Preoperative Serum Caveolin-1 as a Prognostic Marker for Recurrence in a Radical Prostatectomy Cohort

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Abstract

Purpose: Up-regulation of caveolin-1 (cav-1) is associated with virulent prostate cancer, and serum cav-1 levels are elevated in prostate cancer patients but not in benign prostatic hyperplasia. In this study, we evaluated the potential of high preoperative serum cav-1 levels to predict biochemical progression of prostate cancer. The value of the combined preoperative markers, prostate-specific antigen (PSA), biopsy Gleason score, and serum cav-1 for predicting biochemical recurrence was also investigated.

Experimental Design: Serum samples taken from 419 prostate cancer patients before radical prostatectomy were selected from our Specialized Programs of Research Excellence prostate cancer serum and tissue bank. Serum samples were obtained 0 to 180 days before surgery and all patients had complete data on age, sex, race, stage at enrollment, and follow-up for biochemical recurrence. Serum cav-1 levels were measured according to our previously reported ELISA protocol.

Results: Cav-1 levels were measured in the sera of 419 prostate cancer patients; the mean serum level was 4.52 ng/mL (median 1.01 ng/mL). Patients with high serum cav-1 levels had a 2.7-fold ($P = 0.0493$) greater risk of developing biochemical recurrence compared with those with low serum cav-1 levels. Importantly, patients with serum PSA $\geq$ 10 ng/mL and elevated levels of serum cav-1 had 2.44 times higher risk ($P = 0.0256$) of developing biochemical recurrence compared with patients with low levels of cav-1. In addition, high serum cav-1 levels combined with increasing biopsy Gleason score predicted much shorter recurrence-free survival in the group of patients with PSA $\geq$ 10 ng/mL ($P = 0.0353$). Cav-1 was also able to distinguish between high- and low- risk patients with biopsy Gleason score of seven, after adjusting, for patients PSA levels ($P = 0.0429$).

Conclusions: Overall, elevated preoperative levels of serum cav-1 predict decreased time to cancer recurrence. In the subset of patients with serum PSA of $\geq$10 ng/mL, the combination of serum cav-1 and biopsy Gleason score has the capacity to predict time to biochemical recurrence.

In 2005, ~90% of newly diagnosed prostate cancer patients had clinically localized disease (1). Consequently, the majority of patients are treated with curative intent by either radical prostatectomy or radiation therapy. It is well established, however, that 10% to 50% of patients who undergo radical prostatectomy will show biochemical evidence of disease recurrence [prostate-specific antigen (PSA) recurrence] within 5 years of surgery (2, 3). Various clinical variables have been used, singly and in combination (nomograms, tables, etc.), to predict, preoperatively, which patients are likely to fail definitive therapy (4). However, the predictive value of these variables has been thwarted by the vexing biological diversity of clinical prostate cancer. New markers are needed, preferably serum markers that have been mechanistically implicated in the progression of virulent disease. We believe that serum caveolin-1 (cav-1) may be such a marker.

Cav-1 is an important structural/regulatory molecule involved in many aspects of molecular transport and cell signaling (5, 6). Tissue cav-1 is overexpressed in metastatic and in hormone-resistant prostate cancer (7). Overexpression correlates with a shortened interval to disease recurrence following therapy for localized disease and tends to be associated with a high Gleason score pathologically (8–10). Interestingly, cav-1 is secreted by prostate cancer cells (11) and we have developed a sensitive ELISA immunoassay for the detection of cav-1 in the serum (12). In a preliminary study, we documented that prostate cancer patients have a higher serum cav-1 level when compared with age-matched controls with benign prostatic hyperplasia (12).

We report here the utility of a single preoperative measurement of serum cav-1 for predicting disease recurrence in a cohort of 419 prostate cancer patients undergoing radical prostatectomy at our institution.

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Materials and Methods

Study population. The sera of 419 prostate cancer patients were obtained from the Specialized Programs of Research Excellence prostate cancer blood and tissue bank at Baylor College of Medicine. Entry into the study required availability of preoperative serum samples obtained within 6 months of surgery and complete data on age, race, stage at enrollment and follow-up, as well as availability of postoperative serum samples, as this is a part of a larger ongoing investigation. In addition, patients could have had no preoperative therapy. After completion of cav-1 measurements in the serum, it was discovered that seven patients were missing reliable data on their preoperative PSA and/or biopsy Gleason score and/or follow-up information. These patients were included in all analyses that did not require missing data. The preoperative serum collected from 355 patients was at a time period between prostate biopsy and surgery. No information was available in our database on the exact preoperative serum collection timing with respect to biopsy for 64 patients. The mean age of this patient group was 62.6 years (range 42.6-78.9 years); 91.4% were White males, with Hispanics, African-Americans, and Asians comprising 6.0%, 2.4%, and 0.2%, respectively. Mean follow-up time among this group of patients was 52 months, with a median follow-up time of 48 months. Biochemical recurrence is defined throughout this study as serum PSA level of $\geq$0.2 ng/mL on two consecutive measurements, using the first-generation postresection PSA assay (Hybritech, Beckman Coulter, Inc., Fullerton, CA). Patient data were gathered from the Informatics Core using the Specialized Programs of Research Excellence in Prostate Cancer Information System.

Determination of serum cav-1. Cav-1 was determined in the serum samples by the sandwich ELISA protocol developed in our laboratory (12). Briefly, Costar microplate wells were coated with 0.5 μg cav-1 polyclonal antibody (Transduction Laboratories, San Diego, CA) and blocked with TBS buffer containing 1.5% bovine serum albumin and 0.05% v/v Tween 20. Serum samples, calibrators, and controls (50 μL) were added to the well, and 50 μL TBS containing 0.5% v/v Tween 20 was added to each well. The plate was incubated at room temperature for 2 hours with shaking and after extensive washing, 100 μL horseradish peroxidase–conjugated cav-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:200 in blocking buffer was added to each well. The microplate was incubated for 90 minutes at room temperature with shaking, the wells were then washed extensively, and 100 μL 3,3′,5,5′-tetramethylbenzidine substrate solution (Sigma-Aldrich, St. Louis, MO) was added and the blue color was allowed to develop for 20 minutes in the dark. The reaction was stopped by adding 50 μL of 2 N H_2SO_4, and the absorbance was read at 450 nm using a microplate reader (Sunrise Microplate Reader, Tecan US, Inc., Charlotte, NC).

Statistical analysis. Correlations of preoperative serum cav-1 levels with clinical and pathologic variables were evaluated using the Spearman correlation. The predictive value of cav-1 univariately and multivariately with other preoperative clinical and pathologic variables, such as preoperative PSA and biopsy Gleason score, as well as of the interactive terms, were analyzed using the Cox proportional hazards regression model. The minimum *P* value method was used to group patients into "low-level" and "high-level" cav-1 categories (13). The hazard ratio (HR) and 95% confidence intervals (95% CI) were computed for each marker. Kaplan-Meier survival curves were plotted for each risk category. $P < 0.05$ was considered statistically significant. All analyses were done using the SPSS 12.0 software package (SPSS, Inc., Chicago, IL)

Table 1. Preoperative serum cav-1 level correlation with clinical and pathologic variables

	n	Mean (range), %	r^2	*P*
Preoperative cav-1	419	4.5 (0.0-156.7)	—	—
Preoperative PSA (ng/mL)	415	8.6 (0.4-53.2)	0.01	0.9013
Age	419	62.6 (42.6-78.9)	0.02	0.6268
Biopsy Gleason score	412	6.1 (3-9)	−0.06	0.2051
Seminal vesicle invasion	419	7.6%	−0.01	0.8543
Lymph node involvement	419	3.8%	0.08	0.1024
Extraprostatic extension	419	32.7%	0.07	0.1378
Margin positive	419	11.9%	0.06	0.2425
Gleason score	419	6.5 (3-9)	−0.01	0.8342

Table 2. Preoperative serum cav-1 is a univariate and multivariate predictor of decreased biochemical recurrence-free survival

	HR (95% CI)	*P*
Univariate model		
Preoperative cav-1	2.78 (1.003-7.70)	0.0493
Multivariate model		
Preoperative cav-1	2.57 (0.92-7.12)	<0.0704
Ln(PSA)	2.31 (1.60-3.33)	<0.0001
Biopsy Gleason score	1.74 (1.32-2.30)	0.0001

Results

Serum cav-1 levels were measured in 419 prostate cancer patients. The mean cav-1 value was 4.52 ng/mL and the median level was 1.01 ng/mL (range 0.0-156.7 ng/mL). Serum cav-1 levels seemed to have a bimodal distribution, with positive values distributed log normally. The serum cav-1 levels were analyzed for correlation with other pathologic and clinical variables using the Spearman correlation. No statistically significant correlations with clinicopathologic variables were found (Table 1).

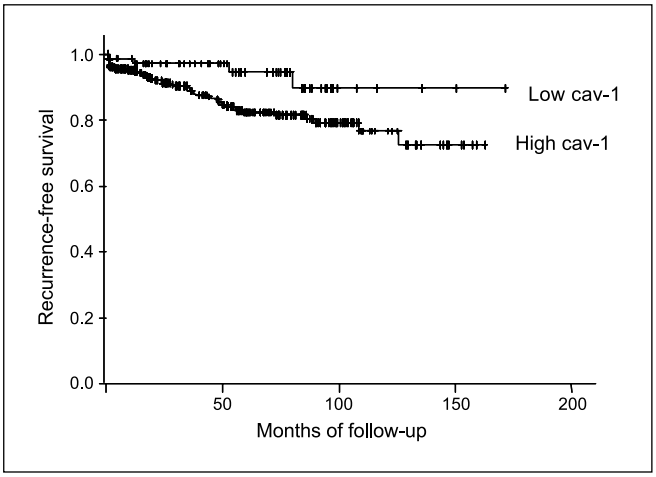

Fig. 1. High expression of cav-1 predicts decreased biochemical recurrence-free survival. This Kaplan-Meier plot illustrates the differences in recurrence-free survival between the low and high groups when separated by cav-1 cut point of 0.13 ng/mL. Patients with high level of cav-1 experienced significantly higher risk of recurrence than those with low levels ($P = 0.0493$).

There were 414 patients with complete follow-up information were included in the analysis of recurrence-free survival (mean follow-up 52.3 months, maximum 171.3 months); 54 patients had PSA recurrence during follow-up. Although it was clear that patients with no or very low levels of cav-1 had a better prognosis, the optimal cutoff was selected using the minimum *P* value method (13). This defined the low cav-1 group as patients with levels of <0.13 ng/mL and the high cav-1 group as those with >0.13 ng/mL. In univariate analysis, the risk of experiencing biochemical recurrence, estimated by HR, was 2.8 times higher ($P = 0.0493$) for the high cav-1 group compared with the low cav-1 group (Table 2). Kaplan-Meier plots illustrate the shorter time to biochemical recurrence following radical prostatectomy in the high cav-1 group compared with low cav-1 group. The 5- and 10-year recurrence-free survival rates were 94.4% and 90.5% for the low cav-1 group compared with 82.0% and 71.8% for the high cav-1 group. This corresponds to a consistent 12% to 21% increased progression-free survival for the low cav-1 group (Fig. 1). When the preoperative serum PSA level and the biopsy Gleason score were incorporated into the multivariate Cox proportional hazard model, the recurrence risk was 2.6 times higher for the high cav-1 group, but this effect was just below the level of significance ($P = 0.0704$; Table 2).

The effect of the serum cav-1 level on biochemical recurrence was further analyzed in patients with more advanced cancers, characterized by PSA of ≥10 ng/mL. The distribution shape remained the same and patients with low cav-1 levels continued to have a better prognosis. A new optimal cutoff of 2.86 ng/mL was identified for this subgroup of patients. Univariately, the estimated risk of recurrence was 2.44 times higher ($P = 0.0256$) in the high cav-1 group (serum cav-1 >2.86 ng/mL) than in its low cav-1 counterpart (serum cav-1 ≤ 2.86 ng/mL; Table 3). Kaplan-Meier plots illustrate that patients in the high cav-1 group had a much shorter time to recurrence than those in the low cav-1 group (Fig. 2). This figure also indicates a 10-year recurrence-free survival rate of 70.3% in the low cav-1 group compared with 47.4% in the high cav-1 group corresponding to a >20% decrease in progression-free survival in the low cav-1 group.

Incorporating the biopsy Gleason score into the Cox proportional hazard model (Table 3), we found that the interaction term between Gleason score and the cav-1 was the most predictive ($P = 0.0353$). This indicates that the biopsy Gleason score was an additional risk factor only in the high cav-1 group. The Kaplan-Meier plot (Fig. 3) illustrates this result by showing the highest recurrence risk in patients with high cav-1 and high biopsy Gleason score (7–9); and lower

Table 3. Preoperative serum cav-1 is a univariate and multivariate predictor of decreased biochemical recurrence-free survival among patients with preoperative PSA of ≥10

	HR (95% CI)	*P*
Univariate model		
Preoperative cav-1	2.44 (1.12-5.34)	0.0256
Multivariate model		
Preoperative Cav-1 × biopsy Gleason score	1.13 (1.01-1.27)	0.0353

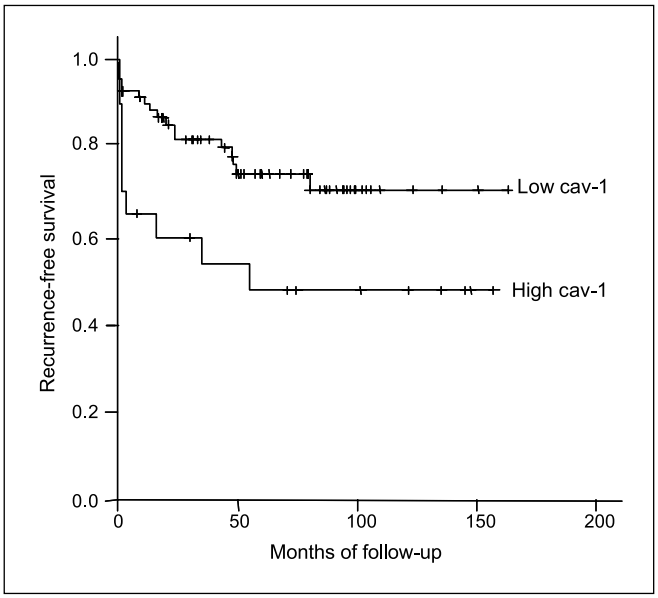

Fig. 2. For patients with PSA of ≥10, high expression of cav-1 is a strong predictor of decreased biochemical recurrence-free survival. For this high-risk patient subgroup, optimal cutoff was determined to be at 2.86 ng/mL. The Kaplan-Meier plot here shows the difference observed in the data.

recurrence risk in the high cav-1 and low biopsy Gleason score (<7) group, and those patients with low cav-1 regardless of the biopsy Gleason score. Recurrence-free survival curve for patients with low PSA (<10) was plotted for reference as well.

For biopsy Gleason 7 patients, the trend was the same: Higher cav-1 was observed in higher-risk patients. The difference in risk of recurrence, estimated by HR, between low and high cav-1 patients with cutoff defined at upper quartile of cav-1 (and confirmed by minimum *P* value method), was not statistically significant ($P = 0.0953$). However, after including preoperative PSA in the model, the

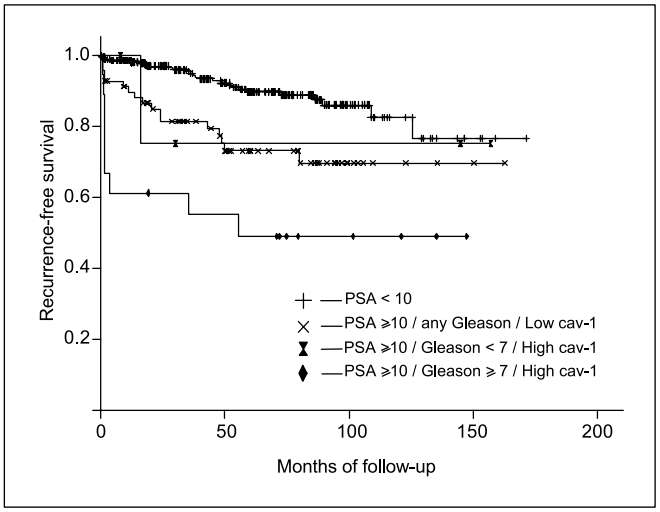

Fig. 3. Cav-1 works with biopsy Gleason score and preoperative PSA to predict biochemical recurrence-free survival. The interaction term between biopsy Gleason score and the cav-1, incorporated into the Cox proportional hazard, was the most predictive of recurrence-free survival among patients with PSA of ≥10 ($P = 0.0353$). This Kaplan-Meier plot illustrates how patients with both high cav-1 (>2.86 ng/mL) and biopsy Gleason score of ≥7 have the poorest prognosis. Curve for patients with PSA < 10 was plotted for reference.

difference became statistically significant (HR, 2.29; P = 0.0429). A patient with cav-1 in the upper quartile had over twice the risk of recurrence of one with cav-1 in the lower three quartiles if their preoperative PSA levels were the same.

Discussion

This study is part of our ongoing efforts to elucidate the biology and to define the clinical usefulness of serum cav-1 in prostate cancer. Although the factors modulating the serum levels of this biomarker remain largely unknown, the current study points out that a single preoperative serum cav-1 determination has prognostic value in a radical prostatectomy cohort. We observed the increase of the risk of biochemical recurrence with high levels of serum cav-1, and so we used the minimum P value method to segregate the patients into low-level and high-level groups. Remarkably, the risk of experiencing a PSA recurrence, estimated by HR, was 2.78 (95% CI, 1.003-7.70) times higher for the high-level cav-1 group (P = 0.0493), (see Fig. 1; Table 2). Incorporating the preoperative serum PSA level and the biopsy Gleason score into the model dropped the effect of cav-1 to just below the level of significance (HR, 2.57; P = 0.0704).

Interestingly, we found that the serum cav-1 levels are particularly important in predicting recurrence-free survival in patients with more advanced disease as defined by the preoperative serum PSA. When only patients with preoperative serum PSA levels of 10 ng/mL or higher were analyzed, cav-1 remained a significant predictor of recurrence-free survival (HR, 2.44; P = 0.0256). Additionally, the cav-1/biopsy Gleason score interaction term was a significant predictor (P = 0.0353). This implies that patients with both a high biopsy Gleason score and a high serum cav-1 level have a higher risk of biochemical recurrence than the remaining patients. Also, a subgroup of biopsy Gleason 7 prostate cancer patients, defined by the upper 25% of serum cav-1 levels, seems to harbor a biologically more aggressive prostate cancer after correction for individual PSA levels. All of these findings are consistent with our previous reports based on tissue up-regulation of cav-1 expression (8).

Notably, the distribution of the serum cav-1 values in the study population was not a normal distribution. About 10% of patients had undetectable serum levels by our sensitive ELISA essay. We can only speculate at this point as to the possible reason for this phenomenon. It is possible that the presence of any cav-1 in the serum is dictated by the genetic background of the individual and that, physiologically, there may be "secretors" and "nonsecretors." Within the secretor population, the specific makeup of the cancer may be contributing to the absolute serum level.

Surprisingly, we could not correlate the serum cav-1 levels with any of a large number of clinical and/or pathologic variables using the Spearman correlation (Table 1). We suggest that the reason is that cav-1 is an independent biomarker causally implicated in disease progression and not simply an epiphenomenon.

Many questions remain. For instance, we do not know the incidence of false-positive and/or false-negative elevated serum cav-1 values vis-à-vis the tumor tissue cav-1 expression. Only a correlative study of tissue and serum levels of cav-1 can answer this question. Likewise, the kinetics of the serum cav-1 has not been worked out, nor do we know what the stability of serum cav-1 is over extended periods of time. Clearly, we are at the beginning of the road leading to the establishment of serum cav-1 as a prognostic marker for prostate cancer. The data presented here suggest that this road is worth pursuing.

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