1.a. **Full Title**: Circulating caveolin-1 levels and prostate cancer incidence

b. **Abbreviated Title (Length 26 characters)**: Caveolin-1 and prostate cancer

2. **Writing Group**:
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   Others are welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __RH__ [please confirm with your initials electronically or in writing]

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3. **Timeline**: All plasma caveolin-1 measurements have already been performed and data analyses will start immediately upon approval of the manuscript proposal. Manuscript preparation is anticipated to be completed by June 2006
4. Rationale:

The precise risk factors for prostate cancer are unknown with both genetic factors and environmental factors likely to be involved (1). African-American men have a much higher incidence and mortality from prostate cancer than white-American men (2). In recent years, efforts to detect and treat prostate cancer have increased dramatically throughout the United States resulting in an apparent increased incidence of the disease and a dramatic rise in the number of radical prostatectomies and irradiation therapy treatments (2, 3). The incidence of prostate cancer has begun to decline (a likely result of saturation of prostate cancer screening), and age-adjusted mortality from prostate cancer (as well as other malignancies) has also recently declined due to unknown factors (2, 4). Unfortunately, the mortality rate remains exceedingly high – a somewhat surprising condition in light of the level of increased utilization of potentially curative treatment modalities over the last decade. One possible explanation for the low impact of prostate cancer therapy thus far is that occult metastases were present at the time of treatment. The treatments currently used for presumably localized disease are indeed exclusively local treatments that are designed to ablate the tumor either surgically or through radiation. Metastatic disease present at the time of treatment would therefore continue to progress. Indeed, the reported failure rate, within 5 years as indicated by rising prostate-specific antigen levels for patients undergoing radical prostatectomy, ranges from 20% (5) to 57% (6), indicating the presence of either local tumor recurrence and/or occult metastasis at the time of treatment.

The assessment of prostate cancer in regard to stage of disease is complicated by both lack of reliable specific tests that differentiate localized disease from early metastatic disease and the highly complex presentation of the local tumor. Localized prostate cancer is exceedingly slow growing and exhibits a remarkable degree of morphologic complexity and histologic heterogeneity. Although a prominent index cancer is typically present, it has long been recognized that prostate cancer is multifocal, usually contains more than one histological grade, and is often juxtaposed and admixed with other benign pathology such as benign prostatic hyperplasia (BPH). Malignant potential is currently most often assessed by the grading system proposed by Gleason (7). Yet examination of radical prostatectomy specimens of non-palpable cancers has revealed that up to 45% of high-grade tumors (Gleason grade 4 or 5) were less than 1 cm³ in volume (8). These clinical data point to the possibility that highly aggressive disease may present early as small tumors and not necessarily evolve in a predictable fashion from low-grade tumors (9). The results of other studies also indicate that although there is a general relationship of tumor volume with metastatic progression, relatively small tumors that are confined to the prostate may also seed metastases (10, 11). These clinical observations have been supported by the results of in vivo experiments that indicate metastases do not necessarily originate from the most abundant clone of the malignant cells at the primary site (12). Overall, the complex morphologic patterns, histologic heterogeneity, and the early manifestations of high malignant potential preclude a straightforward assessment of the metastatic potential of localized prostate cancer and suggest that an additional serum marker besides prostate specific antigen (PSA) could provide useful information.

We have developed new insight into the progression of prostate cancer through investigations of caveolin-1 (cav-1) expression specifically in metastatic disease and propose to validate its use as a biomarker for prostate cancer. Our previous clinical studies in this area have followed a logical progression from the identification of cav-1 overexpression in metastatic prostate cancer (13); the determination of cav-1 as an independent prognostic marker for prostate cancer progression in patients who have recurred following radical prostatectomy (14) and a significant association of increased cav-1 in prostate cancer in African-American men vs. white-American men (15). Our basic research studies have elucidated the mechanism of action
of cav-1 as an anti-apoptotic gene under a variety of clinically relevant circumstances including growth factor deprivation and oncogene overexpression (16-18).

For our clinical studies we have relied on immunohistochemical detection of cav-1 in prostate specimens, such as those obtained at the time of surgery, to analyze cav-1 expression. However, recently we have discovered that cav-1 is secreted by prostate cancer cells and is present in the HDL-3 fraction of human serum at higher levels in prostate cancer patients compared to those without this disease (19). We have used western blotting to document the presence of cav-1 in the serum HDL3 fraction (Figure 1). As demonstrated in this series of 4 western blots, 14 of 16 men with prostate cancer had detectable serum cav-1 whereas only 4 of 16 age matched men without prostate cancer had a signal. Cav-1 migrates with an apparent molecular weight of 22 kD and larger aggregates are typically seen. Our standard of recombinant purified cav-1 has a 6X-His tag and migrates somewhat slower than the authentic cav-1. We have completed a more detailed analysis that confirms this general observation with serum samples from the patient cohort listed in Table I. Since western blotting analysis is semi-quantitative we are in the process of developing a quantitative ELISA for the analysis of cav-1 in serum/plasma.

Our observations are consistent with the view that HDL3 is the principal acceptor of excess cholesterol in the cell’s plasma membrane, and that cav-1 plays a significant role in transporting cholesterol to that site. Caveolae are a major site of cholesterol efflux from cells and cav-1 mediates the intracellular movement of cholesterol (20); in the case of prostate cancer cells caveolin may also be trans-ported out of the cell. Caveolin-1 mRNA expression has been found to be regulated by cholesterol and the level of low-density lipoprotein (21). This may also be relevant to atherosclerosis since a case-control study found that individuals with a history of coronary heart disease (CHD) had an increased risk for prostate cancer (22). A recent report has defined a subset of breast cancer patients with mutations in the cav-1 gene at codon 132 (23). In our studies of cav-1 in several prostate cancer cell lines as well as selected prostate cancer specimens we have not detected any specific mutations in the cav-1 gene, however it is conceivable that genetic mutations of cav-1 may play a role in the development and progression of prostate cancer.

| TABLE I  Characteristics of patients with prostate cancer analyzed for serum cav-1 |
|-----------------|-----------------|-----------------|
| Age             | 63 (48-73)      |                 |
| PSA (Pre-OP)    | 13.1 (3-35)     |                 |
| Gleason Score   | 7               |                 |
| Follow-up (months) | 60.7 (12.5-149) |                 |
| Positive margins | 23% (9/39)      |                 |
| Extracapsular extension | 85% (33/39) |                 |
| Seminal vesicle invasion | 54% (24/39) |                 |

References


5. Main Hypothesis/Study Questions:

**Hypothesis 1:** Caveolin-1 plasma levels are significantly correlated with prostate cancer incidence

**Hypothesis 2:** Caveolin-1 plasma levels in Black men are significantly higher than in White, aged matched men, and this elevation correlates with increased incidence of prostate cancer.

6. Data (variables, time window, source, inclusions/exclusions):
Caveolin-1 measurements were made on Visit 1 plasma samples of prostate cancer cases and a stratified random sample of non-cases. Data will include prostate cancer case status and date of prostate cancer diagnosis. Covariates will include visit 1 age, race, center, BMI, waist circumference, lipids (TC, TG, HDL-C, LDL-C), prevalent diabetes, prevalent hypertension, and years of cigarette smoking, metabolic syndrome status.

The analyses are complicated by the study design, which appears to be a mixture of a couple of approaches. The basic design is a nested case-control design based on post visit 1 incident prostate cancer cases. For this, appropriate selection of controls would have been sampling from the risk set at the time of diagnosis of each case. Using incident cases but non-cases selected at baseline has the potential to introduce biases (Ref. Lubin JH, Gail MH. Biased selection of controls for case-control analyses of cohort studies. Biometrics. 1984 Mar;40(1):63-75.). Further, non-cases were selected using a randomized recruitment strategy that requires modifications to standard analysis techniques in order to produce valid results (Refs. Weinberg CR, Wacholder S. The design and analysis of case-control studies with biased sampling. Biometrics. 1990 Dec;46(4):963-75. Weinberg CR, Sandler DP. Randomized recruitment in case-control studies. Am J Epidemiol. 1991 Aug 15;134(4):421-32.) and it is not clear how these methods would carry over to the incident cases situation. Other minor complications include that
although the cases do appear to be incident cases (without prevalent cancer of any type at baseline), there are some controls who had a history of cancer at baseline. Also, some of the controls had incident prostate cancer in the same time-frame as the cases, and there were a few other ARIC participants who had incident prostate cancer in this time-frame but were not selected as cases or as controls (and so were not assayed for caveolin-1).

Our proposal to deal with these design complications is to do a post hoc conversion of the design into a case-cohort design. The key feature here of the case-cohort design is that a case can also be selected in the cohort. The stratified sampling of non-cases was done by giving each individual a specified probability of being selected, rather than drawing a fixed number of individuals from each stratum. So we can apply exactly the same procedure to the cases, as if they had been part of the sampling frame when the sample was drawn initially. We will then augment the controls with these sampled cases to form the cohort random sample. We will exclude from analyses the controls that had prevalent cancer at baseline.

We will then use the standard ARIC approach to time-to-event analysis in a case-cohort study. That is, we will use proportional hazards models along with Barlow’s method to correct the variance estimates, including using weights to account for the variation in sampling probabilities across the strata. Time will be time from visit 1 to prostate cancer diagnosis or censoring. (Strata are 5-year age groups, with sampling probabilities chosen to be close to the distribution of cases across these strata.) Hypothesis 1 will be investigated using this approach, first investigating the association between cav-1 and prostate cancer without further adjustment and then with adjustment for the covariates listed at the beginning of this section.

For baseline descriptive comparisons of cases and controls, we will use SUDAAN to take into account the sampling procedure used to draw the controls. To test Hypothesis 2 we will use SUDAAN to conduct a two sample t-test to compare cav-1 means between blacks and whites and ANCOVA to examine how this is affected by adjustment for other covariates. We will also investigate interaction between race group and cav-1 on incidence of prostate cancer using time-to-event methods.

7.a. Will the data be used for non-CVD analysis in this manuscript?  __X__ Yes    ____ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used?  ___X__ Yes    ____ No
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?  ____ Yes    __X__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  ____ Yes    ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously
approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

____X__ Yes _______ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

MS#1078: Metabolic Syndrome and Prostate Cancer Incidence
Lead author: Aaron Folsom

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____X__ Yes _____ No

11.b. If yes, is the proposal

____X__ A. primarily the result of an ancillary study (list number* 2001.04__)

____ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ __________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expires.