Caveolin-1 Expression in Clinically Confined Human Prostate Cancer: A Novel Prognostic Marker

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ABSTRACT

We demonstrated previously elevated caveolin-1 expression in metastatic mouse and human prostate cancer cells both in vitro and in vivo. In this study, we analyzed its prognostic value for progression of clinically confined human prostate cancer. Immunohistochemical staining with a caveolin-1-specific antibody was performed on routinely processed paraffin sections from 189 radical prostatectomy specimens. Caveolin-1 immunoreactivity was evaluated in association with patients’ age, race, preoperative prostate-specific antigen, clinical stage, and pathological features including Gleason score, extraprostatic extension, status of surgical margins, and time to disease progression after surgery. Positive caveolin-1 immunostaining was detected in 47 of the 189 cancers (25%) and correlated positively with Gleason score, positive surgical margin, as well as lymph node involvement (P = 0.0071, 0.0267, and 0.0399, respectively). In lymph node-negative cancers (n = 162), caveolin-1 immunoreactivity predicts a shorter time to disease progression after surgery (P = 0.0033, univariate analysis). Multivariate analyses that included caveolin-1 and other prognostic pathological markers identified positive caveolin-1 immunostaining as an independent predictor for time to disease progression (P = 0.0186). Thus, our study establishes caveolin-1 as a novel prognostic marker for clinically confined human prostate cancer.

INTRODUCTION

The introduction of serum PSA screening has led to a dramatic increase in the diagnosis of prostate carcinoma as well as in the number of men undergoing radical prostatectomy in the past decade (1). One of the dilemmas facing urologists today is whether all patients who are aggressively treated by surgery actually benefit from this therapy, which has significant associated morbidity. Up to 16% of the radical prostatectomy specimens contain only small and well-differentiated cancers that may never achieve clinical significance during the lifetime of a patient (2–4). In addition, >50% of cancers that are considered clinically confined prior to the surgery are subsequently found beyond the confines of prostate and thus represent a high risk of progression (5). Some preoperative clinical and pathological indices including PSA levels and the grade of the carcinoma obtained from needle biopsy have been shown to be important predictive indices of pathological and clinical outcome (6–8). However, for those cancers in the middle range of a given index, the predictive power of these indices is diminished greatly. The use of molecular markers to supplement clinical information concerning the biological aggressiveness of a carcinoma may allow better selection among different treatment regimens.

We recently developed a strategy to identify mRNAs that are expressed differentially in cell lines derived from primary versus metastatic mouse prostate cancer using differential display PCR. In using this system, a number of metastasis-related sequences were identified including a cDNA that encodes caveolin-1 (9). Caveolin-1, a M, 22,000 protein is a major structural component of caveolae, specialized plasma membrane invaginations that are abundant in smooth muscle cells, adipocytes, and endothelium. Caveolae appear to mediate molecular transport, cell adhesion, and signal transduction activities in a cell- and context-specific fashion (10). Caveolin-1 was found to be overexpressed in both mouse and human metastatic prostate cancer cells (9). Recently, we documented that suppression of caveolin-1 gene expression with stably transfected antisense caveolin-1 cDNA converted androgen-insensitive metastatic mouse prostate cancer cells to an androgen-sensitive phenotype and that adenovirus-mediated sense caveolin-1 expression blocked androgen sensitivity (11). Caveolin-1, therefore, has been shown to be a metastasis-related gene as well as a candidate gene for hormone-resistant human prostate cancer. Interestingly, recent studies also point to a potential role for caveolin-1 in the resistance of various malignances to multiple antineoplastic agents (12, 13).

In the present study, we investigated the possibility of using caveolin-1 expression as a predictive marker for prostate cancer progression. In this retrospective study, 189 clinically confined prostate cancer specimens obtained from radical prostatectomy were immunostained with a caveolin-1-specific antibody. Expression of caveolin-1 was associated with preoperative clinical information of the patients and with postoperative pathological diagnosis. Our results indicate that caveolin-1 expression is a novel independent prognostic marker for progression in clinically confined human prostate cancer.

MATERIALS AND METHODS

Patients and Prostate Specimens. In this study, 189 prostate cancer radical prostatectomy specimens were obtained in the prostate tissue bank, Methodist Hospital, Baylor College of Medicine (Houston, TX). The patients were selected in a consecutive manner by a statistician, bearing in mind the categorization into the following two groups: those without evidence of prostate cancer progression after 5 years of follow-up; and those with recurrence as determined by the presence of malignant cells in biopsies of the primary tumor, visualization of distant metastases by bone scan, or a serum PSA levels >0.4 ng/ml on two successive measurements (Hybritech, Inc., San Diego, CA). The initial median postoperative follow-up time was 2 months, and the median follow-up intervals within 5 years after the surgery were 6 months for all patients. After surgical removal of the prostate specimens, they were sliced into 5-mm-thick tissue blocks according a procedure described previously (14). The tissue blocks were then fixed in 10% neutral buffered formalin and embedded in paraffin. From each tissue block, H&E sections were made for pathological evaluation. For each patient, one tissue block that was identified as containing the index cancer (the largest cancer in a specimen) was cut into 6-μm sections for caveolin-1 immunostaining. Preoperatively, the patients had a cancer staged according to the Union International Contre Cancer clinical staging system (15) as T1a–c (n = 31), T2a–c (n = 134), and T3a (n = 17). None of these patients received any treatment for cancer before the surgery. Their preoperative PSA levels were recorded. Postoperative pathological findings including status of extraprostatic extension (16), seminal vesicle invasion, lymph node metastases, surgical margin, and Gleason scores were recorded by a single pathologist (T. M. W.). These patients had an average age of 63 years (range, 43–78),
CAVEOLIN-1 AS A PROGNOSTIC MARKER FOR PROSTATE CANCER

Caveolin-1 Immunohistochemistry. The sections were deparaffinized and rehydrated. They were then heated in citrate buffer (0.01 m; pH 6.5) with an 800-Watt microwave oven for 9 min for antigen retrieval. Endogenous peroxidase in sections was inactivated in 2% H$_2$O$_2$ for 10 min. The sections were then blocked in 3% normal goat serum in 0.2 m PBS (pH 7.4), followed by incubation in a rabbit polyclonal antibody to caveolin-1 (Santa Cruz Biotechnology, Santa Cruz, CA). The caveolin-1 antibody was used at a dilution of 1:400 in PBS with 0.5% normal goat serum. The sections were incubated in the primary antibody for 2 h at room temperature. They were then processed following a standard ABC immunostaining procedure with an ABC kit (Vector Lab, Burlingame, CA). Immunoreaction products were visualized in a 3,3'-diaminobenzidine/H$_2$O$_2$ solution. To verify the specificity of the immunoreactions, some sections were incubated in primary antibody preabsorbed by corresponding caveolin-1 peptide (Santa Cruz Biotechnology) at a concentration of 25 µg/ml. The caveolin-1 immunoreactivities were abolished on the sections incubated in the antibody preabsorbed with the caveolin-1 peptide, confirming the specificity of immunostainings. The immunostained sections were evaluated at a power of ×200 under a Zeiss microscope. For each specimen, only the index cancer was evaluated. The entire cancer area was scanned, and an average of 21.76 ± 14.38 (mean ± SD) microscopic fields were evaluated. Positive caveolin-1 staining for a particular specimen was assigned if >50% of the cancer cells in any microscopic field demonstrated caveolin-1-positive granular immunoreaction products in their cytoplasm. All specimens were evaluated without any knowledge of the patients’ clinical information.

Statistical Analysis. The correlation of caveolin-1 immunoreactivity with patients’ clinical and pathological variables was analyzed by the Spearman or Pearson correlation test. The predictive value of caveolin-1 and other clinical and pathological variables such as preoperative PSA, extraprostatic extension, positive surgical margins, and Gleason score were univariately tested in node-negative patients using a Kaplan-Meier actuarial analysis (17) and the log-rank test. In addition, multivariate analysis of caveolin-1 immunostaining and the postoperative pathological markers was performed using the Cox proportional hazards regression model (18). The risk ratio and its 95% confidence interval were recorded for each marker. P < 0.05 was considered statistically significant in all of the analyses. Moreover, Fisher’s exact test was used to test the differences in the incidence rates of caveolin-1 expression in patients of different pathological and racial subgroups. All analyses were performed with a statistical software (Statview version 5.0; SAS Institute, Inc. Cary, NC).

RESULTS

In the prostate cancer specimens analyzed, caveolin-1 immunoreactivity revealed similar localization characteristics as described previously (9). However, the sensitivity of the methodology was improved by the antigen-retrieval treatment in 0.01 m (pH 6.5) citrate buffer by microwave heating. As a result, enhanced immunostaining was observed in the present study. In general, caveolin-1 immunoreactivity was present in all of the cancer tissue specimens and was predominantly localized to some smooth muscle cells and to the endothelial cells of blood vessels in the stroma. Histologically normal glandular epithelia adjacent to cancer cells showed no or weak immunoreactivity for caveolin-1 (Fig. 1A), yet the characteristic granular immunoreaction products were present in the cytoplasm of cancer cells (Fig. 1B). The caveolin-1-positive cytoplasmic granules varied greatly in their abundance, with a higher density being associated with more poorly differentiated cancer (Fig. 1B). In general, caveolin-1 staining within the cancer cell population was heterogeneous, and the proportion of caveolin-1-positive cancer cells varied with individual specimens, ranging from 3.03 to 93.33% and with a median value of 12.50%. According to our criteria (see “Materials and Methods”), 25% of the 189 specimens tested positive for caveolin-1 immunostaining. The incidence of caveolin-1-positive cancers varied with the clinical and pathological variables, as indicated in Table 1.

Correlation of Caveolin-1 Expression with Clinical and Pathological Variables. The data regarding caveolin-1 expression in relation to clinical and pathological features are summarized in Table 1. All clinical and pathological parameters, except preoperative PSA and surgical margin status, were correlated with caveolin-1 expression in the complete set of 189 patients. However, because of incomplete data on every patient specimen, the correlation analysis on preoperative PSA levels and on surgical margin was performed only on 128 and 181 patients, respectively (Table 2).

Among the 189 prostate cancer specimens, 47 (25%) scored positive for caveolin-1 immunoreactivity. The percentage of T1, T2, and T3a cancers with positive caveolin-1 immunoreactivity was 26, 25, and 24%, respectively. No significant correlation between caveolin-1 expression and preoperative clinical stage was found (P > 0.05). When the preoperative PSA levels of the patients were stratified into three groups, <4, 4–10, and >10 ng/ml, the patient group with a <4 ng/ml had the lowest rate of caveolin-1-positive cancers (16% versus 37% in the 4–10 ng/ml group and 30% in the >10 ng/ml group). However, no significant correlation between caveolin-1 expression and preoperative PSA level was found (P < 0.299; Table 2).

Gleason scores for prostatectomy specimens were stratified into well differentiated (Gleason score 3–5; n = 23), moderately differentiated (Gleason score 6–7, n = 143), and poorly differentiated (Gleason score 8–10, n = 23) groups. The caveolin-1-positive cancer rates were 13, 24, and 39% for these groups, respectively. A positive correlation between caveolin-1 and Gleason score was confirmed (correlation coefficient, 0.197; P = 0.0071).

Fig. 1. Caveolin-1 expression as demonstrated by ABC immunohistochemical staining in histologically normal prostate tissue (A) and in adjacent, poorly differentiated prostate cancer (B). Caveolin-1 in normal prostate was primarily localized to some smooth muscle cells and endothelial cells of blood vessels in the stroma with negatively stained glandular epithelia (A). In contrast, caveolin-1 immunostaining was present in the cytoplasm of cancer cells (B). ×200 for both A and B.
In the caveolin-1-positive specimens for which there was more than one Gleason grade pattern, caveolin-1 expression tended to appear in the poorly differentiated one. To further determine whether there is a correlation between Gleason grade and caveolin-1 expression, a total of 77 cancer foci, which included 5 Gleason grade 2, 49 Gleason grade 3, 20 Gleason grade 4, and 3 Gleason grade 5 patterns, were screened from this set of specimens. The caveolin-1 expression rates were 0, 16, 35, and 33% for Gleason grades 2, 3, 4, and 5 cancers, respectively. A significant correlation between caveolin-1 expression and the extraprostatic extension status was found. In the specimens with extraprostatic extension only (n = 46), no significant correlation between caveolin-1 expression and the extraprostatic extension status was found.

Preoperative PSA (ng/ml) was found (Spearman correlation test, respectively. A significant correlation between caveolin-1 expression rates were 0, 16, 35, and 33% for Gleason grades 2, 3, 4, and 5 cancers, respectively. A significant correlation between caveolin-1 expression and extraprostatic extension status was found. In the specimens with extraprostatic extension (n = 46), no significant correlation between caveolin-1 expression and the extraprostatic extension status was found.

The caveolin-1-positive cancer rate was found in metastatic tumor deposits (12 of 21; 57%) relative to their primary tumor counterparts (9 of 21; 43%). Of the 21 patients, 8 of 9 who had caveolin-1-positive primary cancer also had caveolin-1-positive lymph node metastases. Subjectively, the proportion of caveolin-1-positive cancer in the metastatic deposits appeared higher than that in their primary counterpart as well.

The frequency of caveolin-1 expression was higher in the cancers with a positive surgical margin (42%; n = 33) compared with those with negative surgical margins (23%; n = 148), and a positive correlation of caveolin-1 with positive surgical margin status was confirmed (correlation coefficient, 0.177; P = 0.0267; Table 2).

Caveolin-1 expression was somewhat higher in specimens with extraprostatic extension (30%) or seminal vesicle involvement (30%) compared with those without extraprostatic extension (22%) of specimen levels 3 extracapsular extension, Ref. 16; 20%) or seminal vesicle involvement (24%) correspondingly, but these differences were not statistically significant (Table 2). In the 97 patients with extraprostatic extension, 21 also had positive surgical margins, 28 had seminal vesicle involvement, and 2 had both positive margins and seminal vesicle involvement. In the specimens with extraprostatic extension only (n = 46), no significant correlation between caveolin-1 expression and the extraprostatic extension status was found.

In this serial of 189 patients, 166 Caucasian Americans and 11 African Americans were included. The remainder included Hispanic (n = 4), Asian (n = 6), and unknown race (n = 2). The incidence of caveolin-1-positive cancers was higher in African Americans (5 of 11; 45%) than in the Caucasian Americans (42 of 166; 25%), although the difference was not statistically significant in this limited sample set (P = 0.1205, Fisher’s exact test).

Caveolin-1 as a Predictor for Time to Disease Progression.
Analysis of the association of caveolin-1 expression with time to disease progression after surgery was performed on patients with negative lymph nodes. In the 162 patients with negative lymph nodes, 22% of them (36 of 162) were immunoreactive for caveolin-1. The association of caveolin-1 expression with time to disease progression was determined (24%) correspondingly, but these differences were not statistically significant (Table 2). In the 97 patients with extraprostatic extension, 21 also had positive surgical margins, 28 had seminal vesicle involvement, and 2 had both positive margins and seminal vesicle involvement. In the specimens with extraprostatic extension only (n = 46), no significant correlation between caveolin-1 expression and the extraprostatic extension status was found.

When the progression-free probability was plotted versus caveolin-1 staining status, the patients with a caveolin-1-positive cancer had an evident worse prognosis compared with those with a caveolin-1-negative cancer (Fig. 2). The probabilities of being free of progression...
after surgery at 60 months were 0.68 ± 0.042 (mean ± SE) for caveolin-1-negative cancer and 0.43 ± 0.08 (mean ± SE) for the caveolin-1-positive cancer.

Univariate analyses using log-rank test identified positive caveolin-1 (P = 0.0033), Gleason score (P < 0.0001), seminal vesicle involvement (P < 0.0001), positive surgical margins (P < 0.0025), extraprostatic extension (P < 0.0001), or preoperative PSA level (P = 0.0011) as significant prognostic predictors for a shorter time to progression after surgery (Table 3). Age provided no prognostic value in this set of patients.

Multivariate analysis of the same set of patients was performed for caveolin-1, together with many of the commonly used pathological predictors for time to progression using the Cox regression model. The results indicated that positive caveolin-1 is an independent prognostic factor for time to progression (risk ratio, 1.913; P = 0.0186; Table 4). Gleason score (>7), extraprostatic extension seminal vesicle involvement, as well as positive surgical margins, also had independent prognostic value, with a risk ratio of 4.319 (P = 0.0011), 3.016 (P = 0.0004), 2.369 (P = 0.0125), and 1.888 (P = 0.0381), respectively (Table 4).

DISCUSSION

In the United States, ~60% of newly diagnosed prostate cancers are believed to be organ confined (19), yet somewhat <50% of these cancers are found to be confined to prostates, as indicated by final pathological stage after surgical removal of the prostate (5). When the cancer is organ confined, progression-free survival is excellent after radical prostatectomy (20). In contrast, those that are found not to be confined to the prostate upon pathological analysis are not usually curable by surgery alone and have a much poorer prognosis (8). Preoperative PSA levels, clinical stage, and Gleason score have been shown to be useful in predicting clinical outcomes; however, in the middle ranges, these indices lose predictive power. We demonstrated previously that caveolin-1 expression was associated with metastasis in mouse and human prostate cancer (9) and related to the androgen-resistant phenotype in mouse prostate cancer (11). The purpose of the present study was to determine whether caveolin-1 expression of caveolin-1 as determined by immunostaining could provide prognostic value for the patients with clinically confined prostate cancer.

In a large set (n = 189) of prostate cancer specimens, 25% were determined to be positive for cancer cell-specific caveolin-1 immunoreactivity. There was a positive correlation of caveolin-1 expression with positive lymph nodes removed at surgery. It was also found that patients with caveolin-1-positive primary cancer tended to have caveolin-1-positive lymph node metastases. These results, together with our previous study that demonstrated a high (56%) incidence of caveolin-1 expression in metastatic deposits within pelvic lymph nodes (9), indicate a clear association of caveolin-1 expression with development of lymph node metastasis. Once prostate cancer has spread to the regional pelvic lymph nodes, only rarely can it be controlled with any form of local or regional therapy (21). Virtually all patients with positive nodes treated with surgery alone showed clinical evidence of progression within a 10-year period, and at least 70% of this group will eventually die of cancer rather than with it (22). The correlation of caveolin-1 expression with positive lymph node metastases, positive surgical margins, as well as Gleason score/grade of the cancer, indicates that caveolin-1 expression is associated with progression/metastasis of prostate cancer. Although the molecular and cellular underpinnings for this relationship are not well understood, our recent study using a mouse prostate cancer model indicated that caveolin-1 levels were higher in metastatic lesions and metastasis-derived cell lines relative to primary tumors and primary tumor-derived cell lines, respectively (9), and that suppression of caveolin-1 by antisense caveolin-1 vectors converted androgen-resistant metastatic mouse prostate cancer cells to the androgen-sensitive phenotype in vivo (11). In contrast, androviral vector-mediated overexpression of caveolin-1 reestablished androgen resistance in vitro, preventing apoptosis that occurred in control cells after removal of testosterone (11). These data strongly suggest that elevated caveolin-1 expression promotes survival of prostate cancer cells under conditions that can lead to cell death, including those encountered during metastasis and those generated by androgen withdrawal. Our present results further indicate that caveolin-1 expression in the primary tumor is heterogeneous and highly variable. This pattern of expression is similar to other molecular markers of prostate cancer progression (e.g., p53 mutations) that have been shown to occur at low frequency in the primary tumor but at significantly higher frequency in lymph node metastases (reviewed in Ref. 23). Interestingly, both mutations in p53 and overexpression of caveolin-1 have been associated with reduced capacity for apoptosis, supporting the notion that antiapoptotic mutations are important in prostate cancer metastasis. This concept is now further supported by the results from this present study, indicating that caveolin-1 is a positive prognostic marker for a shorter progression-free survival in the clinically localized cancer. Interestingly, two recent reports published shortly after our identification of caveolin-1 overexpression in prostate cancer metastasis (9) and its association with androgen resistance (11) provided strong correlative evidence that caveolin-1 is also associated with the drug resistance phenotype in multiple human carcinoma cell lines (12, 13).

In our study, there was also a trend toward higher incidence of caveolin-1-positive cancers in African American patients (45%) compared with Caucasian American patients (25%). The difference, however, was not statistically significant (P = 0.1205; χ² test), probably because of the relatively small sample size, especially in the black population. It is known that there is a significantly higher rate of prostate cancer incidence and mortality in African Americans compared with Caucasian Americans (24), and in general, prostate cancer in African-American men tends to be more aggressive (25). Socioeconomic, environmental, as well as biological factors have been attributed to the racial differences (24). An obvious question is whether elevated caveolin-1 expression is indeed higher in African-American prostate cancer relative to other ethnic groups and whether

![Table 4 Multivariate analysis of caveolin-1 and pathological parameters to predict recurrence in 162 lymph node-negative cancers](#)

<table>
<thead>
<tr>
<th>Prognostic marker</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>Caveolin-1</td>
<td>1.913 (1.114–3.284)</td>
<td>0.0186</td>
</tr>
<tr>
<td>Gleason score (&gt;7)</td>
<td>4.319 (2.069–9.018)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Extraprostatic extension</td>
<td>3.016 (1.634–5.566)</td>
<td>0.0045</td>
</tr>
<tr>
<td>Positive surgical margins</td>
<td>1.888 (1.036–3.442)</td>
<td>0.0381</td>
</tr>
<tr>
<td>Seminal vesicle involvement</td>
<td>2.369 (1.204–4.662)</td>
<td>0.0125</td>
</tr>
</tbody>
</table>

a CI, confidence interval.

b From Wald χ², Cox regression test.

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Table 3 Univariate analysis for caveolin-1 and pathological parameters to predict time to recurrence in lymph node-negative cases (n = 162)

<table>
<thead>
<tr>
<th>Prognostic marker</th>
<th>P value</th>
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<tr>
<td>Caveolin-1</td>
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<td>Gleason score (&gt;7)</td>
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<tr>
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<td>Preoperative PSA</td>
<td>0.0011</td>
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<tr>
<td>Age</td>
<td>0.078</td>
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a From the log-rank test.
this contributes to the more aggressive profile. A larger scale of study with better population control could help to clarify this issue.

In summary, our present study has demonstrated that caveolin-1 expression, as determined by immunohistochemical staining, is associated with positive lymph nodes and positive surgical margins in clinically confined prostate cancers. The possibility of using caveolin-1 expression in needle biopsies as a preoperative marker to predict pathological stage remains to be investigated. However, this potential approach to the use of caveolin-1 immunostaining may be significantly complicated by the multicentric nature of prostate cancer in most patients (26, 27), the relatively small and potentially disproportionate quantity of cancer tissue in needle biopsies relative to the cancer volume, and the heterogeneity and likely small proportion of caveolin-1-positive cells in the cancer. On the other hand, the present study clearly revealed that caveolin-1 expression in lymph node-negative human prostate cancer predicts a shorter time to disease progression after radical prostatectomy. Multivariate analysis demonstrated that caveolin-1 expression provides additional prognostic value for disease progression over traditional pathological markers. Thus, evaluation of caveolin-1 expression in surgically removed cancer may facilitate clinical decisions regarding the need for the patients to receive adjuvant treatment postoperatively. The association of caveolin-1 with cancer progression also suggests to us that caveolin-1 itself and possibly specific molecular components involved in its regulation or downstream activities represent targets for the development of novel therapies for metastatic prostate cancer.

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