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The Role of Prostate-Specific Antigen in the Chemoprevention of Prostate Cancer

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Abstract An understanding of the natural history of changes in prostate-specific antigen (PSA) may be valuable as a surrogate view of prostate dynamics, as a method to differentiate between benign and malignant growth, and as a means to assess the use of PSA as a tool for monitoring activity of chemoprevention agents. Although PSA appears to be useful as a noninvasive marker of prostatic growth, PSA changes should not be confused with a direct measure of tumor growth. Serum PSA levels are a function of tumor volume but are also influenced by the volume of benign epithelium, grade of carcinoma (if any), inflammation, androgen levels, growth factors, and the extracellular matrix.

The biological functions of PSA in the prostate and in its secretions need to be more completely elucidated in order that PSA measurements may more accurately describe prostate dynamics. The expression of PSA is androgen-regulated. It is one of the most abundant prostate-derived proteins in the seminal fluid. Seminogelin, a major protein in seminal fluid, is cleaved by PSA, and this cleavage is important in the liquefaction of semen. Less is known about other PSA substrates.

Current PSA studies indicate that cancer cases exhibit an early slow linear PSA phase followed by a rapid exponential phase, and that PSA levels begin to increase exponentially approximately 7–9 years before diagnosis. The establishment of age-specific PSA reference ranges (ASRR) and of PSA velocity (PSAV) rates provide elements of a baseline from which prediction models could measure malignant potential of a prostatic carcinoma. Moreover, recent discoveries of different molecular forms of PSA in serum may allow a much more accurate differentiation of benign and malignant growth as well as a more potent measure of the impact of chemoprevention agents.

If PSA doubling time is approximately 2.4–3.0 years and accurately reflects tumor doubling time, and if the average man has less than 0.5 ml of latent prostatic tumor tissue and the average stage T_2 cancer is approximately 4 ml when detected, then the available PSA data suggest that the 3 doublings necessary to change from 0.5–4.0 ml. would take 7–12 years for a typical small volume tumor to reach the size of most stage T_2 tumors. The findings that histologic cancers appear at much younger ages than previously known is disturbing. It indicates that disease initiation may begin sooner than ever thought likely. "Normal" PSA levels for younger men (< 40 years of age) may need to be studied, and an emphasis upon premalignant lesions in this age group may be necessary. Younger men may represent the most appropriate population and premalignant lesions the most relevant clinical factor for prostate cancer chemoprevention studies and trials.

The molecular composition and molecular changes of PSA derived from premalignant lesions have yet to be elucidated, but such investigations may lead to a more complete understanding of the possible progression or transformation of normal prostate cells to premalignancy and subsequently to carcinoma. High grade prostatic intraepithelial neoplasia (PIN) in and of itself does not account for elevated serum PSA levels, but subtle changes in the molecular dynamics of PSA may reveal the influence of androgens and the impact of chemopreventive agents. J. Cell. Biochem. 25S:149–155.

Key words: age-specific PSA reference ranges; complexed PSA; free PSA; prostate-specific antigen; PSA velocity

Prostate specific antigen (PSA) is the most useful tumor marker in clinical practice today [1]. Its use as a test for the early detection of prostate cancer has ushered in a new era of significant advances in prostate cancer detection, diagnosis and treatment. PSA is not specific to prostate cancer, and investigations to improve its sensitivity and specificity suggest methods for the consideration of PSA as a measurable and, perhaps, modulatable intermediate endpoint in prostate cancer chemoprevention studies.

The natural history of PSA changes may be valuable as a surrogate for prostate growth, particularly when considering chemopreven-

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tion strategies to prevent disease initiation or to reverse/retard disease promotion and progression. Although PSA appears to be useful as a noninvasive marker of prostatic growth, PSA changes should not be confused with a direct measure of glandular growth or reduction. Serum PSA levels are a function of tumor volume, but PSA production is also influenced by the volume of benign epithelium, the grade of adenocarcinoma (if any), inflammation, androgen levels, growth factors and the extracellular matrix.

PRODUCTION AND STIMULATION OF PSA

As a serine protease in the kallikrein gene family, PSA presumably has some biological function in the prostate or in its secretions. Seminogelin, a major protein in seminal fluid, is cleaved by PSA, and this cleavage is apparently an important part of the liquefaction of semen [2]. PSA also cleaves one of the six binding proteins of the insulin-like growth factor, IGF BP-3. From in vitro studies, investigators has learned that although IGF BP-3 acts to inhibit IGF-II activity, its cleavage by PSA reverses this inhibition and frees IGF to stimulate proliferation [3,4]. Unfortunately, far less is known of other biological substrates of PSA.

The basement membrane of the acini, basal cells lining the acini, and stromal cells acts as barriers to prevent the escape of PSA into blood circulation. Therefore, the serum levels of PSA are normally maintained below 4 ng/ml, which corresponds to about 10^{-6} of the levels of PSA in seminal fluid [5].

Occurrence of Abnormally Elevated Serum Concentrations of PSA

Prostate cancer can cause a breakdown of the barriers that prevent escape of PSA into the extracellular fluids resulting in elevated PSA levels in the blood. However, elevated serum levels of PSA do not always result from prostate cancer. Benign conditions, such as bacterial prostatitis, urinary retention, and benign prostatic hyperplasia (BPH) may also be the cause of elevated PSA levels. PSA increases approximately in proportion to the volume of prostatic cancer [6]. Although PSA concentrations also increase with the volume of BPH tissue, the average increase is small (0.3 ng/ml per gram of tissue) when compared with that associated with cancer, about 3.5 ng/ml per gram of malignant tissue [7].

Approximately 25% of all men with histopathologic diagnosis of BPH have PSA levels in serum above the commonly used cut-off value of 4.0 ng/ml [1]. In fact, between 4.0 and 9.9 ng/ml, where the largest percentage of elevated PSA results will occur, only about 25% will have prostate cancer. On the other hand, 20–30% of patients with prostate cancer have serum PSA concentrations <4.0 ng/ml at the time of diagnosis, usually made as a result of digital rectal examination (DRE). A baseline or initial PSA level of 2.0–3.0 ng/ml in fact represents a much higher risk of an eventual diagnosis of prostate cancer than does a PSA of 1.0 ng/ml [8]. It is evident that these parameters not only limit the exactitude of PSA testing in the early detection of prostate cancer, but they strongly suggest that participants in prostate cancer chemoprevention trials have PSA levels of no higher than 3.0 ng/ml at entry. This threshold was in fact established for the Prostate Cancer Prevention Trial.

Hormonal Regulatory Mechanism of PSA Expression

Although PSA is the best characterized protein within the human kallikrein family, very little is known about the regulatory mechanism of its expression. Young et al. suggest that PSA mRNA expression is regulated by androgens in a cell-specific fashion [9]. PSA mRNA is localized in the epithelium of the prostate. Northern blot analysis has shown that PSA expression is regulated by androgens in LNCaP cells. In addition, the effect of androgens on PSA expression can be blocked by hydroxyflutamide (a competitive inhibitor of the androgen receptor). Therefore, the androgenic effects of PSA mRNA in LNCaP cells is most likely via the function of the androgen receptor.

Once bound to androgen, the androgen receptor regulates the expression of androgen-responsive genes by binding to their androgen-responsive elements and by interacting with other transcription factors. A notable gene regulated by androgen in prostate cells encodes prostate-specific antigen [10].

Hormones, testosterone (T) and its metabolite in the prostate, dihydrotestosterone (DHT), influence/regulate cell differentiation and prostate growth. T is transformed to DHT by the 5-α reductase enzyme. DHT has a 3-fold greater binding power to the androgen receptor than does T. Moreover, in vitro studies have shown

that hormones up-regulate PSA [9,11]. For chemoprevention agents, therefore, which do not interfere with the androgen receptor, PSA may be a good surrogate marker for disease promotion, progression, or its reversal.

ESTABLISHING BASELINE PARAMETERS OF PSA TO EVALUATE IMPACT OF CHEMOPREVENTION AGENTS

Although PSA has relatively high test sensitivity and specificity for the early detection of prostate cancer, various analytic techniques have been proposed to improve these characteristics. Several of these may be considered as PSA parameters in chemoprevention studies. These methods include calculations of PSA density (PSAD) [12], PSAD adjusted for volume of transition zone [13], analysis of PSA velocity (PSAV) [14], and application of age-specific PSA reference ranges (ASRR) [15]. The calculation of PSAD necessarily involves a transrectal ultrasound (TRUS) examination in order to derive an estimation of prostatic volume. Volume-based PSAD calculations may be justifiable for judging more accurately the presence or absence of prostate cancer, but such procedures would be inappropriate for chemoprevention intermediate end-point evaluations.

Age-Specific PSA Reference Ranges

Age-specific PSA reference ranges (ASRR) represent another method to improve PSA's sensitivity and specificity. Studies have documented the correlation of age to PSA [15–18]. As men age, PSA values increase. This phenomenon has been attributed primarily to increasing prostatic volume. Other factors hypothesized to be responsible for the increase of PSA with age include prostate infections, prostatic infarction, microscopic prostate cancer, and a normal prostatic "aging" process that may allow increased leaking of PSA into serum. Quantifiable evidence has recently been provided of the impact of benign enlargement and inflammation on elevated PSA values among older men with no evidence of prostate cancer [19].

Age-specific PSA reference ranges can contribute data toward the establishment of baseline PSA measurements prior to and during the implementation of chemoprevention trials. Race (by age)-specific PSA reference ranges may provide even more specificity [20].

In a clinical investigation of PSA conducted during Prostate Cancer Awareness Week (PCAW) in 1993–1994, data was compiled from 77,890 records. Two hundred and fifty participating centers tested men in this study which has been described previously [21,22]. Records included men aged 40–79 years of age. Reported cases of prostate cancer were deleted. Records from 1994 of men also tested in 1993 were deleted as were outlier PSA values 20 ng/ml. The cohort was separated into four 10-year age groups by decade. All age groups exhibit a normal age distribution. Statistically significant differences ($P = < 0.00001$) were found among mean PSA values for age groups 40–49, 50–59, 60–69, and 70–79 (Table I). Moreover, variances are significantly different among all four age groups ($P = < 0.00001$). Variances increase significantly in each successively older age group ($P = < 0.00001$). Such variance may necessitate the implementation of chemoprevention studies with younger men (e.g., 30–50 years of age) among whom greater PSA standardization exists.

Observable racial differences exist within the association of age and PSA. A consistent relationship exists between mean age and mean PSA except for blacks (Table II). Whites had the highest mean age (61.4 years) and highest mean PSA (1.63 ng/ml), followed by Asian Americans (59.9 years, 1.58 ng/ml) and then Latinos (57.3 years, 1.41 ng/ml). Blacks had the lowest mean age (55.5 years) and the greatest variance in PSA values (SD = 1.87). Blacks were followed, in descending order of PSA variance, by whites, Asian Americans, and Latinos. Racial variations in PSA may complicate protocol formulae of chemoprevention studies.

Retrospective studies have shown the predictive power of baseline PSA measurements to

TABLE I. Mean Age and PSA Values, by 10-Year Age Groups

Age group (n)	Mean age	Mean PSA (SD)[a]
40–49 (9,383)	45.34	0.83 ng/ml (0.79)
		$P = <0.00001$*
50–59 (24,023)	54.65	1.23 ng/ml (1.33)
		$P = <0.00001$*
60–69 (28,601)	64.45	1.83 ng/ml (1.94)
		$P = <0.00001$*
70–79 (15,693)	73.46	2.31 ng/ml (2.35)

[a]Standard deviation.

* = A statistically significant difference ($P = <0.0001$) was consistently found when comparing the mean PSA and the variance (SD) of each age cohort with the mean PSA and the variance of every other cohort.

TABLE II. Mean Age, Mean PSA (ng/ml), by Race

Race	(n)	Mean age	Mean PSA (SD)[a]
White	(70,772)	61.37	1.63 (1.83)
Black	(4,485)	55.50	1.47 (1.87)
Latino	(1,543)	57.26	1.41 (1.79)
Asian	(900)	59.58	1.58 (1.80)

[a]Standard deviation.

identify men at higher risk of prostate cancer [8,23]. Using a baseline (monoclonal) PSA of ≤1.0 ng/ml (Relative Risk [RR] = 1.0) with a ten-year follow-up, Gann and colleagues graphed the increasing risk of higher baseline PSA values: 1.01–1.50 ng/ml = 2.2 RR; 1.51–2.00 ng/ml = 3.4 RR; 2.01–3.00 ng/ml = 5.5 RR; and 3.01–4.00 ng/ml = 8.6 RR; 4.0–10.0 ng/ml = 22.2 RR [8]. In the above-mentioned PCAW investigation of PSA, using Gann's PSA categories, the percent breakout by age and racial categories is shown in Table III. Although the PCAW population is a self-selected one, estimations can be made from these data of recruitment prospects for chemoprevention trials based on relative risk of baseline PSA.

PSA Velocity

PSA velocity (PSAV) is an important corollary to ASRR. These analytic techniques (PSAV and ASRR) represent two interconnected methods by which to formulate a "moving" baseline of normal PSA values. The Baltimore Longitudinal Study of Aging (BLSA) has provided the most extensive data on PSA velocity and a subsequent diagnosis of prostate cancer [24,25]. The BLSA data demonstrate that men with no prostatic disease exhibited a slow linear increase in PSA levels, approximately 0.04 ng/ml (±0.02) per year, supporting the concept of ASRR and some annual mean PSAV [24]. However, BPH caused a gradual acceleration by age in the rate of change in PSA (60 years of age = 0.07 ng/ml; 70 years = 0.15; 80 years = 0.23). This partially explains the widening of PSA variation with age. In contrast to men with no prostatic disease or with BPH only, cancer cases in the BLSA exhibited an early linear phase (0.10 ng/ml annually) followed by an exponential phase of PSAV prior to diagnosis [24,25]. Moreover, the slope of the exponential phase was much steeper for advanced/metastatic cases than for local/regional cases. On average, the exponential phase of PSA increase began 7–9 years before clinical detection [24].

Intraindividual PSA variation and sampling variability of PSA represent limitations to our current understanding of PSAV. The physiologic intraindividual variation has been estimated to be between 24–30% when the same PSA assay is used on a different day [26]. The sampling interval may also be critical to PSAV calculations. Some investigators have cautioned against PSAV calculations based on only two serial PSA measurements one-year apart [27]. In the above-mentioned clinical investigation of PSA, PSAV calculations indicate correlation with ASRR: mean PSAV increases with age as does the degree of PSAV variance (Table IV). When combined with an analysis of molecular changes in PSA, PSAV could provide an important intermediate endpoint consideration for prostate cancer chemoprevention trials.

Free, Complex and Total PSA

Recent studies have identified several molecular forms of PSA in the serum, and the concentration of these forms has been shown to vary according to the predominant prostatic disease [28–31]. The major proportion of serum PSA (85%) is complexed to α_1-antichymotripsin (ACT), one of the major liver-derived serine protease inhibitors in serum [28,29]. A small fraction (15%) of serum PSA occurs in a free, uncomplexed molecular form. PSA complexed to ACT is higher in men with prostate cancer than in men with benign prostatic hyperplasia (BPH) only. In prostate cancer patients, the proportion of free-to-total PSA is significantly lower than in men with BPH (median ratios, 0.18 versus 0.28, $P = < 0.0001$) [30]. The identification of these molecular forms of PSA and of their relationship to BPH or prostate cancer has led to the formulation of age-specific reference ranges for free, complexed and total PSA [32].

POSSIBLE ANSWERS RAISE ADDITIONAL QUESTIONS

If Age Is Important, When Should Prostate Cancer Chemoprevention Trials Begin?

Because of uncertainty regarding the prevalence and true incidence of prostate cancer, controversy continues to plague the early detection of the disease [33]. However, if a prostatic malignancy can be judged as potentially clini-

TABLE III. Prostate-Specific Antigen (ng/ml) Risk Categories by Age and Race (%)

	PSA (ng/ml)					
	≤1.0	1.01–1.5	1.51–2.0	2.01–3.0	3.01–4.0	4.01–10.0
Age						
40–49	78.6%	12.6%	4.6%	2.7%	0.8%	0.7%
50–59	61.7%	16.6%	8.2%	7.0%	3.1%	3.3%
60–69	43.9%	17.5%	11.1%	12.5%	6.7%	8.3%
70–79	34.6%	15.8%	11.6%	15.2%	9.3%	13.5%
Race						
White	51.2%	16.3%	9.6%	10.3%	5.5%	7.0%
Black	58.2%	15.9%	8.3%	8.1%	4.2%	5.3%
Latino	59.8%	14.7%	7.4%	9.2%	3.9%	5.2%
Asian	53.3%	16.9%	9.2%	8.4%	5.3%	6.9%
All (N)	39,911	12,547	7,326	7,820	4,162	5,283

TABLE IV. Prostate Cancer Awareness Week, 1993–1994 PSA Velocity (ng/ml), by Age

Age	Mean PSAV	SD[a]	Median	75th %
40–49 (1,556)	−0.02	0.2	0	0.1
50–59 (6,878)	0.04	0.7	0	0.2
60–69 (10,701)	0.05	1.0	0	0.2
70–79 (7,185)	0.06	1.2	0	0.3

[a]Statistically significant differences ($P \leq 0.001$) were found between the PSAV variance of all age groups, after log transformation of data.

cally aggressive by histologic grade, morphology and other prognostic variables, most clinicians would agree that radical therapy would offer the most efficient approach and the greatest chance of cure. Prevention strategies would seem inappropriate. However, if all of the promotional steps to malignancy have not occurred, prevention seems wholly reasonable. Current research has significantly broadened our power to predict which tumors have the capacity to manifest aggressive behavior. If one of the malignant steps can be blocked, clinical prostate cancer may be prevented.

The finding that histologic cancers appear at much younger ages than previously known is disturbing [34]. It indicates that initiation factors may begin sooner than ever thought likely, but it also suggests that the span of time during which promotional events occur may be considerable longer as well. Perhaps, the window of opportunity for chemoprevention of prostate cancer has widened. Unfortunately, the history of PSA in younger men (<40 years of age) has not been studied nor have "normal" PSA levels for this age been established. Despite the inconclusiveness that prostatic intraepithelial neoplasia (PIN) is a premalignant lesion of prostate cancer, chemoprevention strategies may well focus upon PIN among younger cohorts of men. But what if any connection can be made between PIN and PSA? What biologic role, if any does PSA play in marking, exacerbating, or controlling PIN? Current research indicates that high grade PIN in and of itself does not account for elevated serum PSA levels and that high-grade PIN has no correlation with PSA ($r = 0.16$) or with PSA density ($r = 0.14$) [35]. However, could PIN-affected PSA levels still be evaluated in terms of molecular composition and by deviation from the norms of ASRR and PSAV?

Potential Chemoprevention Agents for Prostate Cancer: What Are Their Effects Upon PSA

Translational research in the basic science of prostate diseases is moving the chemoprevention laboratory closer to the clinic [36]. Agents whose major effect on prostatic epithelial cells is growth inhibition have been identified: vitamin A and vitamin D (endocrine factors), and transforming growth factor-β (TGF-β). Retinoic acid, the active metabolite of vitamin A, regulates differentiation as well as growth in prostate cells [37]. It has been studied with prostate cancer in vivo, so no indication is yet available of its effect, if any, on PSA [38,39]. Vitamin D, a steroid hormone and not actually a vitamin, has been shown to irreversibly inhibit growth in both benign and malignant prostate cells [40]. However, reports of the effects of 1a,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$] on PSA are somewhat ambiguous. Peehl et al. [40] found that exposure to $1\alpha,25(OH)_2D_3$ did not alter morphology and that keratins associated with the phenotype of secretary cells of the prostatic epithelium were not increased. However, Miller

et al. [41] found that PSA production by LNCaP cells was stimulated by $1\alpha,25(OH)_2D_3$.

At this juncture, no clear answer is available as to what the impact of a given chemopreventive agent is on PSA. One of three things can be hypothesized to happen: PSA production could plateau, it could rise or it could fall. With a given chemopreventive agent, a serum marker could plateau. If cells differentiate (presumably with apoptosis), fewer cells might exist across time, causing PSA perhaps to fall. On the other hand, with some differentiating agents, the amount of PSA produced per cell could increase over time.

Initial prostate cancer chemoprevention studies should evaluate effects of agents on PSA. Such studies should use norms of age-specific PSA reference ranges and PSA velocity to measure possible effects, and should analyze molecular types of PSA before, during, and after the chemoprevention intervention. Confounding variables, such as race, testosterone levels, and PSA density need also to be taken into account.

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