45:10
1774–1780 (1999)

Enzymes and Protein Markers

Human Glandular Kallikrein in Breast Milk, Amniotic Fluid, and Breast Cyst Fluid

Angeliki Magklara,[1] Andreas Scorilas,[1] Carlos López-Otín,[2] Francisco Vizoso,[3] Alvaro Ruibal,[4] and Eleftherios P. Diamandis[1*]

Background: **Human glandular kallikrein (hK2) belongs to the serine protease family of enzymes and has high sequence homology with prostate-specific antigen (PSA). The physiological role of hK2 has not as yet been determined, but there is evidence that it can regulate the proteolytic activity of PSA through processing and activating pro-PSA, an inactive precursor. Thus, it is conceivable that these two secreted proteins may coexist in biological fluids. Currently, hK2 is considered an androgen-regulated and prostate-specific protein. Recently, it has been demonstrated that hK2 is expressed in the breast cancer cell line T-47D after stimulation by steroid hormones, and we reported that hK2 can be detected in a subset of breast tumor extracts. These data suggest that hK2 may be expressed in tissues other than the prostate, such as those in which PSA has already been detected. Because hK2 is a secreted protein, it may be present in various biological fluids.**

Methods: **We analyzed milk samples from lactating women, amniotic fluid from pregnant women, and breast cyst fluid from patients with gross breast cystic disease, using a highly sensitive and specific immunoassay for hK2.**

Results: **hK2 was present in all three biological fluids. We suggest that the female breast may produce hK2 and provide evidence that hK2 may have value as an additional marker for the discrimination between type I and type II breast cysts.**

Conclusions: **The female breast produces hK2 in addition to PSA. More studies are necessary to establish the role of this kallikrein in nondiseased breast, gross breast cystic disease, and breast cancer.**

The human kallikrein gene family is a subgroup of a large family of serine proteases and currently includes three members: tissue kallikrein, human glandular kallikrein (hK2),[5] and prostate-specific antigen (PSA or hK3) *(1, 2)*. PSA is a 33-kDa protease with chymotrypsin-like activity *(3)* and was, until recently, thought to be produced exclusively by epithelial cells of the prostate gland *(4, 5)*. PSA is the most reliable tumor marker available, and it is widely used for prostate cancer diagnosis and management *(6)*. It is now well documented that PSA is also produced by extraprostatic sources *(7, 8)*. Several studies have demonstrated the presence of PSA in the periurethral and perianal glands *(9–11)*; PSA immunoreactivity has also been detected in 30–60% of female breast tumor cytosolic extracts *(12, 13)* and in breast cancer cell lines after stimulation by steroid hormones *(14)*. Furthermore, PSA was found in healthy endometrial tissue *(15)*, in the milk of lactating women *(16)*, in breast cyst fluid *(17)*, and in amniotic fluid *(18, 19)*.

The hK2 gene was identified in 1987 by molecular techniques *(20)*. Shortly afterward, it was demonstrated that the gene is expressed in the prostate *(21)* and the complete cDNA for hK2 was elucidated *(22)*. The deduced amino acid sequence revealed a mature hK2 protein containing 237 amino acids and an approximately 80% sequence identity to PSA. In contrast to PSA, enzyme specificity for hK2 is trypsin-like, with selective cleavage at arginine residues as previously predicted by its amino acid sequence *(23)*.

The striking homology of hK2 to PSA, combined with

[1] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5, and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada M5G 1L5.

[2] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Oviedo, Oviedo, Spain.

[3] Department of Surgery, Hospital de Jove, Gijon, Spain.

[4] Fundacion Tejerina, Madrid, Spain.

*Address correspondence to this author at: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada M5G 1X5. Fax 416-586-8628; e-mail ediamandis@mtsinai.on.ca.

Received March 11, 1999; accepted July 7, 1999.

[5] Nonstandard abbreviations: hK2, human glandular kallikrein; PSA, prostate-specific antigen; BCF, breast cyst fluid; and GCBD, gross cystic breast disease.

the prostate localization of both kallikreins as well as the androgen regulation of both genes, suggested that they may have a close physiological relationship. This is strongly supported by recent findings that hK2 cleaves pro-PSA (244 residues) to generate enzymatically active PSA (237 residues) *(24–26)*. Recombinant hK2, which recently was expressed and purified *(27)*, monoclonal antibodies, and immunological assays developed for hK2 with no cross-reactivity to PSA *(28)* allow direct and reliable studies on this kallikrein.

Considering the potential role of hK2 as a physiological regulator of PSA, we speculated that hK2 may be present, along with PSA, in tissues and biological fluids of nonprostatic origin. Very recent studies identifying the expression of hK2 in the breast carcinoma cell line T-47D after stimulation by steroid hormones *(29)* and in breast tumor extracts and nipple aspirate fluids *(30)* support this hypothesis. Therefore, we analyzed milk from lactating women, breast cyst fluid (BCF) from women with gross cystic breast disease (GCBD), and amniotic fluid with a highly sensitive immunoassay for hK2 that is devoid of cross-reactivity from PSA.

Materials and Methods

SAMPLES

Breast milk was collected from women post delivery and was stored at −20 °C until analysis. The samples were thawed and centrifuged at 12 000*g* for 10 min; after removal of the top lipid layer, the samples were assayed for PSA and hK2. The amniotic fluids analyzed were leftovers from screening programs for fetal abnormalities. They were kept frozen at −70 °C until analysis. The BCFs were obtained by needle aspiration from 104 women with GCBD. The samples were centrifuged at 12 000*g* for 10 min, and the supernatants were stored at −70 °C until analysis.

Our study was approved by the Ethics Committee of Mount Sinai Hospital, Toronto, Ontario, Canada.

IMMUNOASSAYS

The total PSA concentration in all samples was measured by an ultrasensitive time-resolved immunofluorometric assay, as described elsewhere *(31)*. The PSA assay has a detection limit of 0.001 μg/L and has no detectable cross-reactivity to hK2, as previously established *(32)*. A new, time-resolved immunofluorometric assay, recently developed in our laboratory, was used to measure hK2 concentrations *(32)*. Briefly, the hK2 assay uses a mouse monoclonal anti-hK2 capture antibody (supplied by Hybritech Inc., San Diego, CA, and raised against recombinant hK2) immobilized onto polystyrene microtitration wells at a concentration of 4 mg/L in 100 μL of coating buffer per well (total of 400 ng of capture antibody per well). After overnight incubation, the plates were washed and the samples were applied undiluted at a volume of 100 μL, followed immediately by the addition of 50 μL of assay buffer, and incubated at room temperature for 1 h with shaking. After the wells were washed, 100 μL of biotinylated mouse monoclonal detection antibody (code 8311; Diagnostic Systems Laboratories) was added to each well and incubated for 1 h. Wells were washed, and alkaline phosphatase-labeled streptavidin was added, incubated for 15 min, and washed again. The alkaline phosphatase activity was measured by adding the substrate diflunisal phosphate, incubating for 10 min, and then adding a Tb^{3+}-EDTA developing solution. The fluorescence was measured on a Cyberfluor 615 Immunoanalyzer (MDS Nordion). The hK2 assay has a detection limit of 0.006 μg/L and has <0.2% cross-reactivity to PSA *(32)*.

Results

PSA AND hK2 IN MILK

We analyzed 44 milk samples (1 sample per lactating woman), for PSA and 41 samples for hK2 (3 samples were depleted). The time of collection post delivery, the sex of the newborn, and the age of the mother were available for all samples; the statistics for the samples are shown in Table 1.

All but five milk samples contained detectable amounts of PSA, with a median concentration of 0.084 μg/L. Four samples had relatively high concentrations: 11.8, 24, 67, and 111 μg/L. The distribution of PSA concentrations in the milk samples relative to the number of days after delivery, to the sex of the newborn, and to the age of the mother is presented in Fig. 1. Statistical analysis indicated that there was a negative correlation [Spearman correlation coefficient (r_s) = −0.58; P <0.001] between PSA concentration and postdelivery time, which is in accordance with our previous findings *(16)*. However, we found no association between milk PSA and either sex of newborn (Mann–Whitney nonparametric *U*-test; Fig. 1B), or maternal age (Spearman correlation; Fig. 1C).

hK2 was detected in 35 of the 41 samples analyzed. The median concentration was 0.021 μg/L, and the highest concentrations observed were ~2.2–2.7 μg/L (in three samples). In 23 of the samples, the hK2 concentrations were 1.5- to 100-fold lower than the corresponding PSA concentrations. In seven samples, the hK2 concentration was higher than PSA (1.5- to 29-fold), whereas in three samples, the two kallikreins were present in approximately equal concentrations. Finally, in two samples with undetectable PSA, low concentrations of hK2, close to the detection limit of our assay, were measured. The distri-

Table 1. Analysis of PSA and hK2 in milk of lactating women.

Variable	Number of samples	Mean (SD)	Median	Range
PSA, μg/L	44	5.2 (19.5)	0.084	0–111
hK2, μg/L	41	0.35 (0.66)	0.021	0–2.67
Days post-delivery	44	22 (46)	5	2–243
Mother's age, years	44	31 (4.1)	31	20–39

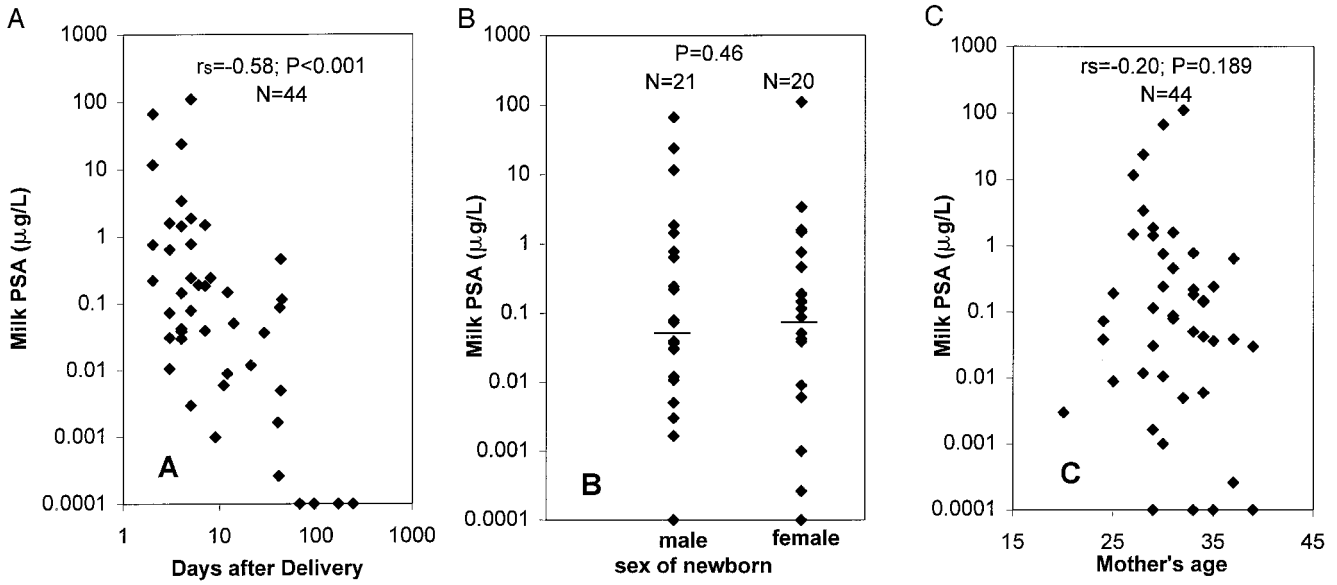

Fig. 1. PSA concentrations in 44 milk samples obtained at various times post delivery (*A*) and plotted according to the sex of newborn (*B*) and maternal age (*C*).

In *A* and *C*, the Spearman correlation coefficient (r_s) was used to determine the correlation between the two variables. In *B*, the *horizontal lines* indicate the median PSA concentration for each gender; *P* was determined by Mann–Whitney *U*-test. Three twin pregnancies were excluded from this analysis.

bution of hK2 concentrations according to the number of days after delivery, to the sex of the newborn, and to the age of the mother is presented in Fig. 2. We found no statistically significant correlation between hK2 concentration and either the age of the mother or the sex of the newborn, as is the case for PSA. However, hK2 concentration correlates negatively ($r_s = -0.48$; $P < 0.001$) with postdelivery time, suggesting that similar to PSA, hK2 concentrations in the milk of lactating women decline with time after delivery. We found a strong positive correlation between PSA and hK2 concentrations in milk ($r_s = 0.79$; $P < 0.001$; Fig. 3).

PSA and hK2 in amniotic fluid

Amniotic fluid samples (n = 116) from different gestational ages (11–25 weeks of gestation as well as terminal) were assayed for PSA and hK2. PSA was detected in all samples, with concentrations of 0.001–2 µg/L; one sample

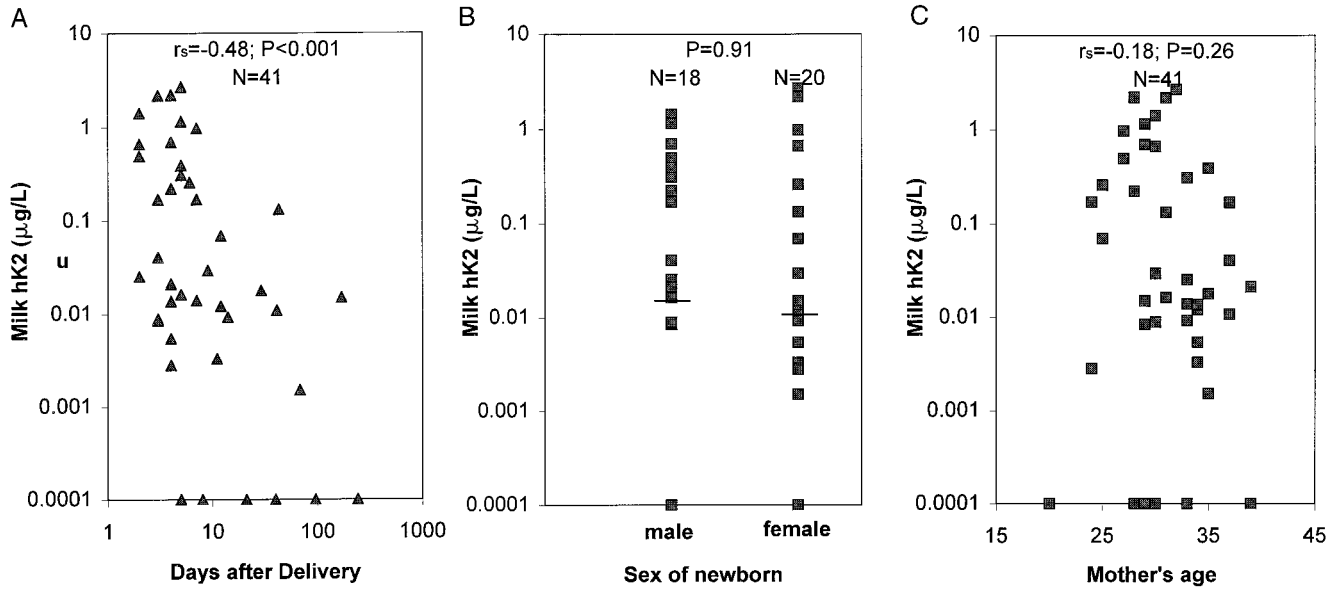

Fig. 2. hK2 concentrations in 41 milk samples obtained at various times post delivery (*A*) and plotted according to the sex of newborn (*B*) and maternal age (*C*).

In *A* and *C*, the Spearman correlation coefficient (r_s) was used to determine the correlation between the two variables. In *B*, the *horizontal lines* indicate the median hK2 concentration for each gender; *P* was determined by Mann–Whitney *U*-test. Three twin pregnancies were excluded from this analysis.

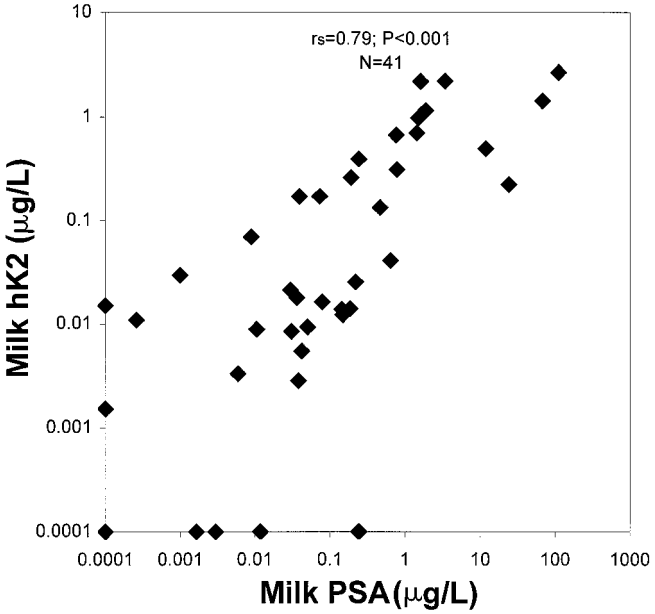

Fig. 3. Correlation between PSA and hK2 concentrations in 41 milk samples.

Spearman correlation coefficient (r_s) was used to determine the correlation between the two variables. The regression equation is: $y = 0.021x + 0.236$, where y is hK2, and x is PSA.

had a concentration of 185 μg/L. The 10th, 25th, 50th, 75th, and 90th percentiles for the PSA concentrations are shown in Fig. 4. There was a positive correlation between PSA and gestational age (data not shown), as reported previously *(18, 19)*.

hK2 was detected in 31 (27%) of the 116 samples examined. The highest concentration observed was 0.38 μg/L in the sample that had the highest PSA concentration (185 μg/L). Because most of the amniotic fluids had

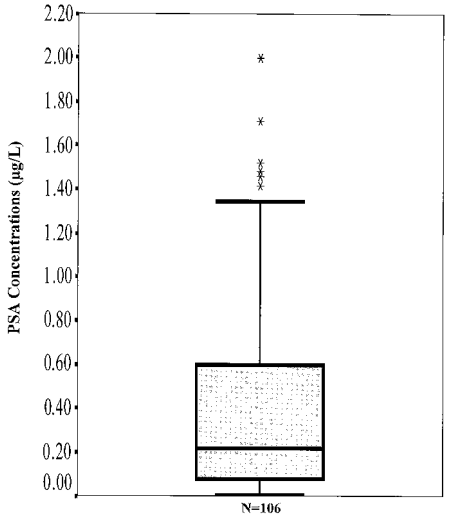

Fig. 4. Distribution of PSA concentrations in amniotic fluid.

The *box and whiskers* display the 10th, 25th, 50th, 75th, and 90th percentiles. *, outliers.

Table 2. Analysis of PSA in BCF samples.

	PSA, μg/L		
	All samples (n = 103)	**Type I cysts (n = 85)**	**Type II cysts (n = 18)**
Percentile			
0	0.001	0.001	0.001
5	0.003	0.004	0.002
25	0.020	0.021	0.017
50	0.076	0.075	0.076
75	0.564	0.573	0.474
95	14.068	13.361	11.763
100	42.674	42.674	16.705
Mean ± SE	1.70 ± 0.56	1.75 ± 0.65[a]	1.46 ± 0.94[a]

[a] There was no difference in PSA concentration between type I and type II cysts ($P = 0.65$ by Mann–Whitney *U*-test).

no detectable hK2, no statistical analysis was deemed necessary.

PSA and hK2 in BCF

According to the K^+/Na^+ ratio, the 103 samples were distributed into two groups: type I cysts (with ratio ≥1.5) and type II cysts (with ratio <1.5). Eighty-five BCF samples (82%) were found to belong to type I cysts and 18 (18%) to type II cysts.

The PSA concentrations ranged from below the detection limit of our assay in two samples to 42.7 μg/L. The frequency distribution of values along with their means and medians in all fluids as well as in type I (apocrine) and in type II (flattened) cysts separately are shown in Table 2. Because the distribution of PSA concentrations was not gaussian, the analysis of differences between the two cyst types was performed with the nonparametric Mann–Whitney *U*-test. There was no statistically significant difference in PSA concentrations between the two groups ($P = 0.65$).

The hK2 concentration in cyst fluids ranged from below the detection limit, in eight samples, to 21.2 μg/L, with a mean of 1.03 ± 0.26 μg/L. Using the Mann–Whitney *U*-test, we found a statistically significant differ-

Table 3. Analysis of hK2 in BCF samples.

	hK2, μg/L		
	All samples (n = 103)	**Type I cysts (n = 85)**	**Type II cysts (n = 18)**
Percentile			
0	0.002	0.003	0.002
5	0.004	0.005	0.003
25	0.019	0.021	0.010
50	0.101	0.136	0.017
75	0.715	0.773	0.237
95	6.120	6.309	2.045
100	21.201	21.201	2.783
Mean ± SE	1.03 ± 0.26	1.19 ± 0.313[a]	0.27 ± 0.16[a]

[a] There was a statistically significant difference in hK2 concentration in type I vs type II cysts ($P = 0.02$ by Mann–Whitney *U*-test).

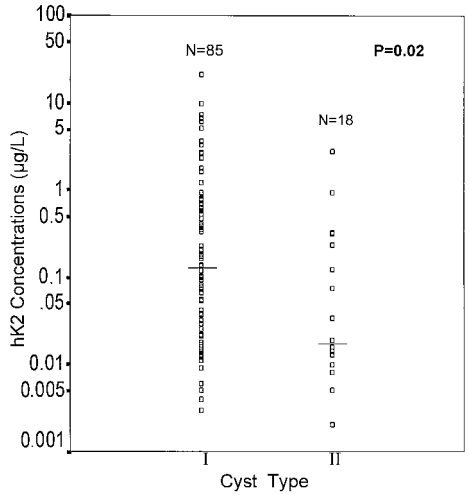

Fig. 5. Relationship between hK2 concentration and cyst type.
The hK2 concentrations in 103 breast fluids are plotted according to cyst type. *P* was determined by the Mann–Whitney *U*-test.

ence between the hK2 values in the two cyst types (P = 0.02; Table 3 and Fig. 5).

No correlation was observed between either PSA or hK2 and the K^+/Na^+ ratio (data not shown). However, there was a significant correlation between PSA and hK2 concentrations when all samples were considered (r_s = 0.37; P <0.001) and when type I cysts were considered separately (r_s = 0.39; P <0.001). There was no significant correlation between PSA and hK2 concentrations in type II cysts (r_s =0.20; P = 0.43; Fig. 6).

Discussion

hK2 is a serine protease closely related to PSA. hK2 is primarily, if not exclusively, expressed in the prostate gland. It recently has been reported that the breast carcinoma cell line T-47D can produce and secrete hK2 after steroid hormone stimulation *(29)*. This cell line was reported previously to produce substantial amounts of PSA when stimulated by steroids *(14)*. hK2 has also been detected in breast tumor extracts and in nipple aspirate fluid, a common breast secretion *(30)*. These findings suggest that the hK2 gene is expressed in breast tissue.

To verify this hypothesis, we first examined milk from lactating women. The concentrations of circulating steroids are increased during pregnancy, and they could induce the production of this kallikrein. Yu and Diamandis *(16)* have shown that PSA is present in considerable concentrations in human milk. We found that the vast majority of the milk samples examined contained variable concentrations of hK2, ranging from <0.006 to 2.67 μg/L. Because hK2 is undetectable in serum from healthy females *(32)*, we can rule out the possibility of hK2 diffusion from the circulation into the milk and assume that it is secreted by breast epithelial cells. There was no significant correlation between the hK2 concentration in milk and the age of the mother or the gender of the newborn, but we found a declining trend with postdelivery time (Fig. 2) for both PSA and hK2 concentrations *(16)*. This is likely attributable to the decrease of steroid hormone concentrations in maternal serum after removal of the placenta. The physiological role of hK2 in human breast milk currently is unknown. hK2 may serve as regulator of PSA activity, for which an involvement in the growth of healthy breast tissue has been proposed *(16)*, or it may have a physiological activity of its own because it has been shown to cleave insulin-like growth factor-binding proteins even more rapidly than PSA *(33)*.

The presence of hK2 in amniotic fluid was verified in only a small proportion of samples, and the concentrations were relatively low. This is a notable difference

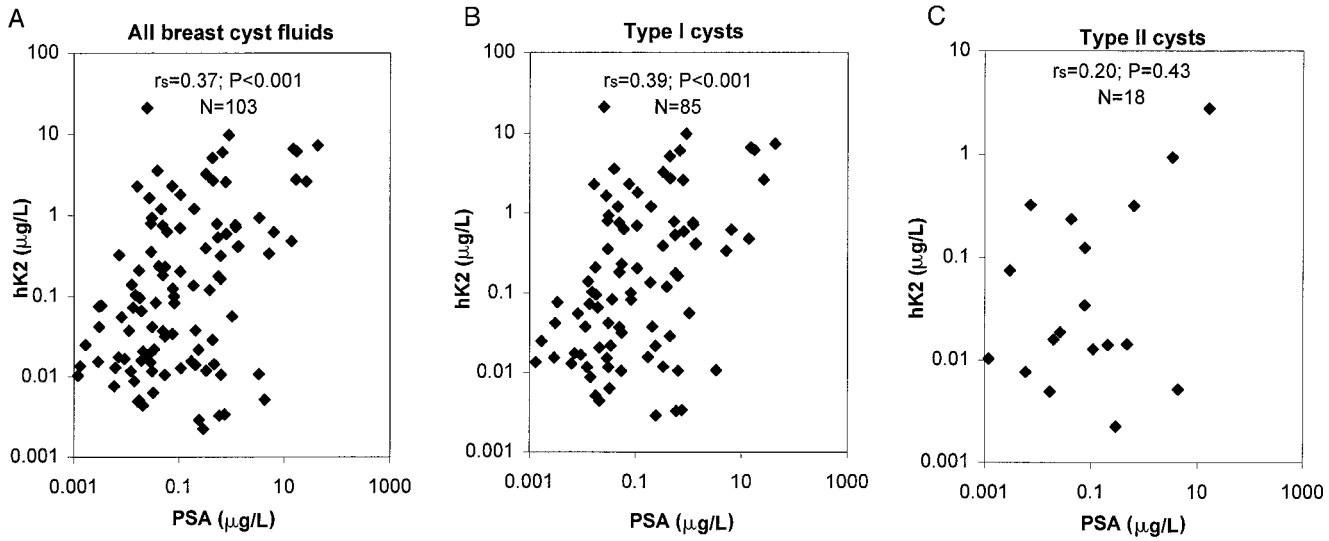

Fig. 6. Correlation between PSA and hK2 concentrations in all BCFs (*A*), in type I cysts (*B*), and in type II cysts (*C*).
Spearman correlation coefficient (r_s) was used to determine the correlation between the two variables.

between PSA and hK2, which seem to coexist in all other breast secretions. The source of hK2, as well as that of PSA, in amniotic fluid remains unclear.

In this study, we report for the first time that women with GCBD produce and accumulate in the BCF relatively large amounts of hK2. This finding, in conjunction with our findings of hK2 present in nipple aspirate fluid and milk, further suggests that the breast epithelium secretes hK2, an enzyme that was originally defined as prostate specific. Although breast cysts by themselves are not considered precancerous lesions, they have been associated with a higher risk for developing breast cancer *(34, 35)*. Taking into account that benign and malignant breast diseases could have some common pathogenetic factors, the analysis of BCF, in which the metabolic products of the cells lining the cysts accumulate, could provide additional information about the environment of the breast tissue, regarding focal lesions prone to malignant transformation. Examination of human BCF reveals two major subgroups of cysts: type I, with a high K^+/Na^+ ratio and large amounts of dehydroepiandrosterone sulfate, lined with apocrine epithelium and resembling the intracellular microenvironment; and type II, with a low K^+/Na^+ ratio and lower amounts of dehydroepiandrosterone sulfate, lined with flattened epithelium. A third population with intermediate K^+ and Na^+ concentrations has also been reported *(36)*.

In the present study, no significant difference was found in the mean values of PSA concentrations between the two cyst subgroups, which is in accordance with the data of Lai et al. *(37)* and Filella et al. *(38)*; nevertheless, we and others have reported that there is a small difference in the mean PSA concentrations in the two types of cysts *(39, 40)*. On the other hand, hK2 not only shows a wide range of concentrations in BCF (Table 3) but a differential distribution of values as well, which is consistent with the presence of distinct subpopulations of cysts. The fact that hK2 is detected at higher concentrations in type I cysts (Fig. 5) suggests that the epithelium lining this group of cysts may be especially active in hK2 production because of its high content of steroid hormones, mainly androgens. This observation is interesting because it might provide some clues to understanding the mechanisms that determine the high risk for breast cancer associated with these cysts. Activation of this proteolytic enzyme in the apocrine microenvironment, probably in a similar manner to that proposed for hK2 activation in breast cancer cells in culture *(29)*, could initiate events leading to proliferative breast diseases. hK2 may be an additional marker for discriminating between the two types of cysts, for monitoring GCBD, and for the prognosis of the disease (depending on the cyst subtype). Furthermore, human breast cancer cells secrete and have membrane receptors for insulin-like growth factor-I, suggesting that mitogenesis, cell proliferation, and tumor growth may result from an autocrine or paracrine effect of insulin-like growth factor-I *(41, 42)*. Wang et al. *(43)* have identified insulin-like growth factor-I in BCF. hK2 has been shown to cleave insulin-like growth factor-binding proteins, which suggests a role similar to that proposed for PSA *(33)*. The significant correlation between hK2 and PSA in all the samples tested suggests that the factor(s) that control their production and/or the mechanism by which they enter the BCF are similar.

In summary, considering the fact that the inactive precursor of PSA, pro-PSA, is rapidly converted to active PSA by hK2, suggesting an important in vivo regulatory function by hK2 on PSA activity, we hypothesized that hK2 may exist in biological fluids in which PSA has already been detected, such as milk, BCF, and amniotic fluid. We report here the consistent presence of hK2 in breast milk and BCF at concentrations that strongly support production from the breast tissue, possibly after steroid stimulation. This finding is in accordance with the notion that PSA and hK2 coexist in biological tissues, correlating strongly and possibly acting synergistically. The PSA and hK2 concentrations in breast secretions are not always proportionate, as is the case for seminal plasma *(32)*. However, the PSA/hK2 ratio in milk was 1.5–100, with a median of 2, whereas in BCF the median was 1; in seminal plasma, hK2 is present at concentrations 100- to 500-fold lower than PSA *(32)*. It appears that both kallikreins are compartmentalized in breast and prostate tissue and secretions, but their concentrations in women do not differ as greatly as in men. More studies are needed to evaluate whether hK2 has some prognostic/diagnostic value in benign and malignant breast disease. Clearly, the concurrent expression of PSA and hK2 in the female breast is of high interest and their physiological role in this tissue needs further investigation.

We thank Cathy Buller for collecting milk samples and Herb Yu for assistance in the statistical analysis. We are grateful to Dr. R. Wolfert from Hybritech Inc., San Diego, CA, for providing one hK2 antibody.

References

1. Clements JA. Current perspectives on the molecular biology of the renal tissue kallikrein gene and the related tissue kallikrein gene family [Review]. Biol Res 1998;31:151–9.
2. Berg T, Bradshaw RA, Carretero OA, Chao J, Chao L, Clements JA, et al. A common nomenclature for members of the tissue (glandular) kallikrein gene families. Agents Actions Suppl 1992;38:19–25.
3. Ban Y, Wang MC, Watt KW, Loor R, Chu TM. The proteolytic activity of human prostate-specific antigen. Biochem Biophys Res Commun 1984;123:482–8.
4. Papsidero L, Kuriyama M, Wang MC, Horoszewicz J, Leong SS, Valenzuela L, et al. Prostate antigen: a marker for human prostate epithelial cells. J Natl Cancer Inst 1981;66:37–42.
5. Aumüller G, Seitz J, Lilja H, Abrahamsson PA, von der Kammer H, Scheit KH. Species- and organ-specificity of secretory proteins derived from human prostate and seminal vesicles. Prostate 1990;17:31–40.

6. Diamandis EP. Prostate specific antigen—its usefulness in clinical medicine [Review]. Trends Endocrinol Metab 1998;9:310–6.
7. Diamandis EP, Yu H. New biological functions of prostate specific antigen? J Clin Endocrinol Metab 1995;80:1515–7.
8. Diamandis EP, Yu H. Non-prostatic sources of prostate-specific antigen. In: Oesterling JE, ed. Prostate-specific antigen: the best prostatic tumor marker. The Urologic Clinics of North America, Vol. 24. Philadelphia, PA: WB Saunders, 1997:275–82.
9. Frazer HA, Humphrey PA, Burchette JL, Paulson DF. Immunoreactive prostatic specific antigen in male periurethral glands. J Urol 1992;147:246–8.
10. Iwakiri J, Granbois K, Wehner N, Graves HCB, Stamey T. An analysis of urinary prostatic specific antigen before and after radical prostatectomy: evidence for secretion of prostatic specific antigen by the periurethral glands. J Urol 1993;149:783–6.
11. Elgamal AA, Van de Voorde W, Van Poppel H, Lauweryns J, Baert L. Immunohistochemical localization of prostate-specific markers within the accessory male sex glands of Cowper, Litre and Morgagni. Urology 1994;44:84–90.
12. Yu H, Diamandis EP, Sutherland DJA. Immunoreactive prostate-specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. Clin Biochem 1994;27:75–9.
13. Diamandis EP, Yu H, Sutherland DJA. Detection of prostate-specific antigen immunoreactivity in breast tumors. Breast Cancer Res Treat 1994;32:301–10.
14. Zarghami N, Grass L, Diamandis EP. Steroid hormone regulation of prostate-specific antigen gene expression in breast cancer. Br J Cancer 1997;75:579–88.
15. Clements J, Mukhtar A. Glandular kallikrein and prostatic specific antigen are expressed in the human endometrium. J Clin Endocrinol Metab 1994;78:1536–9.
16. Yu H, Diamandis EP. Prostate specific immunoreactivity in milk of lactating women. Clin Chem 1994;41:54–60.
17. Diamandis EP, Yu H, López-Otín C. Prostate specific antigen-a new constituent of breast cyst fluid. Breast Cancer Res Treat 1996; 38:259–64.
18. Yu H, Diamandis EP. Prostate specific antigen immunoreactivity in amniotic fluid. Clin Chem 1995;41:204–10.
19. Melegos DN, Yu H, Allen LC, Diamandis EP. Prostate-specific antigen in amniotic fluid of normal and abnormal pregnancies. Clin Biochem 1996;29:555–62.
20. Schedlich LJ, Bennets BH, Morris BJ. Primary structure of a human glandular kallikrein gene. DNA 1987;6:429–37.
21. Chapdelaine P, Paradis G, Tremblay RR, Dube JY. High level of expression in the prostate of a human glandular kallikrein mRNA related to a prostate-specific antigen. FEBS Lett 1988;236: 205–8.
22. Young CY, Andrews PE, Montgomery BT, Tindall DJ. Tissue-specific and hormonal regulation of human prostate specific glandular kallikrein. Biochemistry 1992;31:818–24.
23. Mikolajczyk SD, Millar LS, Kumar A, Saedi MS. Human glandular kallikrein, hK2, shows arginine-restricted specificity and forms complexes with plasma protease inhibitors. Prostate 1998;34: 44–50.
24. Kumar A, Mikolajczyk SD, Goel AS, Millar LS, Saedi MS. Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. Cancer Res 1997;57:3111–4.
25. Lövgren J, Rajakoski K, Karp M, Lundwall A, Lilja H. Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. Biochem Biophys Res Commun 1997;238:549–55.
26. Takayama TK, Fujikawa K, Davie EW. Characterization of the precursor of prostate specific-antigen. Activation by trypsin and by human glandular kallikrein. J Biol Chem 1997;272:21582–8.
27. Kumar A, Goel AS, Hill TM, Mikolajczyk SD, Millar LS, Kuus-Reichel K, et al. Expression of human glandular kallikrein, hK2 in mammalian cells. Cancer Res 1996;56:5397–402.
28. Finlay JA, Evans CL, Day JR, Mikolajczyk SD, Millar LS, Kuus-Reichel K, et al. Development of monoclonal antibodies specific for human glandular kallikrein (hK2): development of a dual antibody immunoassay for hK2 with negligible prostate-specific antigen cross-reactivity. Urology 1998;51:804–9.
29. Hsieh ML, Charlesworth C, Goodmanson M, Zhang S, Seay T, Klee GG, et al. Expression of human prostate-specific glandular kallikrein protein (hK2) in the breast cancer cell line T47-D. Cancer Res 1997;57:2651–6.
30. Black MH, Magklara A, Obiezu C, Levesque MA, Sutherland DJA, Tindall DJ, et al. Expression of a prostate associated protein, human glandular kallikrein (hK2) in breast tumors and in normal breast secretions. Br J Cancer 1999;in press.
31. Ferguson RA, Yu H, Kalyvas M, Zammit S, Diamandis EP. Ultrasensitive detection of prostate specific antigen by a time-resolved immunofluorometric assay and the Immulite immunochemiluminescent third generation assay: potential applications in prostate and breast cancers. Clin Chem 1996;42:675–84.
32. Black MH, Magklara A, Obiezu C, Melegos D, Diamandis EP. Development of an ultrasensitive immunoassay for human glandular kallikrein with no cross-reactivity from prostate-specific antigen. Clin Chem 1999;45:790–9.
33. Dubé JY, Tremblay RR. Biochemistry and potential roles of prostatic kallikrein hK2. Mol Urol 1997;1:279–85.
34. Haagensen CD, Boduan C, Haagensen DE. Breast carcinoma, risk and detection. Philadelphia, PA: WB Saunders, 1981.
35. Ciatto S, Biggeri A, Rosselli Del Turco M, Bartoli D, Iossa A. Risk of breast cancer subsequent to proven gross cystic disease. Eur J Cancer 1990;26:555–7.
36. Dogliotti L, Orlandi F, Torta M, Buzzi G, Naldoni C, Mazzotti A, et al. Cations and dehydroepiandrosterone-sulfate in cyst fluid of pre- and menopausal patients with gross cystic disease of the breast. Evidence for the existence of subpopulations of cysts. Eur J Cancer Clin Oncol 1986;11:1301–7.
37. Lai LC, Erbas H, Lennard TW, Peaston RT. Prostate-specific antigen in breast cyst fluid: possible role of prostate-specific antigen in hormone-dependent breast cancer. Int J Cancer 1996; 66:743–6.
38. Filella X, Molina R, Alcover J, Menendez V, Gimenez N, Jo J, et al. Prostate-specific antigen detection by ultrasensitive assay in samples from women. Prostate 1996;29:311–6.
39. Mannello F, Bocchiotti G, Bianchi G, Marcheggiani F, Gazzanelli G. Quantification of prostate-specific antigen immunoreactivity in human breast cyst fluids. Breast Cancer Res Treat 1996;38:247–52.
40. Borchert GH, Yu H, Tomlinson G, Giai M, Roagna R, Ponzone R, et al. Prostate specific antigen molecular forms in breast cyst fluid and serum of women with fibrocystic breast disease. J Clin Lab Anal 1999;13:75–81.
41. Furlanetto RW, DiCarlo JN. Somatomedin-C receptors and growth effects in human breast cells maintained in long-term tissue culture. Cancer Res 1984;44:2122–8.
42. Zapf J, Froesch ER. Pathophysiological and clinical aspects of the insulin-like growth factors. Horm Res 1986;24:160–5.
43. Wang DY, Hamed H, Mockridge CI, Fentiman IS. Radioimmunoassayable insulin-like growth factor-I in human breast cyst fluid. Eur J Cancer Clin Oncol 1989;25:867–72.