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Urinary α HGF, IGFBP3 and OPN as diagnostic and prognostic biomarkers for prostate cancer

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Abstract

Aim: Serum PSA screening for prostate cancer (PCa) is controversial. Here, we identify three urinary biomarkers – α HGF, IGFBP3 and OPN – for PCa screening and prognostication.

Methods: Urinary α HGF, OPN and IGFBP3 from healthy men ($n = 19$) and men with localized ($n = 65$) and metastatic ($n = 36$) PCa were quantified via ELISA. Mann–Whitney nonparametric t -test and the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) analyses were used to analyze associations.

Results: Mean α HGF and IGFBP3 levels were significantly elevated in PCa patients versus controls ($p = 0.0006$ and $p = 0.0012$, respectively), and the area under the curve of the receiver operating characteristic curve (indicator of diagnostic accuracy) for α HGF and IGFBP3 was 0.75 and 0.74, respectively. OPN levels were significantly higher in metastatic groups ($p = 0.0060$) versus localized and controls (area under the curve = 0.68).

Conclusion: Urinary α HGF and IGFBP3 exhibit the capacity for diagnostic discrimination for PCa, whereas OPN may indicate presence of metastatic disease.

Executive summary

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Background—PSA is the most widely used prostate cancer (PCa) biomarker for screening and prognostication, but it is limited in its capacity to resolve between the presence and absence of disease and the extent of disease.

Urinary proteins have great potential over serum biomarkers and urinary nucleic acids as they are able to functionally interrogate the prostatic tumor.

*a*HGF, IGFBP3 and OPN are potential urinary biomarkers that have been shown to be increased in other media such as plasma and serum.

Methods— Urine samples of patients enrolled in NCI protocols were used in this study: localized PCa (n = 65), metastatic PCa (n = 36) and normal men (n = 19).

Samples were run in duplicate via ELISA kits for each biomarker. The protein levels were normalized for urinary creatinine.

Statistical analysis was performed using the Mann–Whitney nonparametric t-test to compare medians and Kruskal–Wallis nonparametric analysis of variance tests to compare the means. Receiver operating characteristic curve analysis with area under the curve was based on 95% CI.

Results— *a*HGF and IGFBP3 concentrations were significantly different between the control and the localized group, and between the control and the metastatic group. Concentrations were also significantly higher in the PCa group compared with the non-PCa group.

With the chosen thresholds, the sensitivity of IGFBP3 is the highest at 98% with a specificity of 58%. The positive-predictive value for *a*HGF is 93%.

OPN concentrations were significantly different between localized and metastatic groups, and healthy and metastatic groups.

OPN has a high negative-predictive value for having metastatic versus localized disease (78%).

Discussion—This study suggests the value of measuring urinary levels of *a*HGF and IGFBP3 for the diagnosis of PCa, and OPN as a discriminator of localized or metastatic PCa. Our findings will need to be further validated in larger studies with matched controls.

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) analysis highlights the interactions between the three biomarkers, and other molecules such as CD44 and FN1 that play important roles in tumorigenesis.

Future directions may include the development of a multiplex of biomarkers to better characterize the heterogeneity of PCa.

Keywords

biomarker; HGH; OPN; prostate cancer; urine

Prostate cancer (PCa) is the second leading cause of cancer death among men in the USA [1]. PSA is the most widely used PCa biomarker for screening and prognostication [2]. PSA is produced by prostatic glandular epithelial cells and thus may be detected in healthy controls, typically at levels below 4.0 ng/ml. Because PSA is produced by prostatic epithelia, elevated PSA levels may be associated with cancer, benign prostatic hypertrophy and prostatic infection.

The limitations of PSA in regard to sensitivity and specificity are well described. For example, in a study comparing 540 cases of PCa with 1034 controls, PSA cutoff values of 3, 4 and 5 ng/ml could not yield sensitivities higher than 59% [3]. Data from 9459 men treated with placebo and biopsy in the Prostate Cancer Prevention Trial demonstrated that 17% of men with a normal digital rectal examination and low PSA (1.1–2.0 ng/ml) were discovered to have PCa, with 12.5% of the patients with a PSA of <0.5 ng/ml harboring high-grade cancer [4]. Furthermore, as many as 65–70% of patients presenting with PSA ranging between 4 and 10 ng/ml will have a negative biopsy result [5]. These data highlight the limitations of PSA as a screening modality.

PSA has also been used as a prognostic or predictive marker in patients diagnosed with PCa. The PSA level and the rate of rise or decline have been evaluated in this regard. Unfortunately, low or undetectable PSA levels may still occur in metastatic disease, and high levels of PSA do not necessarily equate to metastatic disease [6,7]. As a single biomarker, PSA has a diminished capacity for resolving between the presence and absence of disease and the extent of disease (localized vs metastatic).

PCA3 is a RNA urine marker that was recently approved by the US FDA for patients with elevated PSAs and negative biopsy. However, there is still controversy as to its ability to predict tumor stage and Gleason score. TMPRSS2:ERG is a fusion transcript that has been studied in combination with PCA3 and was found to increase the area under the curve (AUC) from 0.66 to 0.75 in a study of 471 men [8]. Although these biomarkers are promising, protein urinary markers have more potential for functionally interrogating the tumor and do not require exfoliated cancer cells to be present for detection.

Urinary biomarkers have great potential over serum markers for the detection of PCa as prostatic products are directly secreted into the urinary tract, theoretically increasing the possibility of detection. Although urinary nucleic acids have been investigated as PCa biomarkers, it is likely that analysis of secreted or shed proteins may provide a more effective way to functionally interrogate prostate tumors [9]. Although much work has focused on identifying a single biomarker, it is conceivable that a combination of markers may provide improved diagnostic and prognostic accuracy, thus alleviating unnecessary procedures, cost and morbidity [10].

Here, we report the evaluation of three specific proteins as biomarkers for PCa: *aHGF*, *IGFBP3* and *OPN*. These proteins have yet to be identified as urinary biomarkers in PCa, but have been shown to play key roles in PCa initiation and progression. HGF is a pleiotropic cytokine that has been implicated in angiogenesis, adhesion, migration, invasion and proliferation of PCa cells [11]. Elevated quantities of activated HGF have been detected in serum of PCa patients [12]. Similarly, elevated levels of c-Met, the tyrosine kinase receptor for HGF, have been detected in the urine of PCa patients [13,14], suggesting the importance of the HGF–c-Met axis in PCa. *IGFBP3*, a component of the IGF system, has been reported to be involved with cellular differentiation, survival and proliferation; recent studies have shown higher levels of plasma *IGFBP3* to correlate with an increased likelihood of harboring PCa [15]. Finally, *OPN*, also known as *SPP1*, is an extracellular matrix protein with a number of diverse roles, including blood vessel formation and tumorigenesis [16].

OPN has been found to have significantly increased expression at the mRNA and protein levels in patients with aggressive PCa [17]. We chose to study these three biomarkers in urine based on prior reports suggesting increased expression in serum of PCa patients, as well as the known importance of these pathways in PCa progression. We further investigated protein–protein interactions using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database to examine if these three biomarkers are in the same tumorigenic pathway and how this may help explain our clinical findings and results [101]. We hypothesized that the levels of these three proteins in the urine would allow a discrimination of the presence or absence of PCa and would provide a method for determining the extent of disease.

Methods

Patients & urine processing

The prospective clinical studies 02-C-0064, 04-C-0257 and 09-C-0195 were approved by the Institutional Review Board of the National Cancer Institute (NCI; MD, USA). Informed consent was obtained from each patient or normal control. Urine samples were obtained in 65 patients with localized PCa (n = 12 low risk; n = 24 intermediate risk; n = 29 high risk) on protocol 02-C-0064. For patients with localized disease, risk stratification followed the D'Amico criteria [18]. Low risk included patients with Gleason ≤ 6, PSA <10 ng/ml and clinical stage ≤ T2a. Intermediate risk was defined as T2b, PSA between 10 and 20 ng/ml or Gleason = 7 with no high-risk features. High risk was defined as PSA ≥ 20 ng/ml, clinical stage T3 or Gleason ≥ 8. 35% of patients with localized cancer received hormonal therapy. Two patients from protocol 02-C-0064 were classified as metastatic for analysis, owing to the presence of lymph node metastasis. The clinical characteristics of the patients included with localized PCa are listed in Table 1. Urine samples from patients with metastatic PCa were acquired from patients enrolled in protocol 04-C-0257 and protocol 09-C-0195 (n = 36). Patients with advanced disease who did not respond to castration or hormonal therapy (either leuprolerin or bicalutamide) were enrolled in this study. Urine samples from males with no evidence of PCa were obtained for comparison (n = 19). After collection, urine was centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant was aliquoted and stored at –20°C until use.

ELISA quantification

Samples were thawed on ice 3 h prior to assay. Quantification of biomarkers in urine specimens was determined via ELISAs. ELISA kits for OPN (R&D Systems, MN, USA) and IGFBP3 (R&D Systems) were used according to manufacturer's instructions. Samples were plated in a 96-well format in duplicate, after which polyclonal antibody against IGFBP-3 or OPN conjugated to horseradish peroxidase was added. The substrate solution (hydrogen peroxide/teramethylbenzidine) was then added to each well and incubated for 30 min, and then subsequently quenched with sulfuric acid. *sHGF* levels in urine specimens were measured using ELISA kits according to the manufacturer's protocol (Immuno-Biological Laboratories Co., Ltd, Japan). Undiluted test samples were added onto the precoated plate in duplicate and then incubated at 37°C with a labeled antihuman HGF IgG antibody conjugated with horseradish peroxidase. Chromogen substrate solution was added

to each well, incubated at room temperature and then quenched with sulfuric acid. For all kits, plates were read at an absorbance of 450 nm on a Victor™ 3 Plate reader (Perkin Elmer, MA, USA). The extrapolated absorbance was analyzed using MasterPlex Readerfit ELISA software (Hitachi, MA, USA) and the concentration was determined following a 4-parameter logistic curve fit according to the manufacturer's recommendation. Urinary creatinine was measured with the Bayer DCA 200+ Analyzer (Germany). Protein levels in urine were normalized to urinary creatinine for each sample to account for possible compromised kidney function.

Statistical analysis

All analyses were performed with GraphPad Prism version 5.0 (CA, USA). Differences between risk classification groups and the association between biomarkers and categorical clinicopathologic variables were analyzed using the Mann–Whitney nonparametric t-test (two-tailed) to compare medians between the groups. Kruskal–Wallis nonparametric analysis of variance tests were used to compare the means. Receiver operating characteristic curve analysis was used to analyze the diagnostic accuracy of each biomarker. The AUC was estimated based on a 95% CI.

Results

To determine the diagnostic utility of *a*HGF, IGFBP3 and OPN in the setting of PCa, we first analyzed the results of each candidate biomarker for a comparison of presence or absence of PCa (metastatic and localized cases combined) followed by an examination of differences between all three groups (healthy control, localized PCa, metastatic PCa).

The mean levels of *a*HGF were 111.3 pg/mg (median: 96.92 pg/mg; range: 27.0–250.0) for control, 235.2 pg/mg (median: 215.8; range: 24.6–668.0) for localized cancer and 220.8 pg/mg (median: 181.6; range: 20.9–1010.0) for the metastatic cancer groups (Figure 1A & Table 2). The comparison of urinary *a*HGF levels between the healthy control subjects and the PCa subjects was statistically significant (Mann–Whitney nonparametric t-test; $p = 0.0006$; Table 3). Furthermore, *a*HGF concentrations were significantly different between the control and the localized group ($p = 0.0006$) and between the control and the metastatic group ($p = 0.0059$; Table 3). The Kruskal–Wallis test confirmed that there was a significant difference between all three groups ($p = 0.0020$) in regard to urinary *a*HGF concentrations (Table 2). The AUC of the receiver operating characteristic curve, which is associated with the diagnostic accuracy of *a*HGF between control and PCa groups, was 0.75 ($p = 0.0006$; Figure 1).

For IGFBP3, the mean for controls was 0.648 ng/mg (median: 0.1582; range: 0–4.282), localized cancer was 0.8862 ng/mg (median: 0.5219; range: 0–7.045) and metastatic cancer was 1.353 ng/mg (median: 0.5132; range: 0.1095–6.256) (Figure 2A & Table 2). Similar to our results with *a*HGF, normalized IGFBP3 levels were significantly higher in the PCa group compared with the non-PCa group using the Mann–Whitney nonparametric t-test ($p = 0.0012$; Table 3). When the PCa group was analyzed separately, IGFBP3 was significantly higher in the localized group ($p = 0.0024$) and the metastatic group ($p = 0.0024$) as compared with the control (Table 3). The three groups were statistically different according

to the Kruskal–Wallis test ($p = 0.0041$; Table 2). The diagnostic accuracy of IGFBP3 is comparable to that of *a*HGF with an AUC of 0.74 ($p = 0.0012$; Figure 2).

The mean level of OPN for the control was 532.4 ng/mg (median: 323.5; range: 51.0–1559.0), while the levels for localized and metastatic groups were 613.6 ng/mg (median: 459.1; range: 2.3–2716.0) and 1024.0 ng/mg (median: 901.1; range: 123.6–3304.0), respectively (Figure 3A & Table 2). Unlike the diagnostic abilities of *a*HGF and IGFBP3, OPN showed prognostic potential. The mean normalized OPN levels for healthy men, men with localized cancer and men with metastatic cancer were significantly different from each other as demonstrated by the Kruskal–Wallis non parametric analysis of variance test ($p = 0.0160$; Table 2). Furthermore, the urinary OPN levels were significantly different between healthy and metastatic groups ($p = 0.0060$), as well as between the localized and metastatic groups ($p = 0.0086$; Table 3). The AUC for distinguishing between localized and metastatic groups was 0.68 ($p = 0.0030$; Figure 3). Therefore, individuals with higher urinary OPN were significantly more likely to have disseminated disease.

To clarify whether these results were useful in the context of other known clinical prognostic factors, we evaluated each biomarker for correlations with PSA, clinical stage and Gleason score in the localized PCa cohort. The clinical characteristics of patients with the localized form of PCa are presented in Table 1. Interestingly, urinary *a*HGF, IGFBP3 and OPN did not show any significant positive or negative correlation to any of these accepted prognostic criteria.

For further comparison, we generated ‘gates’ using 1 standard deviation above/below the pooled average using a previously published method [19]. A threshold value was chosen that would maximize the sensitivity of the test, at the risk of decreased specificity. As described in Table 4, a threshold of 79 for *a*HGF and 53 pg/mg for IGFBP3 was used as the cutoff for diagnosis of PCa. For OPN a threshold of 628 pg/mg was used as the threshold for the diagnosis of meta-static PCa versus localized cancer. With the chosen thresholds, the sensitivity of IGFBP3 is the highest at 98% with a specificity of 58% (Table 4). The sensitivity and specificity of *a*HGF is comparable at 85% and 32%, respectively, and a positive-predictive value of 93%. OPN has a high negative-predictive value for having metastatic vs localized disease at 78%.

To examine if these three biomarkers are related in the context of cancer signaling, we studied general protein–protein interactions using the STRING database (Figure 4) [101]. We were able to identify several well-known intermediary angiogenic factors, such as VEGFA and MMP2. In addition, all three molecules were experimentally validated to interact with CD44 and FN1 (Figure 4), suggesting that CD44 and FN1 may play key roles in prostate tumor initiation and progression. A number of other molecules implicated in tumor–stroma interactions, invasiveness and metastasis were identified in this analysis.

Discussion

Since the introduction of PSA screening, epidemiological studies have shown increased PCa diagnosis rates, but its beneficial impact on survival has been inconclusive [20]. Novel

biomarkers for PCA with higher sensitivity are needed, but most helpful would be biomarkers that could provide prognostic benefit as well. Urine is an ideal medium for evaluation of bio-markers as it is obtained noninvasively [10]. Our urinary evaluation of *aHGF*, *IGFBP3* and *OPN* showed that *aHGF* and *IGFBP3* exhibit the capacity for diagnostic discrimination, whereas *OPN* may have the ability to stratify localized and metastatic disease. HGF, also known as SCF, is a pleiotropic cytokine that has been demonstrated to be overexpressed in multiple cancers and in particular has been shown to be involved in growth, angiogenesis, proliferation, adhesion, migration and invasion of PCa cells [11]. Our results of finding significant overexpression of *aHGF* in PCa urine samples as compared with controls is supported by evidence of similar findings in serum and plasma HGF biomarker studies [13,21]. In particular, the mechanism by which *aHGF* is elevated can be associated with the pathway involving its receptor, c-met, and proteolytic enzymes, HGFA and matriptase, which cleave HGF to form a biologically active heterodimer [12]. c-met, a HGF tyrosine kinase receptor, has been shown to be significantly elevated in the urine of metastatic PCa patients, whereas we have found that *aHGF* is overexpressed in the urine of PCa patients without differentiation between meta-static and localized phenotype. This suggests that *aHGF* levels increase to initiate the cancer phenotype, which perhaps subsequently leads to the stimulation of *c-met* gene upregulation and *c-met* overexpression in more aggressive stages [22]. In addition, HAI-1 expression was found to be much higher in patients with distant metastasis and hormone-refractory PCa than in nonmetastatic patients [23]. Inhibition of HGFA by HAI-1 in patients with more progressive disease may support our findings that suggest *aHGF* cannot discriminate between localized and metastatic disease.

IGFBP3 is a component of the IGF system, which has been shown to modulate the action of IGFs, leading to diverse biological effects including cellular proliferation, survival and differentiation [24]. Although previous findings on circulating IGFBP3 levels in PCa have been contradictory with several studies reporting IGFBP3 to have anticancer properties [25–27], more recent studies, including a larger study including over 2000 men, have attributed higher levels of plasma IGFBP3 to higher risk for PCa, which was consistent with our findings in urine [15,28–30]. The incongruence of studies evaluating the importance of IGFBP3 in PCa diagnosis may reflect variable tumorigenic mechanisms affecting different populations of people. For instance, one study found that the A-202C single nucleotide polymorphism for *IGFBP-3* was associated with increased risk for prostate and breast cancer in African-American patients but not in Caucasian and Asian individuals [31]. Further investigations into the genetic effects on PCa tumorigenic pathways and IGFBP3 levels, differential detection of IGFBP3 detection in the urine and blood, and differences of IGF levels in the urine and its interactions with IGFBP3, are warranted.

The last biomarker investigated in this study was OPN, otherwise known as SPP1, which is an extracellular matrix protein with diverse roles, including bone mineralization, immunity, blood vessel formation and tumorigenesis [17]. Unlike the results observed for *aHGF* and *IGFBP3*, our study found levels of urinary OPN to be significantly higher in metastatic samples compared with localized disease and normal samples, which confirm previous findings that have linked OPN to a malignant phenotype [32]. OPN has been shown to play an important role in disease progression through the inhibition of apoptosis, induction of

proliferation, and promotion of invasion and migration of cells through interactions with the CD44 and integrin receptors [33–35]. Recently, *TMPRSS2* fusion with the *ERG* gene, which is found in 40–80% of PCas, was shown to be associated with cancer aggressiveness, recurrence of PCa and death [36–39]. *ERG* was shown to promote the transcription of OPN, and the detection of the *TMPRSS2-ERG* gene fusion was highly associated with OPN overexpression [39]. This process of *TMPRSS2-ERG* gene fusion may explain why patients with more aggressive tumors may overexpress OPN. While OPN has been identified as a plasma marker [40,41] and has been shown to be overexpressed in PCa tissue biopsies [42], it has not been pursued as a urinary biomarker until this report.

By using STRING analysis, we were able to link the three candidate biomarkers via CD44 and FN1. CD44 is a transmembrane glycoprotein that allows cell–cell and cell–matrix interactions, and is a stem cell marker for PCa [43,44]. Although the role of CD44 in PCa development and progression is controversial, several studies have implicated CD44 in cell migration, invasion and proliferation [45]. FN1 is a multifunctional glycoprotein in the extra-cellular matrix that promotes tumor migration and invasion [46,47]. FN1 can interact with integrin receptors, the CD44 receptor and other molecules to initiate a cascade of reactions involving cell adhesion, growth, migration and differentiation [48–50]. STRING highlights the important roles that IGFBP3, HGF and OPN may play in tumorigenesis, presumably through their interactions with various molecules such as VEGF, MMP2, CD44, and FN1.

Importantly, there have been studies that highlight the interplay between HGF and OPN during tumorigenesis and tumor progression. Medico *et al.* showed that stimulation of MLP-29 mouse embryo liver cells with HGF led to the induction of *OPN* gene transcription [51]. HGF upregulation of OPN expression was dependent on the activation of the PI3K pathway in epithelial cells [52] and HGF was shown to mediate the cells' invasive phenotype by promoting OPN interaction with the CD44 receptor [51]. These two studies attribute HGF-mediated invasiveness to the expression of OPN. This supports our findings and suggests that molecules, such as HGF, are secreted by cells early during the process of tumorigenesis, and as cancers acquire metastatic characteristics, OPN and other genes are upregulated to promote invasion and migration, and inhibit apoptosis.

The perfect threshold for PSA, which should prompt a biopsy, has been debated in the literature. The Physicians Healthy Study showed PSA with a cutoff value of 4.0 ng and sensitivity of 46% [53]. Our individual biomarkers for *α*HGF and IGFBP3 showed comparably higher sensitivities with the chosen thresholds for normal versus PCa. OPN has a sensitivity of 65% for discriminating between metastatic and localized disease. A larger sample size will be needed to verify thresholds. The combination of PSA with these biomarkers may yield even higher sensitivities.

There are several limitations to our study. One potential limitation is the relatively small patient cohort pulled from a larger group of PCa patients who were enrolled in research protocols at the NIH. Importantly, baseline differences of these biomarker levels before diagnosis of PCa in individuals were not controlled for in this study. Despite this, we were able to find statistically significant differences in biomarker levels between the three groups.

Further validation of our thresholds and results in a larger sample size is warranted with controls selected to match demo graphics and preexisting conditions. Second, the research protocols only included individuals who were to undergo treatment for active PCa and did not include normal controls. The normal healthy controls in our study were volunteers who did not undergo biopsies or PSA screening for PCa and were also not matched for age. Third, we analyzed urine supernatant obtained after centrifugation rather than whole urine using a technique that has been used in previously published papers [14]. Previous studies have found that the use of sediment may falsely elevate biomarker levels and recommended using supernatant for ELISAs [54]. Future studies may warrant direct comparisons between urine supernatant and whole urine. Last, we used commercially available ELISA kits that we did not validate prior to running our study. Although we selected assays that have been tested and validated for reproducibility by the manufacturers, there may be differences between kits produced by different companies, which may limit comparisons between published reports. This is a recognized limitation of ELISAs that will need to be kept in mind when conducting multicenter trials. We tried to minimize interassay variability by designating one technician to perform all of the ELISAs for a specific biomarker. Samples were also run in duplicate to try to limit intra-assay variability. We also selected kits for IGFBP3 [55], OPN [56] and *a*HGF [12] that have been used in previously published reports.

Our results suggest that urinary *a*HGF and IGFBP3 can be used to differentiate between individuals with and without cancer, while OPN levels can be used to identify those with more aggressive disease. Our study showed no significant correlation of *a*HGF, IGFBP3 or OPN levels with patient baseline PSA, Gleason score and clinical stage within the localized cohort. Longitudinal analyses over time and throughout radiation or chemotherapy treatment would be a valuable addition to describing these biomarkers in relation to having any predictive capacity. Other important factors, such as the presence of benign hyperplasia, should be analyzed to determine the specificity of these markers. Future studies may build on these findings to identify a multiplex panel of biomarkers that could be used in the clinic to better reflect the heterogeneity of PCa.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Website

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Future perspective

The longstanding controversial role of PSA as a biomarker for PCa represents a need for caution in choosing future biomarkers. Rising bio markers must strive for multimedial conformation via serum, plasma, tissue biopsy and urine. A single biomarker is rarely able to correlate with disease status, prognosis and remittance. However, with the combination of biomarkers, proteomic profiling of cancers may eventually achieve this gold standard. As new technology emerges with the ability to amass biomarkers into a clinically relevant product, a new frontier for early detection and monitoring of disease will be within reach.

Figure 1. aHGF levels in men with prostate cancer.

(A) aHGF levels were compared in the urine of normal volunteers, and nonaggressive and aggressive PCa disease patients. The mean for each group is shown along with $\pm$ standard error (median not shown). (B) The ROC curve analysis for PCa versus non-PCa patient samples has an AUC of 0.75 with a 95% CI of 0.65–0.85 ($p = 0.0006$). (C) Subgroup ROC curve for non-PCa versus localized cancer had an AUC of 0.76 ($p = 0.0006$) and (D) subgroup ROC curve for non-PCa versus metastatic cancer had an AUC of 0.73 ($p = 0.006$). AUC: Area under the curve; PCa: Prostate cancer; ROC: Receiver operating characteristic.

Figure 2. IGFBP3 levels in men with prostate cancer.

(A) Urinary IGFBP3 mean concentrations with standard errors (median not shown) in the healthy controls, localized and metastatic disease groups were compared. (B) Diagnostic accuracy based on the ROC curve analysis of PCa patients versus healthy controls. Demonstrates an AUC of 0.74 (95% CI: 0.58–0.88; $p = 0.001$). (C) Subgroup analyses revealed an AUC of 0.73 ($p = 0.003$) for localized versus normal and an AUC of 0.75 ($p = 0.002$) for metastatic versus normal. (D) Subgroup ROC curve for non-PCa versus metastatic PCa. AUC: Area under the curve; PCa: Prostate cancer; ROC: Receiver operating characteristic.

Figure 3. OPN levels in men with prostate cancer.

(A) Mean OPN levels of urinary samples $\pm$ standard error in the normal, localized and metastatic disease groups are compared (median not shown). (B) ROC curve analysis for localized versus metastatic groups of PCa patients with an AUC of 0.68 (95% CI: 0.56–0.79; $p = 0.003$). AUC: Area under the curve; PCa: Prostate cancer; ROC: Receiver operating characteristic.

Figure 4. Direct (physical) and indirect (functional) interactions between HGF, OPN (SPP1), IGFBP3 and other molecules.

Generated by the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) 9.0 informatics database [101]. The top 20 associations were calculated with respect to probabilities reported from genomic context, high-throughput experiments, coexpression and previous knowledge, and corrected for the probability of random observations. The different colors of lines and circles are for visual enhancement and have no specific meaning.

Table 1.

Clinical and pathologic characteristics of localized prostate cancer patients.

Characteristic	Overall (n = 65)	Low (n = 12)	Mid (n = 24)	High (n = 29)
PSA (ng/ml), mean (range)	14.9 (0.5–190)	5.8 (4.5–8.8)	9.4 (4.3–19.3)	23.1 (0.5–190)
Gleason score, n (%)				
5–6	15 (23.1)	12 (100.0)	2 (8.3)	1 (3.5)
7	27 (41.5)	0 (0)	22 (91.7)	5 (17.2)
8–10	23 (35.4)	0 (0)	0 (0)	23 (79.3)
Clinical stage, n (%)				
T1	30 (46.2)	8 (66.7)	14 (58.3)	8 (27.6)
T2a	14 (21.5)	4 (33.3)	5 (20.8)	5 (17.2)
T2b	5 (7.7)	0 (0)	2 (8.3)	3 (10.3)
T2c	4 (6.2)	0 (0)	3 (12.5)	1 (3.5)
T3	12 (18.5)	0 (0)	0 (0)	12 (41.4)
Hormones, n (%)				
Previous hormone therapy	35 (53.8)	1 (8.3)	9 (37.5)	25 (86.2)

Patients with localized prostate cancer classified by risk as determined by Gleason score, PSA and Tumor–Node–Metastasis staging.

Table 2.

The analyzed mean, median and range values for each biomarker.

	Mean	Median	Range	p-value
<i>Normalized aHGF (pg/mg)</i>				
Control	111.3	96.92	27.0–250.0	0.002
Localized	235.2	215.8	24.6–668.0	
Metastatic	220.8	181.6	20.9–1010.0	
<i>Normalized IGFBP3 (ng/mg)</i>				
Control	0.648	0.1582	0–4.282	0.0041
Localized	0.8862	0.5129	0–7.045	
Metastatic	1.353	0.5132	0.1095–6.256	
<i>Normalized OPN (ng/mg)</i>				
Control	532.4	323.5	51.0–1559.0	0.016
Localized	613.6	459.1	2.3–2716.0	
Metastatic	1024	901.1	123.6–3304.0	

The mean, median and range values were analyzed for each biomarker. Kruskal–Wallis nonparametric analysis of variance test showed significant differences in the means between the three groups.

Table 3.

Mann–Whitney nonparametric t-test was performed to study differences between groups.

Comparison groups	p-value		
	<i>aHGF</i>	<i>IGFBP3</i>	<i>OPN</i>
Control All cancers (localized and metastatic cancers)	0.0006	0.0012	0.016
Control Localized cancer	0.0006	0.0024	0.541
Control Metastatic cancer	0.0059	0.0024	0.006
Localized cancer Metastatic cancer	0.7065	0.4773	0.0086

There were significant differences between control and all cancer groups as well as control and metastatic cancer for all three biomarkers. aHGF and IGFBP3 showed significant differences between control and localized cancer groups, while OPN showed significant differences between localized and metastatic cancer groups.

Table 4.

The sensitivity, specificity, positive-predictive value and negative-predictive value, and area under the curve are enumerated for the three biomarkers for prostate cancer.

Biomarker	Cutoff	Sensitivity	Specificity	PPV (%)	NPV (%)	AUC
aHGF	>79 pg/mg	0.85	0.32	93	46	0.75
IGFBP3	>53 pg/mg	0.98	0.58	90	80	0.74
OPN	>628 ng/mg	0.65	0.36	48	78	0.68

For aHGF and IGFBP3, the values were calculated for prostate cancer versus nonprostate cancer. Similarly, OPN parameters were based on localized versus metastatic disease groups.
AUC: Area under the curve; NPV: Negative-predictive value; PPV: Positive-predictive value