

NONPROSTATIC SOURCES OF PROSTATE-SPECIFIC ANTIGEN

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Prostate-specific antigen (PSA) is a valuable tumor marker for the diagnosis and management of prostate cancer. The name was intended to underscore the tissue specificity of this protein because it was believed to be produced exclusively by the epithelial cells of the prostate gland. This knowledge was established on the basis of initial studies in which PSA immunoreactivity detected by immunohistochemistry or immunoassay was found only in normal or malignant prostatic tissue.⁵³ Because of the clinical significance of PSA, however, the study of this molecule has increased substantially in the last 10 years. It is now clear that PSA is not tissue specific. In this review, we focus on the most recent findings of PSA in nonprostatic tissues, and we discuss the possible physiologic role of PSA and its potential for clinical applications in diseases other than those of the prostate.

BRIEF OVERVIEW OF PSA IN PROSTATIC TISSUE

History

PSA was discovered in seminal plasma and was named γ -*seminoprotein* by a group of Japanese investigators who were searching for a semen marker in the late 1960s.²⁴ A few years later another group isolated a similar protein from semen and called it *protein E₁* based on its electrophoretic mobility.⁴¹ In 1978, Sensabaugh⁶⁶ characterized this protein biochemically with the use of an immunoelectrophoretic method and named it *p30* based on its molecular weight. Because of its *in vitro* stability, immunogenicity, and tissue specificity, *p30*

was considered a useful semen marker for identifying rape victims or for other criminal and forensic investigations.²²

PSA was found in the prostate by Wang et al in 1977⁷³ and later was isolated from this tissue by the same group.⁷² Because the protein was identified only in prostatic tissue at that time, it was named *prostate-specific antigen*. Wang et al also showed that PSA was present in seminal plasma and that it was similar to the PSA found in the prostate.⁷¹ Although it has not been accepted universally that PSA is the only protein under discussion, consensus has been reached that γ -seminoprotein, protein E₁, p30, and PSA represent the same protein.⁴⁸ PSA later was identified in male serum by the same group who found PSA in the prostate.⁵⁵ Shortly after the finding of PSA in serum, the relationship between serum PSA levels and prostatic disease was investigated. Results from clinical studies indicated possible increases in serum PSA in patients with prostate cancer and possible changes in serum PSA in association with the cancer metastasis or recurrence as well as with patient's response to treatment and survival.^{19, 37, 55} These preliminary observations triggered enormous interest in the utility of serum PSA in diagnosis and management of prostate cancer. Currently, PSA is one of the most valuable and widely used tumor markers.

PSA Protein and Gene

The molecular and biochemical features of PSA have been addressed in several recently published reviews.^{9, 28, 48} Briefly, PSA is a monomeric mole-

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cule with a single polypeptide chain that has 237 amino acids and several disulphide bonds. PSA is a 33 kd glycoprotein and is composed of 93% amino acids and 7% carbohydrates. The sugar components in the protein vary slightly and the variation results in different isoelectric points.

The PSA gene is located on the long arm of chromosome 19 and spans 6 kd in length, including 5 exons, 4 introns, 2 promoter sites, and an untranslated 3' end. One of the promoter sites contains an androgen-response element, and the transcription of the PSA gene is up-regulated by androgen through the androgen receptor.^{61, 77} In vitro experiments also indicate that some growth factors may be involved in the transcription of the PSA gene.²⁷ Several different PSA gene transcripts (mRNAs) have been seen, but only one is considered useful for protein translation. The rest are thought to be products of alternative splicing during gene transcription.²⁹

The PSA gene (now known as *KLK3*) has 80% homology with the human glandular kallikrein 1 gene (now known as *KLK2*) and 68% homology with the tissue (renal/pancreatic) kallikrein gene (now known as *KLK1*).^{8, 62, 65} Owing to the extensive homology between the PSA and the *KLK2* genes, the product of *KLK2* has been speculated to be able to cross-react with PSA antibodies,^{46, 57} which may give rise to false-positive results for the serum PSA test. The *KLK2* protein, however, has not been isolated from natural sources, although it is known to be present in seminal plasma. Recent experiments, using recombinant *KLK2* protein, suggest that some PSA antibodies cross-react with the *KLK2* protein, but others are specific for PSA.⁴⁴ No *KLK2* protein-specific antibodies have been found, however. Preliminary assays for *KLK2* suggest that this protein is present at very low levels in serum.

Protein Function

PSA is a serine protease with chymotrypsin-like enzymatic activity.⁴² Two seminal vesicle proteins and fibronectin are believed to be the substrates of PSA in semen, and cleavage of these proteins by PSA results in semen clot liquefaction. Other studies suggest that PSA is able to digest one of the insulin-like growth factor-binding proteins (IGFBP-3),¹⁰ which in turn indirectly regulates the function of IGFs.¹¹ It is known that IGFs have mitogenic effect for normal and cancer cells and are involved in cell transformation.^{3, 35} It also has been speculated that PSA may inactivate the function of protein C inhibitor in semen when binding to it.⁶ As a serine protease, PSA has certain sequence homology with other serine proteases, including 56% homology with γ -nerve growth factor and 53% homology with epidermal growth factor-binding protein,⁴⁵ but it is unknown if PSA has functions similar to these molecules. Recently, others have speculated that PSA may activate trans-

forming growth factor- β ,³¹ may cleave parathyroid hormone-related peptide,¹² or may release vasoactive peptides from a precursor protein.²⁰

Molecular Forms of PSA

PSA in semen has two major molecular forms, one of which is the intact polypeptide chain called *native form*. The other, called *nicked form*, has a cleavage at amino acids Lys 145 and Lys 146.⁴³ A small amount of PSA in semen also binds to protein C inhibitor. Several molecular forms of PSA exist in serum, including free PSA, α_1 -antichymotrypsin-bound PSA (PSA-ACT complex), and α_2 -macroglobulin-bound PSA.^{5, 42} The PSA-ACT complex is the major molecular form of PSA in serum. All these forms are believed to be enzymatically inactive. Most current PSA assays can detect free PSA and PSA-ACT.⁴⁸ PSA assays that detect only free PSA have been developed, and the ratio of free to total PSA appears to provide a slightly better sensitivity and specificity for diagnosis of prostate cancer.⁵⁶

PSA IN NONPROSTATIC TISSUES

Periurethral Glands

The periurethral gland is the first female tissue that was suggested to be able to produce PSA. PSA production by this gland is based on the findings of immunohistochemical studies that show positive stainings for PSA in male and female periurethral glands^{30, 52, 58, 67} and of PSA in urine of male prostate cancer patients who have had their prostate removed.³⁰ Further histologic studies indicate that the tissue structure of the female periurethral gland is similar to that of the male prostate before puberty, but the gland remains underdeveloped throughout the whole life because of the lack of androgenic stimulation.⁷⁵ Therefore, the female periurethral gland can be considered the "female prostate."^{1, 2} An interesting animal experiment suggests that endoderm-derived urethral epithelium from newborn female mice would develop into a prostate-like tissue producing a PSA-like protein if the cells were allowed to grow in the body of male mice.⁴

Breast

Breast Cancer Tissue

In 1993, PSA immunoreactivity in breast cancer cytosolic extracts, prepared for measurement of steroid hormone receptors, was discovered accidentally in our laboratory while we were evaluating the analytical specificity of a new ultrasensitive PSA assay developed for monitoring prostate cancer patients after radical prostatectomy. PSA im-

munoreactivity in nonprostatic tissues usually was thought to be due to the cross-reaction of polyclonal antibodies to non-PSA proteins. This possibility was excluded, however, because similar immunoreactivity was seen with double-monoclonal PSA assays.¹⁸ Moreover, the possible existence of PSA in breast cancer was suggested by the molecular weight of PSA immunoreactive species in the cytosols. The molecular weight determined by gel electrophoresis and gel filtration was identical to that of PSA in seminal plasma or to the free form of PSA in male serum. Finally, PSA mRNA was detected in breast cancer tissues that were positive for PSA protein measured by immunoassay.⁵¹ In contrast, tissues negative for PSA protein were negative for PSA mRNA.

Because PSA concentrations in breast cancer cytosols are relatively low compared with the levels in male prostate, the number of samples that have detectable PSA may vary depending on the sensitivity of the PSA assay used. Using a PSA assay that has a detection limit of 0.01 $\mu\text{g/L}$, we found PSA levels at or higher than 0.03 ng/mg of total protein in about 30% of breast cancer cytosol samples.

Breast Cancer Cells

Several breast cancer cell lines are able to produce PSA after the cells are incubated with androgens, progestins, glucocorticoids, and mineralocorticoids.⁸⁵ The stimulation of PSA production by these steroid hormones is time- and dose-dependent. The molecular weight of PSA produced by these breast cancer cells is identical to seminal PSA or to PSA from the prostate cancer cell line LNCaP. PSA mRNA is readily identified in these cells lines after the stimulation by steroids.⁵¹ Estrogens do not have the ability to stimulate the cells to produce PSA but can impair the stimulating effect of androgens.⁸⁵ The production of PSA is observed only in breast cancer cell lines that have steroid hormone receptors (MCF-7 or T47-D), but for the cells that do not possess the receptors (BT-20) no PSA is produced after similar stimulation.⁸⁵ For as-yet-unknown reasons, however, many cell lines that are positive for receptors are not able to produce PSA after steroid hormone stimulation.

Other Breast Tissues

PSA also has been found in normal breast tissues (specimens from cosmetic breast-reduction surgery) and breast tissues from benign breast disease. The percentage of women who have detectable levels of PSA in their breast tissues is significantly higher in patients with benign breast disease (60%) than in normal women (30%), but the positivity rates are not different between breast cancer patients and normal women.⁷⁸ The molecular weight of PSA in these specimens is similar to the one found in seminal plasma (33 kd).

Breast Fluids

PSA is detectable in all tested breast fluids, including milk,⁸⁰ cyst fluid,¹⁷ and nipple aspirate fluid.⁶⁴ PSA concentrations in these fluids vary widely from undetectable to 5000.0 $\mu\text{g/L}$. PSA levels in milk decrease with postdelivery time but are not affected by the mother's age or the baby's gender. Free PSA is the major molecular form of PSA in milk. PSA-ACT complex also is present in milk, but accounts for less than 25%.⁸⁰ In cyst fluid, PSA levels vary between less than 0.01 and 82 $\mu\text{g/L}$, and free PSA and PSA-ACT complex are present in roughly equal proportions.¹⁷ Even wider variations of PSA concentrations are seen in nipple aspirate fluid (0–5000 $\mu\text{g/L}$); the molecular forms of PSA in this fluid are free PSA and PSA-ACT, in roughly equal proportions.

PSA Regulation and Function in Breast Tissue

Based on the results of cell culture experiments, we concluded that the PSA gene expression in breast cancer cells is up-regulated by androgens, glucocorticoids, and progestins. The regulation requires androgen, glucocorticoid, and progesterone receptors, respectively (unpublished data). Estrogen not only does not up-regulate but also can interfere with the effect of androgen. The exact mechanism is unknown. Because all these observations are based on in vitro experiments using breast cancer cell lines, it remains unknown which steroid hormones are involved in the PSA gene expression and PSA protein production in normal breast cells and during pregnancy. There is indirect evidence, however, for PSA production in normal breast tissue induced by progesterone. We have analyzed normal breast tissues from 11 healthy women who underwent cosmetic breast reduction surgery for PSA. Of these women, only one had very high levels of PSA in the tissue; that woman was also the only one who was on oral contraceptives that contained high levels of progestin.⁸⁴ Clinical studies have shown that the presence of PSA in breast cancer cytosols is associated with the presence of estrogen and progesterone receptors. Although we did not examine the relationship between androgen receptors and PSA status in the clinical study, it is known that androgen receptors are present in breast cancer cytosols³⁸ and are positively correlated with estrogen and progesterone receptors.³⁶

Interestingly, PSA is associated only with estrogen receptor (ER) or progesterone receptor (PR) by their dichotomous status, that is, the presence or absence of these proteins. No correlation could be seen between the levels of PSA and ER or PR. This phenomenon may suggest that the associations between PSA and ER or PR are indirect, and the key linkage between PSA and the receptors is the ligands that bind to the receptors and activate PSA gene transcription. Therefore, the amounts of

receptors present in the cells at and above certain levels are not the key factors for PSA gene transcription. ER and PR positivity in breast cancer increase with age of the patients, but PSA positivity decreases slowly with age.⁸¹ The opposite direction of correlation between age and PSA or receptors may indicate that PSA and the receptors are associated with the steroid hormones released from the ovary because the production of ovarian hormones is an age-dependent phenomenon.

The enzymatic activity and physiologic role of PSA in breast tissue remains undetermined. In seminal plasma, PSA is able to proteolyze IGFBP-3.¹⁰ An inverse correlation between PSA and IGFBP-3 also is seen in the serum of prostate cancer patients.³² Based on this information, we have examined PSA levels in breast cancer cytosols in association with IGFBP-3. No correlation or association, however, was seen between PSA and IGFBP-3 in our preliminary study.⁸⁷ The relationships between PSA and other members of the IGF family, IGF-I, IGF-II, and IGFBP-1, also was analyzed, but no association was found.

The fact that PSA is present in milk and amniotic fluid and that PSA levels in amniotic fluid change with gestational age suggests that PSA may play a role in fetal and newborn development.

The relationship between PSA and IGFBP-3 indicates a possible regulatory pathway for IGFs. In an *in vitro* study, it was found that IGF-I could activate androgen receptors, resulting in androgen receptor-mediated gene transcription.¹³ The PSA gene is one of those genes up-regulated by androgen via androgen receptors.⁷⁷ Taken together, these data suggest that it may be likely that there is a regulatory loop on IGF-I through PSA and IGF binding proteins. IGF-I increases the production of PSA through androgen receptors, and PSA degrades IGFBP-3, causing release of IGF-I from its binding protein. It is believed that IGF binding proteins control the bioavailability of IGFs through regulation of their transportation and binding to IGF receptors. Whether the regulation results in enhanced or suppressed action of IGF depends on the type of tissues involved.³⁵ Because of this tissue-specific regulation, it is difficult to speculate whether the loop is up- or down-regulating the action of IGFs.

Other interesting findings about IGF include estrogen-regulated expression of IGFs and their binding proteins in breast tissue⁴⁷ and involvement of the IGF family in cell transformation and apoptosis.^{56, 76}

Clinical Applications

The presence of PSA in breast cancer cells and its association with steroid hormone receptors indicated potential clinical utilities of this protein in breast cancer. Preliminary clinical studies did suggest the possibility of PSA being a favorable indicator of prognosis. It was found that PSA presence was associated with early stage of the disease

and small size of the tumor, and that patients with PSA-positive breast cancer could have longer survival than those with PSA-negative cancer. Furthermore, the reduced risk for relapse by PSA was sustained after other major clinical and pathologic factors were controlled in the analysis.⁸⁶ Survival analysis in subgroups of patients indicated that PSA status may help to identify further patients with favorable prognosis among those who were node positive or estrogen-receptor negative.

One recent study on breast-nipple aspirate fluid found that PSA levels in this fluid were significantly lower in women with high risk of breast cancer than in women with low risk of the disease.⁶⁴ All these observations, however, are based on a small number of patients. Large-scale, well-designed studies are needed to confirm these findings.

It still is not clear through what biologic mechanism PSA is a good prognostic indicator for breast cancer. One possible interpretation is that PSA can be produced only by well-differentiated cancer cells. Being a product of androgen regulation through their receptors, the presence of PSA indicates the presence of the regulatory system, which is a sign of good differentiation of cancer cells. Moreover, PSA in breast cancer cells indicates not only the existence but also the functional status of the system. Based on the observation of 30% ER-positive patients who do not respond to endocrine treatment,¹⁶ it is thought that these receptors are mutant products that are not functional.⁷⁴ Therefore, the physical existence of these receptors does not necessarily indicate their functional status. From this point of view, PSA could be a more direct and useful marker for this system, because it is placed downstream from the function of the ER and PR.

The other possibility is the regulatory balance between androgens and estrogens in the target cell. Because PSA is an androgen up-regulated protein in the prostate, the impact of androgen on breast cancer needs to be considered. The androgen receptor is present in breast cancer and is correlated with ER and PR. Cell-culture experiments show that androgen could inhibit the proliferation of breast cancer cells¹⁵ and counteract the effect of estrogen.¹⁴ In addition, androgen has been used effectively in the treatment of some breast cancer patients.⁵⁹ Our cell-culture study demonstrates that PSA production in breast cancer cells can be induced by androgens.⁸⁵ Based on this evidence, the presence of PSA in breast cells may suggest an androgenic suppression of estrogen effect that is believed to play an essential role in the development and progression of breast cancer.^{25, 26, 31}

Amniotic Fluid

In addition to milk and other breast fluids, amniotic fluid is a female fluid containing detectable

levels of PSA.⁷⁹ Free PSA and PSA-ACT complex are found in the fluid, but the former is the major component. The median concentration of PSA in amniotic fluid increases between gestational weeks 14 and 21 and then seems to level off or decrease until parturition. The presence and change of PSA concentration in amniotic fluid seem to have an impact on serum PSA in pregnant women. Serum PSA is significantly higher in pregnant women than in nonpregnant women, and changes in serum PSA of pregnant women match well the changes of PSA in amniotic fluid.⁵⁰ Extremely low or high levels of PSA in amniotic fluid at a specific gestational age may be linked to fetal abnormalities.⁵⁰ The source and physiologic role of PSA in amniotic fluid remain unknown, but the levels are not related to fetal gender. The relationship between PSA and IGFBP-3 or other members of IGF family in amniotic fluid has not been examined.

Female Serum

Using conventional PSA assays with a detection limit of 0.1 $\mu\text{g/L}$ or higher, it is possible to find detectable levels of PSA in less than 10% of female serum.^{23, 34, 63, 69, 70} Because of a lack of interest, the real identity of PSA immunoreactivity in female serum has never been studied. It was assumed that this immunoreactivity was an artifact that was caused by cross-reaction of polyclonal anti-PSA antibodies to nonspecific proteins or by the presence of heterophilic antibodies or anti-PSA antibodies.

Based on our studies, the percentage of female serum which is positive for PSA increased when we use an increasingly sensitive PSA method (detection limits between 0.01 and 0.001 $\mu\text{g/L}$). This suggests that trace amounts of PSA are present in the majority of female serum.⁷⁸ The molecular form of PSA in normal female serum as determined by gel chromatography is similar to the one in male serum, that is, mainly PSA-ACT complex.⁷⁸

The close relationship between PSA and steroid hormone regulation prompted us to study changes in serum level of PSA in association with the phase of the menstrual cycle. Sera from four apparently healthy women were collected every 3 to 5 days during the cycle. Except for one woman who had unmeasurable serum PSA, PSA levels in the other three women changed consistently with menstrual cycle. The level of PSA was low during days 10 to 23 and then increased steadily to reach peak at the end of the cycle or the beginning of the next. This change was interpreted as PSA stimulation by progesterone, because we already know from tissue culture experiments that this steroid stimulates PSA production (unpublished data).

In the prostate, androgen up-regulates the expression of PSA, and the regulation of PSA production by androgen also is observed in the cultured breast cancer cells. Based on this, it is interesting to know if PSA is elevated in the serum of women who have high levels of androgen. Our

recent study shows that serum PSA is increased significantly in hirsute women and that PSA levels are correlated positively with the levels of testosterone and 3α -androstane diol glucuronide (unpublished data). A potential clinical utility of serum PSA in hirsutism is suggested.

The source of PSA in female serum remains undetermined, but several possibilities exist based on preliminary observations. Serum PSA in pregnant women could come from amniotic fluid, because PSA levels increase during pregnancy and change with gestational age of the fetus. Amniotic fluid has a 20 to 40 times higher PSA level than serum of pregnant women. Obviously, this source should be taken into account only if women are pregnant. It is reasonable to speculate that breast tissue is an important source of serum PSA in women. When we measured PSA in matched pre- and postsurgical sera of breast cancer patients, however, we found no change in serum PSA after surgery and no correlation in PSA levels between breast tissue and serum.²¹ These observations, however, may have several limitations. First, the PSA assay we used in the study was not sensitive enough to detect minute changes of PSA in female serum. Second, serum PSA can come from normal and cancerous breast tissues. Thus, the presence and removal of the tumor tissue may not affect the levels of PSA in serum. Our most recent (unpublished) data, however, do suggest that removal of breast cancer could result in decrease of serum PSA levels if a more sensitive PSA assay is used.

It is possible that PSA in female serum arises from other sources such as the ovaries, endometrium, or lungs, but the frequency of these tissues having detectable PSA is relatively low. Although PSA also is produced by the female periurethral gland, the amount that enters blood circulation is thought to be minimal. Thus, PSA from this gland is not thought to be a major source of PSA in female serum.

Recently, we observed changes in the molecular form of PSA in female serum in association with disease status. For healthy women and postoperative patients with breast cancer, the major molecular form of PSA in serum is PSA-ACT complex, whereas for preoperative women with breast cancer, the major form is free PSA.⁴⁹ This observation is preliminary, however. The explanation for this phenomenon and its potential clinical utility remain unknown.

Ovary, Endometrium, and Other Tissues

PSA is detectable in some ovarian cancer cytosols, but the frequency of detection in primary ovarian cancer is much lower (3%) than the frequency of detection in breast cancer (30%).⁸³ Ovarian cancer metastatic from breast origin also has detectable PSA, provided that the primary tumor produces it. We have measured such specimens from three women, and two of them had detectable PSA. Of the three patients, clinical informa-

tion was available for one PSA-negative patient and one PSA-positive patient. Interestingly, the patient with PSA-positive cancer, which was negative for steroid hormone receptors, responded well to the endocrine treatment and survived many years after distant metastasis. The patient with PSA-negative cancer, however, was positive for the receptors, and she died shortly after the relapse.⁸³ Although this observation is based on only two patients, it is consistent with our finding of PSA being a favorable prognostic indicator in primary breast cancer.⁸⁶

Clements and Mukhtar⁷ reported that PSA mRNA is detectable in endometrial tissues, along with two other members of the kallikrein gene family. Other tissues that have been reported to have PSA immunoreactivity by immunohistochemistry include kidney tissue, pituitary tissue,⁸ normal axillary and perineal apocrine sweat glands, and some apocrine foci in fibrocystic breast tissues.⁵⁴ Most of the immunohistochemical findings, however, are believed to be due to the cross-reaction of polyclonal antibody to nonspecific antigens, because the results were not reproducible if a monoclonal antibody was used.⁵⁴

Other Cancers

Some primary lung cancer tissues from men and women contain low but detectable amounts of PSA.^{39, 40} Other cancer tissues that are found to have trace amounts of detectable PSA in our study include kidney, adrenal, and colon.⁴⁰ It also has been reported that serum PSA levels were increased in a woman with renal cell carcinoma.⁶⁰ Van Krieken⁶⁸ found PSA in salivary gland carcinomas, and Papotti et al⁵⁴ reported carcinomas in sweat glands and breast apocrine glands having false-positive staining for PSA.

SUMMARY

The name *prostate-specific antigen* has been given to a protein that now is known not to be prostate-specific; however, prostatic tissue does produce extremely high levels of PSA and secretes it into the seminal plasma. Seminal plasma contains about 1 million $\mu\text{g/L}$ of PSA and is the richest source of PSA reported. The biologic fluid with the second highest PSA concentration, however, is nipple aspirate fluid from the female breast (up to about 5000 $\mu\text{g/L}$), and the third is milk from lactating women (up to 300 $\mu\text{g/L}$). Male serum PSA is usually less than 4 $\mu\text{g/L}$. In nonprostatic tissues, PSA exists mainly in its free molecular form, but PSA-ACT complex is also present in most of the fluids that contain PSA, such as breast secretions and amniotic fluid. The gene expression and protein production of PSA in nonprostatic tissues are under the regulation of steroid hormones via their receptors. Androgens, glucocorticoids, and progestins up-regulate the PSA gene expression, resulting in an increase of protein production. Estrogen by

itself seems to have no effect on PSA regulation, but it can impair PSA production induced by androgen. It remains unknown whether PSA is enzymatically active and what is the physiologic role of PSA in nonprostatic tissues. It is speculated that PSA may be involved in the regulation of growth factors. Measuring PSA in breast cancer cytosol, breast-nipple aspirate fluid, and female serum may have potential clinical utilities, including breast cancer prognosis, breast cancer risk assessment, and evaluation of androgen excess. Further studies are needed to identify the exact function and regulation of PSA in nonprostatic tissues and to explore the clinical application of this protein.

References

1. Ablin RJ: Prostate-specific antigen and the female prostate. *Clin Chem* 35:507-508, 1989
2. Armbruster DA: Prostate-specific antigen: Biochemistry, analytical methods, and clinical application. *Clin Chem* 39:181-195, 1993
3. Baserga R: The insulin-like growth factor I receptor: a key to tumor growth? *Cancer Res* 55:249-252, 1995
4. Boutin EL, Battle E, Cunha GR, et al: The response of female urogenital tract epithelia to mesenchymal inductors is restricted by the germ layer origin of the epithelium: Prostatic inductions. *Differentiation* 48:99-105, 1991
5. Christensson A, Lilja H: Complex formation between protein C inhibitor and prostate-specific antigen in vitro and in human semen. *Eur J Biochem* 220:45-53, 1990
6. Christensson A, Laurell CB, Lilja H: Enzymatic activity of the prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur J Biochem* 194:755-763, 1990
7. Clements J, Mukhtar A: Glandular kallikreins and prostate-specific antigen are expressed in the human endometrium. *J Clin Endocrinol Metab* 78:1536-1539, 1994
8. Clements JA: The glandular kallikrein family of enzymes: Tissue-specific expression and hormonal regulation. *Endocr Rev* 10:393-419, 1989
9. Clements JA: The human kallikrein gene family: A diversity of expression and function. *Mol Cell Endocrinol* 99:C1-6, 1994
10. Cohen P, Graves HCB, Peehl DM, et al: Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. *J Clin Endocrinol Metab* 75:1046-1053, 1992
11. Cohen P, Peehl DM, Graves HCB, et al: Biological effects of prostate specific antigen as an insulin-like growth factor-binding-3 protease. *J Endocrinol* 142:407-415, 1994
12. Cramer SD, Chen Z, Peehl DM: Prostate specific antigen (PSA) inactivates parathyroid hormone-related protein-stimulated cAMP accumulation in mouse osteoblasts. *Proc American Association for Cancer Research* 37:243, 1996
13. Culig Z, Hobisch A, Cronauer MV, et al: Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. *Cancer Res* 54:5474-5478, 1994
14. Dauvoid S, Geng SC, Levesque C, et al: Additive inhibitory effects of an androgen and the antiestrogen EM-170 on estradiol-stimulated growth of human

- ZR-75-1 breast tumors in athymic mice. *Cancer Res* 51:3131-3135, 1991
15. de Launoit Y, Dauvois S, Dufour M, et al: Inhibition of cell cycle kinetics and proliferation by the androgen 5 α -dihydrotestosterone and antiestrogen N, n-butyl-N-methyl-11-[16' α -chloro-3', 17B-dihydroxy-estra-1', 3', 5'-(10')triene-7'a-yl] undecanamide in human breast cancer ZR-75-1 cells. *Cancer Res* 51:2797-2802, 1991
 16. Muss HB: Endocrine therapy for advanced breast cancer: A review. *Breast Cancer Res Treat* 21:15-26, 1992
 17. Diamandis EP, Yu H, Lopez-Otin C: Prostate specific antigen: A new constituent of breast cyst fluid. *Breast Cancer Res Treat* 38:259-264, 1996
 18. Diamandis EP, Yu H, Sutherland DJA: Detection of prostate-specific antigen immunoreactivity in breast tumors. *Breast Cancer Res Treat* 32:301-310, 1994
 19. Edson Pontes J, Chu TM, Slack N, et al: Serum prostate antigen measurement in localized prostatic cancer: Correlation with clinical course. *J Urol* 128:1216-1218, 1982
 20. Fichtner J, Graves HCB, Thatcher K, et al: Prostate specific antigen releases a kinin-like substance on proteolysis of seminal vesicle fluid that stimulates smooth muscle contraction. *J Urol* 155:738-742, 1996
 21. Giai M, Yu H, Katsaros D, et al: Comparison of prostate specific antigen in the serum of normal women and women with breast cancer before or after surgery. *Br J Cancer* 72:728-731, 1995
 22. Graves HCB, Sensabaugh GF, Blake RT: Postcoital detection of male-specific protein. Application to the investigation of rape. *N Engl J Med* 312:338-343, 1985
 23. Graves HCB, Wehner N, Stamey TA: Ultrasensitive radioimmunoassay of prostate-specific antigen. *Clin Chem* 38:735-742, 1992
 24. Hara M, Koyanagi Y, Inoue T, et al: Some physicochemical characteristics of gamma-seminoprotein: an antigenic component specific for human seminal plasma. *Japanese Journal of Legal Medicine* 25:322-324, 1971
 25. Harris JR, Lippman ME, Veronesi U, et al: Breast cancer. *N Engl J Med* 327:319-328, 1992
 26. Henderson BE, Ross RK, Pike MC: Hormonal chemoprevention of cancer in women. *Science* 259:633-638, 1993
 27. Henttu P, Vihko P: Growth factor regulation of gene expression in the human prostatic carcinoma cell line LNCaP. *Cancer Res* 53:1051-1058, 1993
 28. Henttu P, Vihko P: Prostate-specific antigen and human glandular kallikrein: Two kallikreins of the human prostate. *Ann Med* 26:157-164, 1994
 29. Henttu P, Lukkarinen O, Vihko P: Expression of the gene coding for human prostate-specific antigen and related hGK-1 in benign and malignant tumors of the human prostate. *Int J Cancer* 45:654-660, 1990
 30. Iwakiri J, Grandbois K, Wehner N, et al: An analysis of urinary prostate specific antigen before and after radical prostatectomy: Evidence for secretion of prostate specific antigen by the periurethral glands. *J Urol* 149:783-786, 1993
 31. Jordan VC, Murphy CS: Endocrine pharmacology of antiestrogens as antitumor agents. *Endocr Rev* 11:578-610, 1990
 32. Kanety H, Madjar Y, Dagan Y, et al: A. Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: Correlation with serum prostate-specific antigen. *J Clin Endocrinol Metab* 77:229-233, 1993
 33. Killian CS, Corral DA, Kawinski E, et al: Mitogenic response of osteoblast cells to prostate specific antigen suggests an activation of latent TGF- β and a proteolytic modulation of cell adhesion receptors. *Biochem Biophys Res Commun* 192:940-947, 1993
 34. Klee GG, Dodge LA, Zincke H, et al: Measurement of serum prostate specific antigen using IMx prostate specific antigen assay. *J Urol* 151:94-98, 1994
 35. Krywichi RF, Yee D: The insulin-like growth factor family of ligands, receptors, and binding proteins. *Breast Cancer Res Treat* 22:7-19, 1992
 36. Kuenen-Boumeester V, Van Der Kwast TH, van Putten WLT, et al: Immunohistochemical determination of androgen receptors in relation to oestrogen and progesterone receptors in female breast cancer. *Int J Cancer* 52:581-584, 1992
 37. Kuriyama M, Wang MC, Lee C, et al: Use of human prostate-specific antigen in monitoring prostate cancer. *Cancer Res* 41:3874-3876, 1981
 38. Lea OA, Kviunslund S, Thorsen T: Improved measurement of androgen receptors in human breast cancer. *Cancer Res* 49:7162-7167, 1989
 39. Levesque MA, Yu H, D'Costa M, et al: Immunoreactive prostate specific antigen in lung tumors. *J Clin Lab Anal* 9:375-379, 1995
 40. Levesque MA, Yu H, D'Costa M, et al: Prostate specific antigen expression by various tumors. *J Clin Lab Anal* 9:123-128, 1995
 41. Li TS, Beling CG: Isolation and characterization of two specific antigens of human seminal plasma. *Fertil Steril* 24:134-143, 1973
 42. Lilja H: Structure, function, and regulation of the enzyme activity of prostate-specific antigen. *World J Urol* 11:188-191, 1993
 43. Lilja H, Christensson A, Dahlen U, et al: Prostate-specific antigen in serum occurs predominantly in complex with α_1 -antichymotrypsin. *Clin Chem* 37:1618-1625, 1991
 44. Lovgren J, Piironen T, Overmo C, et al: Production of recombinant PSA and HK2 and analysis of their immunologic cross-reactivity. *Biochem Biophys Res Commun* 213:888-895, 1995
 45. Lundwall A, Lilja H: Molecular cloning of human prostate specific antigen cDNA. *FEBS Lett* 214:317-322, 1987
 46. Malm J, Lilja H: Biochemistry of prostate specific antigen, PSA. *Scand J Clin Lab Invest Suppl* 55:15-22, 1995
 47. Manni A, Badger B, Wei L, et al: Hormonal regulation of insulin-like growth factor II and insulin-like growth factor binding protein expression by breast cancer cell in vivo: Evidence for stromal epithelial interactions. *Cancer Res* 54:2934-2942, 1994
 48. McCormack RT, Rittenhouse HG, Finlay JA: Molecular forms of prostate-specific antigen and the human kallikrein gene family: A new era. *Urology* 45:729-744, 1995
 49. Melegos D, Diamandis EP: Diagnostic value of molecular forms of prostate specific antigen for female breast cancer. *Clin Biochem* 29:193-200, 1996
 50. Melegos DN, Yu H, Allen LC, et al: Prostate specific antigen in amniotic fluid of normal and abnormal pregnancies. *Clin Biochem* 29:555-562, 1996
 51. Monne M, Croce CM, Yu H, et al: Molecular characterization of prostate-specific antigen messenger RNA expressed in breast tumors. *Cancer Res* 54:6344-6347, 1994
 52. Nowels K, Kent E, Rinsho K, et al: Prostate specific antigen and acid phosphatase-reactive cells in cystitis

- cystica and glandularis. *Arch Pathol Lab Med* 112:734-737, 1988
53. Oesterling JE: Prostate specific antigen: A critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. *J Urol* 145:907-923, 1989
 54. Papotti M, Paties C, Peveri V, et al: Immunocytochemical detection of prostate-specific antigen (PSA) in skin adnexal and breast tissues and tumors. *Basic Applied Histochemistry* 33:25-29, 1989
 55. Papsidero LD, Wang MC, Valenzuela LA, et al: A prostate antigen in sera of prostatic cancer patients. *Cancer Res* 40:2428-2432, 1980
 56. Partin AW, Oesterling JE: The clinical usefulness of prostate specific antigen: Update 1994. *J Urol* 152:1358-1368, 1994
 57. Peehl DM: Prostate specific antigen role and function. *Cancer* 75:2021-2026, 1995
 58. Pollen JJ, Dreilinger A: Immunohistochemical identification of prostatic acid phosphatase and prostate specific antigen in female periurethral glands. *Urology* 23:303-304, 1984
 59. Pritchard KJ: The use of endocrine therapy. *Hematol Oncol Clin North Am* 3:765-805, 1989
 60. Pummer K, Wirnsberger G, Purstner P, et al: False positive prostate specific antigen values in the sera of women with renal cell carcinoma. *J Urol* 148:21-23, 1992
 61. Riegman PH, Vlietstra RJ, van der Korput J, et al: The promotor of the prostate-specific antigen gene contains a functional androgen responsive element. *Mol Endocrinol* 5:1921-1930, 1991
 62. Riegman PHJ, Vlietstra RJ, van der Korput JAGM, et al: Characterization of the prostate-specific antigen gene: A novel human kallikrein-like and gene. *Biochem Biophys Res Commun* 159:95-102, 1989
 63. Rock RC, Chan DW, Bruzek D, et al: Evaluation of a monoclonal immunoradiometric assay for prostate-specific antigen. *Clin Chem* 33:2257-2261, 1987
 64. Sauter ER, Daly M, Lenahan K, et al: Prostate specific antigen in nipple aspirate fluid correlate with breast cancer risk [abstract]. *Proc American Association for Cancer Research* 37:213, 1996
 65. Schedlich LJ, Bennetts BH, Morris BJ: Primary structure of a human glandular kallikrein gene. *DNA* 6:429-437, 1987
 66. Sensabaugh GF: Isolation and characterization of a semen-specific protein from human seminal plasma: A potential new marker for semen identification. *J Forensic Sci* 23:106-115, 1978
 67. Tepper SL, Jagirdar J, Heath D, et al: Homology between the female paraurethral (Skene's) glands and the prostate. *Arch Pathol Lab Med* 108:423-425, 1984
 68. Van Krieken JH: Prostate marker immunoreactivity in salivary gland neoplasms. *Am J Surg Pathol* 17:410-414, 1993
 69. Vessella RL, Noteboom J, Lange PH: Evaluation of the Abbott IMx automated immunoassay of prostate-specific antigen. *Clin Chem* 38:2044-2054, 1992
 70. Vihko P, Kurkela R, Ramberg J, et al: Time-resolved immunofluorometric assay of human prostate-specific antigen. *Clin Chem* 36:92-95, 1990
 71. Wang MC, Valenzuela LA, Murphy GP, et al: A simplified purification procedure for human prostate antigen. *Oncology* 39:1, 1982
 72. Wang MC, Valenzuela LA, Murphy GP, et al: Purification of a human prostate specific antigen. *Invest Urol* 17:159-163, 1979
 73. Wang MC, Valenzuela LA, Murphy GP, et al: Tissue specific and tumor specific antigens in human prostate. *Federation Proceeding* 36:1254, 1977
 74. Wei LL: Transcriptional activation of the estrogen receptor. *Clin Chem* 39:341-345, 1993
 75. Wernert N, Albrecht M, Sesterhenn I, et al: The "female prostate": Location, morphology, immunohistochemical characteristics and significance. *Eur Urol* 22:64-69, 1992
 76. Westley BR, May FEB: Insulin-like growth factors: The unrecognized oncogenes. *Br J Cancer* 72:1065-1066, 1995
 77. Young CYF, Montgomery BT, Andrews PE, et al: Hormonal regulation of prostate-specific antigen messenger RNA in human prostatic adenocarcinoma cell line LNCaP. *Cancer Res* 51:3748-3752, 1991
 78. Yu H, Diamandis EP: Measurement of prostate specific antigen level in the serum of females and prostatectomized males with an ultrasensitive immunoassay technique. *J Urol* 153:1004-1008, 1995
 79. Yu H, Diamandis EP: Prostate specific antigen immunoreactivity in amniotic fluid. *Clin Chem* 41:204-210, 1995
 80. Yu H, Diamandis EP: Protease prostate specific antigen in milk of lactating women. *Clin Chem* 41:54-60, 1995
 81. 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* 27:75-79, 1994
 82. Yu H, Diamandis EP, Levesque M, et al: Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue. *Breast Cancer Res Treat* 40:171-178, 1996
 83. Yu H, Diamandis EP, Levesque MA, et al: Ectopic production of prostate specific antigen by a breast tumor metastatic to the ovary. *J Clin Lab Anal* 8:251-253, 1994
 84. Yu H, Diamandis EP, Monne M, et al: Oral contraceptive-induced expression of prostate-specific antigen in the female breast. *J Biol Chem* 270:6615-6618, 1995
 85. Yu H, Diamandis EP, Zarghami N, et al: Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines. *Breast Cancer Res Treat* 32:291-300, 1994
 86. Yu H, Giai M, Diamandis EP, et al: Prostate specific antigen is a new favourable prognostic indicator for women with breast cancer. *Cancer Res* 55:2104-2110, 1995
 87. Yu H, Levesque MA, Mohammad JK, et al: Associations between insulin-like growth factors and their binding proteins and other prognostic indicators in breast cancer. *Br J Cancer* 74:1242-1247, 1996

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