

A Precursor Form of Prostate-specific Antigen Is More Highly Elevated in Prostate Cancer Compared with Benign Transition Zone Prostate Tissue

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ABSTRACT

Prostate-specific antigen (PSA) is a widely used serum marker for prostate cancer (PCa), but in the critical diagnostic range of 4–10 ng/ml it has limited specificity for distinguishing early PCa from benign prostatic hyperplasia (BPH). PSA in serum is comprised of a variety of both “free” and “complexed” forms that have been used to improve the specificity of PSA for prostate cancer detection. We previously reported that pro PSA (pPSA), the zymogen or precursor form of PSA, is a component of free PSA in the serum of PCa patients. In the current study, we examined prostate tissues to understand the origin and specificity of pPSA. PSA was immuno-affinity purified from matched sets of prostate tissues including peripheral zone cancer (PZ-C); peripheral zone noncancer; and benign tissue from the transition zone (TZ), the primary site of BPH within the prostate. We found that pPSA is differentially elevated in PZ-C, but is largely undetectable in TZ. N-terminal sequencing revealed that the pPSA was comprised primarily of [–2]pPSA and minor levels of [–4]pPSA, containing pro leader peptides of 2 and 4 amino acids, respectively. The median value of pPSA was 3% in PZ-C and 0% (undetectable) in TZ ($P < 0.0026$). No pPSA was detected in 13 of 18 transition zone specimens (72%), but only 2 of the 18 matched cancer specimens (11%) contained no measurable pPSA. These results demonstrate that pPSA is more highly correlated with prostate cancer than with BPH. The pPSA in serum may represent a more cancer-specific form of PSA that could help distinguish prostate cancer from BPH.

INTRODUCTION

The measurement of serum PSA² is widely used for the screening and early detection of prostate cancer (1–3). PSA is highly compartmentalized within the normal prostate gland, which contains PSA levels approximately one million-fold higher than in the serum (4). In men with prostatic disease, increased amounts of PSA enter the blood, most likely due to disruption of this strict compartmentalization, leading to an elevation of circulating PSA. Serum PSA that is measurable by current clinical immunoassays exists primarily as either the “free” or “noncomplexed” form or in a complex with α_1 -antichymotrypsin (ACT; “complexed PSA”) (5, 6). From 70 to 95% of the measurable total serum PSA is complexed with ACT (4). In patients with a mildly elevated PSA, the ratio of free to total PSA in serum has been demonstrated to significantly improve the discrimination of prostate cancer (PCa) from benign prostatic hyperplasia (BPH), with higher ratios correlating with a lower risk of prostate cancer (7, 8). The biological mechanism resulting in the increased ratio of serum free PSA in patients with BPH is unknown.

It is generally assumed that the free PSA in serum is enzymatically

inactive because it would otherwise complex with endogenous serum protease inhibitors. Free forms of PSA may include mature, inactive PSA (9) and various forms of clipped, inactivated PSA (10, 11) as well as precursor or zymogen forms of PSA (12). The molecular forms comprising “free” PSA in the serum of prostate cancer patients may differ from those in BPH patients. Previously, we reported that pro PSA (pPSA) was a component of the free PSA in PCa serum (12). Identification of free PSA forms in the serum of BPH patients has not been feasible because the lower levels of PSA are insufficient for current analytical techniques. To determine whether specific molecular forms of pPSA or clipped PSA were preferentially associated with prostate cancer or BPH tissues, we examined matched sets of tissues harvested from patients undergoing radical prostatectomy. From each patient samples of prostate cancer and adjacent noncancerous tissues from the peripheral zone (PZ-C and PZ-N, respectively) as well as a sample of benign transition zone tissue (TZ) were selected for analysis. Most cancers are localized to the peripheral zone (13), whereas pathological BPH occurs almost exclusively in the transition zone of the prostate (14). We have previously reported that TZ tissues exhibiting nodular hyperplasia (pathological BPH) contain elevated levels of a specific, clipped form of PSA, called BPSA (BPH-associated form of PSA), when compared with levels in both cancerous and noncancerous PZ tissues (15). We now report that pPSA is associated primarily with PZ prostate cancer tissues but not with benign TZ tissues, indicating that an assay specific for pPSA in serum may help further distinguish PCa from BPH.

MATERIALS AND METHODS

Sample Selection. Samples were obtained from the Baylor Specialized Program of Research Excellence in Prostate Cancer Tissue and Serum Bank, which is comprised of frozen tissue harvested at radical prostatectomy according to a previously described protocol (16). Matched TZ, PZ-N, and PZ-C tissue specimens were randomly selected from patients with either small (total gland mass <25 g) or large (total prostate gland mass >50 g) prostatectomy specimens. Cancer specimens were comprised of at least 80% cancer as determined by analysis of hematoxylin and eosin-stained sections of whole-mount radical prostatectomy specimens in the area surrounding each specimen core. Patients found to have transition zone cancer were excluded from the study. The presence of nodular BPH in the TZ was determined in a blinded fashion by macroscopic and low-power magnification microscopic examination of whole-mount tissue slides. In addition, eight transition zone specimens obtained during transurethral resection of the prostate (TURP) at Brotman Medical Center (Culver City, CA) were analyzed. Samples were immediately frozen at -70°C and stored until analysis.

Isolation of PSA from Prostate Tissue. Prostate tissues were frozen in liquid nitrogen, pulverized to a fine powder, and homogenized in PBS containing a protease inhibitor cocktail (Complete, Boehringer Mannheim, Indianapolis, IN). Samples were then centrifuged to remove cell debris, and the supernatant solution was filtered through a $0.2\ \mu\text{m}$ membrane. The supernatant solution was passed over two immunoaffinity columns in series, a column containing the hK2-specific mAb HK1G586.1, and the PSA-specific mAb, PSM773. HK1G586.1 has been shown to have no cross-reactivity with PSA (17, 18). PSM773 has been shown previously to have no cross-reactivity with hK2 and to have specificity for mature, clipped, and precursor forms of PSA (19–21). The columns were washed with 40 volumes of PBS containing 0.1%

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² The abbreviations used are: PSA, prostate specific antigen; ACT, α_1 -antichymotrypsin; PCa, prostate cancer; BPH, benign prostatic hyperplasia; pPSA, pro PSA; TZ, benign transition zone tissue; PZ-C, peripheral zone cancer; PZ-N, peripheral zone noncancer; TURP, transurethral resection of the prostate; HIC-HPLC, high performance hydrophobic interaction chromatography.

reduced Triton X-100 and bound protein eluted with 100 mM glycine (pH 2.5) containing 200 mM sodium chloride. The eluant was immediately neutralized with 10% vol/vol 1 M Tris (pH 8.0).

Immunoassay of PSA. The concentration of PSA in the extracts was determined by Tandem-MP PSA and Tandem-MP_{free} PSA assays (Hybritech, San Diego, CA). The percentage of inactive PSA was determined by measuring the level of noncomplexed PSA in the original extract and comparing it to the level of noncomplexed PSA remaining after incubation with female serum for 3 h at 37°C. Incubation with female serum allowed active PSA to complex with serum proteins. The total protein in the extracts was determined using the Bradford protein assay (Pierce, Rockford, IL).

High Performance Hydrophobic Interaction Chromatography (HIC-HPLC) of PSA. HIC-HPLC was performed using a polypropylaspartamide column (PolyLC, Western Analytical Products, Temecula, CA). The column was 4.6×250 mm in length with a 1000 Å pore size. Samples were applied in 1.5 M ammonium sulfate and eluted with a gradient. Buffer A contained 1.2 M sodium sulfate and 25 mM sodium phosphate (pH 6.3). Buffer B contained 50 mM sodium phosphate and 5% v/v 2-propanol. The gradient was 0–35% B for 1 min, 30–80% B for 12 min, and then isocratic at 80% B for 2 min before equilibration in Buffer A. High-sensitivity peak detection was obtained with a Varian model 9070 scanning fluorescence detector using an excitation of 232 nm and emission of 334 nm to detect the tryptophan residues in protein.

Amino Acid Sequencing of PSA. N-terminal sequence of the samples was performed through 9 cycles on a PE-Applied Biosystems model 492 amino acid sequencer (Perkin-Elmer, Applied Biosystems Division, Foster City, CA). Purified PSA and peaks collected by HIC-HPLC were applied directly to polyvinylidene difluoride membranes using the Prosorb cartridges (PE-Applied Biosystems), washed 3 times with 0.1 ml 0.1% trifluoroacetic acid, and applied to the model 492 sequencer.

Statistical Analysis. All statistical analyses were performed using SAS software. The median percentages of pPSA in TZ, PZ-N, and PZ-C tissues were evaluated for statistical significance using the nonparametric Kruskal-Wallis test.

RESULTS

The Identification of Precursor Forms of PSA in Prostate Tissues. Total PSA was immunoaffinity purified from the tissue extracts using the PSA-specific mAb, PSM773. Fig. 1 shows the HIC-HPLC profile of the affinity-purified PSA from the PZ-C of patient #1 (Table 1). PSA typically elutes at 10 min under the conditions used. The identities of inactive forms of PSA in this peak were determined by performing HIC-HPLC after incubation of the purified PSA with ACT (Fig. 2) and after sequencing individual fractions to confirm their identities. Peak 1 in Fig. 2A is the inactive, clipped ACT that is formed during complex formation with PSA (22). Peak 2 represents the residual excess active ACT remaining after incubation. Peak 3 is the PSA-ACT complex, and peak 4 comprises a mixture of inactive forms of PSA that failed to complex with ACT. Under these conditions, the

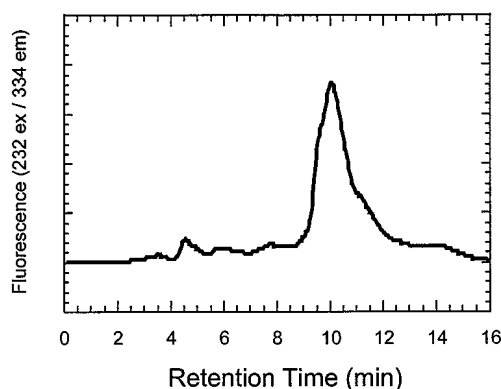


Fig. 1. HIC-HPLC profile of immunoaffinity-purified PSA isolated from PZ-C tissues.

Table 1 The percentage of pPSA and inactive PSA in matched prostate tissues

No.	Prostate volume	Peripheral zone Noncancer		Peripheral zone Cancer		Transition zone	
		pPSA (%)	Inactive PSA (%)	pPSA (%)	Inactive PSA (%)	pPSA (%)	Inactive PSA (%)
1	118	21	54	35	59	3.6	57
2	90	2.5	38	5.4	44	0	38
3	72	19	40	3.3	35	10	48
4	62	4	33	3.8	31	2.5	26
5	60	0	24	0.5	34	0	27
6	54	0	44	0	39	0	28
7	54	0	31	8	29	0	42
8	53	1	29	2.6	35	0	46
9	53	3.5	29	1	40	0	32
10	51	4	27	3	39	1	36
11	24	0	46	11	48	0	51
12	24	0	29	1.2	28	0	23
13	23	0	43	0	26	0	35
14	22	7	28	17	26	0	36
15	21	0	37	3.6	31	0	31
16	18	3.4	47	1	37	0	65
17	17	6	41	4	38	3	42
18	17	1	39	4	33	0	38
Mean		3.9	36.6	5.6	36.2	1.1	38.9
Median		1.5	37.5	3.0	35	0	40.4

PSA-ACT complex eluted at 8 min and was clearly resolved from the residual, unreactive forms of PSA, which remained at 10 min.

N-terminal sequencing of peak 4 revealed multiple PSA forms, including [−2]pPSA, [−4]pPSA, [+5]PSA, PSA clipped at Lys145 and Arg85, and mature unclipped PSA. Fig. 2B shows the relative elution positions of these different forms of inactive PSA. The front shoulder of the inactive PSA peak contained primarily mature PSA, PSA clipped at Lys145 and Arg85, and PSA clipped after Gly4. The second half of the inactive PSA peak contained the majority of the [−2]pPSA. The small peak eluting at 12 min contained the [−4]pPSA. The retention time of [−4]pPSA has been reported previously (12). The [−2] and [−4] forms of pPSA are truncated forms of pPSA. proPSA is normally expressed with a heptapeptide pro leader sequence consisting of APLILSR. The [−2]pPSA contains the SR pro dipeptide on the N-terminal isoleucine of mature PSA, and [−4]pPSA contains an ILSR leader peptide. As indicated by their inability to form a complex with ACT, each of these clipped or truncated forms of pPSA was enzymatically inactive. The [−2]pPSA constituted 65% of the inactive PSA in this sample, and [−4]pPSA was 6%. Other samples of purified PSA were incubated with ACT as described above and showed varying percentages of the different inactive forms of PSA (data not shown).

The Distribution of pPSA in Prostate Tissues. The distribution of the different percentages of clipped PSA and pPSA in the 18 sets of matched tissues was determined by N-terminal sequencing of whole immuno-affinity purified PSA. The clips at [+5]PSA, Lys145, and Arg85 showed no significant trends in the PZ-N, PZ-C, and TZ tissues (data not shown). However, [−2]pPSA was elevated in almost all of the PZ-C and in many of the PZ-N tissues but was largely undetectable in the TZ (<0.2% pPSA). The [−2] form of pPSA was the predominant form of pPSA observed in the purified PSA, although very minor levels of other proforms could be detected in some samples.

Table 1 shows the percentage of [−2]pPSA in the 18 sets of matched tissue specimens. When grouped according to tissue type, levels of [−2]pPSA were significantly higher in PZ tissues than in TZ tissues (Fig. 3). Only 5 TZ samples showed measurable pPSA. Ten of eighteen TZ were shown to have pathological nodular BPH (15), but they were not statistically different in their levels of pPSA from the eight TZ tissues without nodules. The median value of pPSA in TZ was 0, compared with 1.5% for PZ-N and 3.0% of the total PSA for

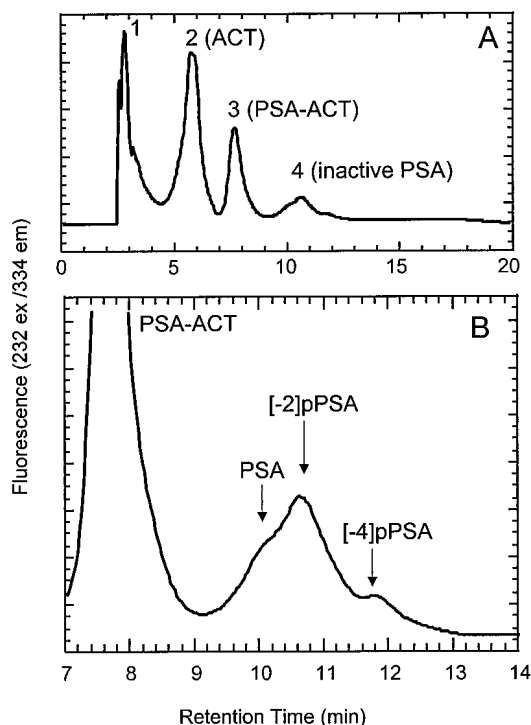


Fig. 2. HIC-HPLC profile of the reaction mixture of PZ-C PSA incubated with excess ACT. A, Peak 1 is inactive ACT which has been cleaved by PSA at the P1 reactive site, Leu358. Peak 2 is the residual excess active ACT. Peak 3 is the covalent PSA-ACT complex. Peak 4 is the inactive PSA that did not react with ACT. B, Enlargement of A showing the PSA-ACT and inactive PSA peaks. Different fractions of the inactive PSA peak were collected and analyzed by N-terminal sequencing, as indicated by the arrows.

PZ-C ($p < 0.0026$). The mean values for TZ, PZ-N, and PZ-C were 1.1, 3.9, and 5.6% of the total PSA, respectively. The mean values of total PSA in the three tissues, as determined by immunoassay, were 15.6, 12.4 and 9.0 μg PSA per mg of total protein in the TZ, PZ-C, and PZ-N, respectively. Eight samples of transition zone tissue, obtained during TURP for BPH, also revealed no detectable pPSA (data not shown).

Table 1 also shows the percentage of total inactive PSA in each sample as determined by immunoassay after incubation with female serum (see "Materials and Methods"). The pPSA in PZ-N and TZ showed no statistical correlation with the percentage of inactive PSA, whereas the pPSA in PZ-C showed a mild correlation ($p < 0.012$). The median percentages of inactive PSA for PZ-N, PZ-C, and TZ were 38, 35, and 37%, respectively.

DISCUSSION

These results show that truncated forms of pPSA were differentially elevated in the peripheral zone of the prostate, the site where most cancers are localized. By contrast, little or no pPSA was found in the transition zone, which is the site of pathological BPH and the site where prostatic enlargement occurs. Because both PCa and BPH result in increased release of PSA into the serum (4), the detection of pPSA in serum may add specificity to the diagnosis of prostate cancer in men with mildly elevated PSA.

These tissue studies add important new insight into our previous work, in which we reported that pPSA is a component of the PSA in PCa serum (12). In that work we identified [-4]pPSA by HIC-HPLC as a component of the free PSA in PCa serum. The serum may also have contained [-2]pPSA, the form we have found most abundantly in tissues, but [-2]pPSA cannot be readily distinguished from mature forms of PSA by HIC-HPLC (see Fig. 2) and requires sufficient

quantities of protein for sequence analysis to confirm the identity of these species. Therefore, the analysis of serum PSA forms is limited by the necessity of using serum with highly elevated levels of PSA, typically from 100 to 1000 ng/ml or higher.

Because high PSA levels are found primarily in patients with prostate cancer but not BPH, it remains unknown whether pPSA can be detected in the serum of patients with BPH only. However, high levels of PSA are released into the blood during TURP, and a recent study analyzed the PSA in TURP serum and found no pPSA (23). This is consistent with our finding that pPSA is largely absent in the transition zone (Fig. 3). In the same study, attempts were made to detect pPSA in the PSA purified from PCa serum by probing Western blots with an antibody raised against the N-terminal propeptide moiety of pPSA. However, this aspect of those studies remains inconclusive in light of our findings because the antibodies were not specific for the [-2] and [-4] pPSA forms we have found in the tissues and serum (12). Comprehensive analysis of serum samples awaits the availability of immunoassays that can specifically detect the [-2] and [-4] pPSA forms.

The current study of prostate tissues provides some evidence for the origin of pPSA in serum. Because transition zone tissues contain little or no pPSA, it is more likely that any pPSA found in the serum is derived from cancerous prostate PZ tissue. This distinction between PZ and TZ becomes critical in the early stages of prostate cancer in which comparable levels of total serum PSA may be derived from both cancer and BPH. In the diagnostic gray zone of 4–10 ng/ml, the measurement of pPSA in serum may help distinguish PCa from BPH.

The reasons for the higher levels of these truncated pPSA forms in the PZ are not known. PSA, like most tissue kallikreins, is secreted from cells as the precursor form (21, 24, 25), but it has not been established how or where it is converted to the enzymatically active form *in vivo*. The truncated forms of pPSA are likely to result from post-translational proteolytic modification and may reflect an altered biochemical pathway. An analysis of seminal plasma PSA, as in Fig. 2, found no detectable pPSA forms (data not shown). Because the homologous prostate kallikrein, hK2, has been shown to activate pPSA *in vitro*, it has been speculated that hK2 may be involved in the *in vivo* regulation of PSA activity (21, 26, 27). hK2 is currently the subject of studies to determine its relationship to prostate cancer (17).

In these studies, we simultaneously purified the hK2 from the tissue extracts by immunoaffinity chromatography using an hK2-specific

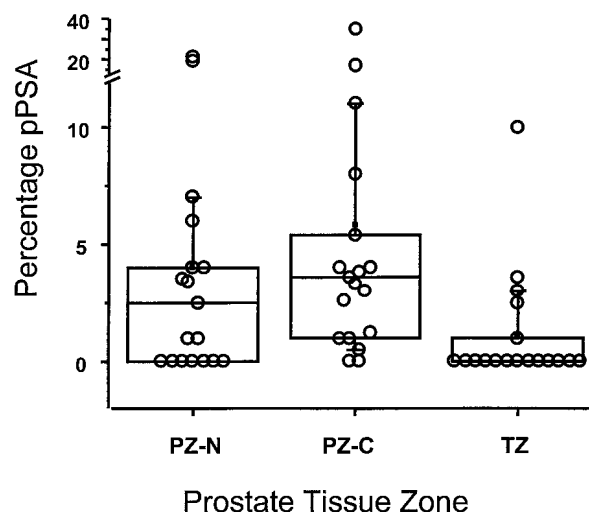


Fig. 3. Box plots of [-2]pPSA in 18 matched sets of prostate tissues. The top and bottom of the box denote the 25th and 75th percentile values, the center bar denotes the median, and the error bars show the 5th and 95th percentile values.

mAb. hK2 was present at 2–4% of the PSA (data not shown) and is largely inactive in tissue extracts due to internal peptide bond cleavage, as shown previously (28). The [–2] form of pPSA contains the same N-terminal amino acids as [–2]phK2, SRIVGGWEC, whereas the [–4] pro forms are different from each other. Any significant contamination by [–2]phK2 was ruled out by the use of both hK2-specific and PSA-specific mAbs, together with the observation that hK2 represents only a minor percentage of the PSA in these extracts.

Monoclonal antibodies specific for different pPSA forms are currently under development. If the levels of pPSA in serum reflect the presence of pPSA in prostate cancer PZ tissues but not in the transition zone, then an immunoassay that measures pPSA may improve the discrimination of prostate cancer from BPH. The combination of an assay for cancer-associated pPSA, together with an assay for the nodular BPH-associated form of PSA that we recently reported (15), may enhance our ability to distinguish cancer from BPH.

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REFERENCES

- Catalona, W. J., Smith, D. S., Ratliff, T. L., Dodds, K. M., Coplen, D. E., Yuan, J. J., Tetros, J. A., and Andriole, G. L. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N. Engl. J. Med.*, 324: 1156–1161, 1991.
- Oesterling, J. E. Prostate-specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. *J. Urol.*, 145: 907–923, 1991.
- Labrie, F., Dupont, A., Suburu, R., Cusan, L., Tremblay, M., Gomez, J. L., and Emond, J. Serum prostate specific antigen as pre-screening test for prostate cancer. *J. Urol.*, 147: 846–851, 1992.
- Rittenhouse, H. G., Finlay, J. A., Mikolajczyk, S. D., and Partin, A. W. Human kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. *Crit. Rev. Clin. Lab. Sci.*, 35: 275–368, 1998.
- Lilja, H., Christensson, A., Dahlen, U., Matikainen, M. T., Nilsson, O., Pettersson, K., and Lovgren, T. Prostate-Specific Antigen in Serum Occurs Predominantly in Complex with α_1 -antichymotrypsin. *Clin. Chem.*, 37: 1618–1625, 1991.
- Stenman, U. H., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K., and Alfthan, O. A complex between prostate specific antigen and α_1 -antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res.*, 51: 222–226, 1991.
- Catalona, W. J., Partin, A. W., Slawin, K. M., Brawer, M. K., Flanigan, R. C., Patel, A., Richie, J. P., deKernion, J. B., Walsh, P. C., Scardino, P. T., Lange, P. H., Subong, E. N., Parson, R. E., Gasior, G. H., Loveland, K. G., and Southwick, P. C. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA*, 279: 1542–1547, 1998.
- Woodrum, D. L., Brawer, M. K., Partin, A. W., Catalona, W. J., and Southwick, P. C. Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. *J. Urol.*, 159: 5–12, 1998.
- Zhang, W. M., Leinonen, J., Kalkkinen, N., Dowell, B., and Stenman, U. H. Purification and characterization of different molecular forms of prostate-specific antigen in human seminal fluid. *Clin. Chem.*, 41: 1567–1573, 1995.
- Chen, Z., Chen, H., and Stamey, T. A. Prostate specific antigen in benign prostatic hyperplasia: purification and characterization. *J. Urol.*, 157: 2166–2170, 1997.
- Noldus, J., Chen, Z., and Stamey, T. Isolation and characterization of free form prostate specific antigen (f-PSA) in sera of men with prostate cancer. *J. Urol.*, 158: 1606–1609, 1997.
- Mikolajczyk, S. D., Grauer, L. S., Millar, L. S., Hill, T. M., Kumar, A., Rittenhouse, H. G., Wolfert, R. L., and Saedi, M. S. A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. *Urology*, 50: 710–714, 1997.
- McNeal, J. E. Origin and evolution of benign prostatic enlargement. *Invest. Urol.*, 15: 340–345, 1978.
- McNeal, J. Pathology of benign prostatic hyperplasia: insight into etiology. *Urol. Clin. North Am.*, 17: 477–486, 1990.
- Mikolajczyk, S. D., Millar, L. S., Wang, T. J., Wolfert, R. L., Rittenhouse, H. R., Marks, L. S., Song, W., Wheeler, T. M., and Slawin, K. M. “BPSA,” a specific molecular form of free prostate specific antigen, is found predominantly in the transition zone of patients with nodular BPH. *Urology*, 55: 1–5, 2000.
- Wheeler, T. M., and Lebovitz, R. M. Fresh tissue harvest for research from prostatectomy specimens. *Prostate*, 25: 274–279, 1994.
- Finlay, J. A., Evans, C. L., Day, J. R., Payne, J. K., Mikolajczyk, S. D., Millar, L. S., Kuus-Reichel, K., Wolfert, R. L., and Rittenhouse, H. G. Development of monoclonal antibodies for human glandular kallikrein (hK2): development of a dual antibody immunoassay for hK2 with negligible prostate-specific antigen cross-reactivity. *Urology*, 51: 804–809, 1998.
- Grauer, L. S., Finlay, J. A., Mikolajczyk, S. D., Pusateri, K. D., and Wolfert, R. L. Detection of human glandular kallikrein, hK2, as its precursor form and in complex with protease inhibitors in prostate carcinoma serum. *J. Androl.*, 19: 407–411, 1998.
- Wang, T. J., Linton, H. J., Sokoloff, R. L., Grauer, L. S., Rittenhouse, H. G., and Wolfert, R. L. Antibody specificities for PSA and PSA fragments by SDS-PAGE Western blot analysis. *Tumor Biol.*, 20: 79–85, 1999.
- Finlay, J. A., Day, J. R., and Rittenhouse, H. G. Polyclonal, and monoclonal antibodies to prostate-specific antigen can cross-react with human kallikrein 2, and human kallikrein 1. *Urology*, 53: 746–751, 1999.
- Kumar, A., Mikolajczyk, S. D., Goel, A. S., Millar, L. S., and Saedi, M. S. Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. *Cancer Res.*, 57: 3111–3114, 1997.
- Christensson, A., Laurell, C. B., and Lilja, H. Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur. J. Biochem.*, 194: 755–763, 1990.
- Hilz, H., Noldus, J., Hammerer, P., Buck, F., Luck, M., and Huland, H. Molecular heterogeneity of free PSA in sera of patients with benign and malignant prostate tumors. *Eur. Urol.*, 36: 286–292, 1999.
- Khan, A. R., and James, M. N. G. Molecular mechanisms for the conversion of zymogens to active proteolytic enzymes. *Protein Sci.*, 7: 815–836, 1998.
- Clements, J. The glandular kallikrein family of enzymes: tissue specific expression and hormonal regulation. *Endocr. Rev.*, 10: 393–419, 1989.
- Takayama, T. K., Fujikawa, K., and Davie, E. W. Characterization of the precursor of prostate-specific antigen-activation by trypsin and by human glandular kallikrein. *J. Biol. Chem.*, 272: 21582–21588, 1997.
- Lovgren, J., Rajakoski, K., Karp, M., Lundwall, A., and Lilja, H. Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. *Biochem. Biophys. Res. Commun.*, 238: 549–555, 1997.
- Mikolajczyk, S. D., Millar, L. S., Marker, K. M., Rittenhouse, H. G., Wolfert, R. L., Marks, L. S., Charlesworth, M. C., and Tindall, D. J. Identification of a novel complex between human kallikrein 2 and protease inhibitor-6 in prostate cancer tissue. *Cancer Res.*, 59: 3927–3930, 1999.