**Advances in Brief**

### Elevated Caveolin-1 Levels in African-American versus White-American Prostate Cancer

Guang Yang, Josephine Addai, Michael Ittmann, Thomas M. Wheeler, and Timothy C. Thompson

Departments of Urology [G. Y., J. A., T. M. W., T. C. T.], Pathology [M. I., T. M. W.], Molecular and Cellular Biology [T. C. T.], and Radiology [T. C. T.], Baylor College of Medicine, Houston, Texas 77030

**Abstract**

Clinical studies suggest that African-American (AA) prostate cancer patients manifest a more aggressive form of the disease compared with white prostate cancer patients. However, the biological underpinnings of this potential difference remain unresolved. To address this issue, we used specific quantitative immunostaining protocols to determine whether a panel of biomarkers related to apoptosis including caveolin-1, bcl-2, p53, and c-myc were differentially expressed in AA versus white prostate cancer patients with similar clinical and pathological characteristics. We further attempted to correlate biomarker positivity with proliferation-related markers including Ki-67 labeling index and apoptotic index. Interestingly, our results indicated that only the incidence of caveolin-1 staining was significantly different between these two ethnic/racial groups of prostate cancer patients. The incidence of caveolin-1 staining in white patients was 17% compared with 39% in AA patients ($P = 0.0048$; Fisher's exact test). In addition, the percentage of caveolin-1-positive prostate cancer cells was also higher in moderately differentiated (Gleason score 6) prostate cancer patients in AA versus white patients. Because caveolin-1 has been shown previously to demonstrate antiapoptotic activities in prostate cancer cells, our results suggest that differences in caveolin-1 expression may in part underlie the apparent differences in the clinical virulence of this disease in AA versus white prostate cancer patients.

**Introduction**

Both the age-adjusted incidence and mortality rates of prostate cancer in AA men have been reported to be significantly higher than those in white-American men (1, 2). Socioeconomic factors may partially explain these differences because at diagnosis AA tend to have higher clinical stage (3) and more poorly differentiated cancer (4). However, AA patients who have equal access to medical service and have similar pathological stage at diagnosis as their white counterparts still have significantly higher rates of recurrence after radical prostatectomy and increased overall death rate from this disease (5, 6). Overall, the clinical data suggest that intrinsic differences in the biological activity of the cancer per se and/or the host response to it underlie the more virulent nature of AA versus white prostate cancer. Thus far, studies designed to identify discriminating biomarkers in AA versus white patients that may predispose toward or contribute to more aggressive AA prostate cancer have been limited and in general inconclusive (reviewed in Refs. 7–9). We have identified previously caveolin-1 expression as being up-regulated in metastatic prostate cancer (10) and as a biomarker that has independent prognostic value in recurrent prostate cancer after radical prostatectomy (11). We have further shown that caveolin-1 can promote survival through antiapoptotic activities in prostate cancer cells in vitro and in vivo under adverse conditions, such as withdrawal of testosterone (12). In this study, we used specific immunostaining protocols to determine whether caveolin-1 or other biomarkers including bcl-2, p53, and c-myc are differentially expressed in AA versus white prostate cancer. We further attempted to correlate biomarker positivity with proliferation-related markers, i.e., KiI (percentage of Ki67-positive cancer cells) and AI (number of apoptotic cells per 1000 cancer cells).

**Materials and Methods**

**Patients and Prostate Specimens.** Prostate cancer specimens from 71 AA and 71 white (non-Hispanic) men that had clinically confined cancers and had undergone radical prostatectomy were obtained. None of these patients had previously received neoadjuvant hormone therapy. This set of specimens was matched for pathological stage by a statistician such that each group had 49 patients with organ-confined disease, 14 with extraprostatic invasion only and 8 with seminal vesicle invasion, and their cancers had similar average Gleason scores (6.35 for the AA and 6.46 for the whites). The two groups of patients also had comparable average ages (62.00 for the AA and 62.14 for the whites) and preoperative prostate-specific antigen levels (5.2 in the AA and 8.2 in the whites). Statistical comparisons in pathological stage, Gleason score, and

---

Received 2/11/00; revised 6/26/00; accepted 6/29/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grants CA50588, CA68814, and Specialized Programs of Research Excellence P50-58204 from the National Cancer Institute.

2 To whom requests for reprints should be addressed, at Scott Department of Urology, Baylor College of Medicine, 6560 Fannin, Suite 2100, Houston, TX 77030. Phone: (713) 799-8718; Fax: (713) 799-8712; E-mail: timothyt@bcm.tmc.edu.

3 The abbreviations used are: AA, African American(s); KiI, Ki67 labeling index; AI, apoptotic index.
Table 1 Ethnic/racial differences in biomarkers and their correlation with KiI and AI in pathologically matched white versus African-American prostate cancers

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Whitea</th>
<th>Incidence/total n+ (%)</th>
<th>Median (range)</th>
<th>Pb</th>
<th>AAa</th>
<th>Incidence/total n+ (%)</th>
<th>Median (range)</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caveolin-1</td>
<td>12/71</td>
<td>(16.90)</td>
<td></td>
<td></td>
<td>26/71</td>
<td>(39.43)</td>
<td></td>
<td>0.0048</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>5/62</td>
<td>(8.06)</td>
<td></td>
<td></td>
<td>3/58</td>
<td>(5.17)</td>
<td></td>
<td>0.7183</td>
</tr>
<tr>
<td>c-Myc</td>
<td>27/71</td>
<td>(38.02)</td>
<td></td>
<td></td>
<td>34/71</td>
<td>(47.88)</td>
<td></td>
<td>0.3091</td>
</tr>
<tr>
<td>p53</td>
<td>11/65</td>
<td>(16.92)</td>
<td></td>
<td></td>
<td>16/69</td>
<td>(23.18)</td>
<td></td>
<td>0.396</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KiIa</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole group</td>
<td>65</td>
<td>3.086 (0.163–21.200)</td>
<td></td>
<td></td>
<td>62</td>
<td>2.943 (0.314–20.048)</td>
<td></td>
<td>0.815</td>
</tr>
<tr>
<td>Caveolin-1-positive</td>
<td>11</td>
<td>4.429 (0.163–13.321)</td>
<td></td>
<td></td>
<td>25</td>
<td>2.508 (0.314–16.571)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caveolin-1-negative</td>
<td>54</td>
<td>2.548 (0.429–21.200)</td>
<td>0.178</td>
<td>37</td>
<td>3.238 (0.500–20.048)</td>
<td>0.600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2-positive</td>
<td>5</td>
<td>3.086 (2.026–7.036)</td>
<td></td>
<td></td>
<td>3</td>
<td>3.429 (0.960–3.657)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2-negative</td>
<td>51</td>
<td>2.857 (0.163–21.200)</td>
<td>0.509</td>
<td>49</td>
<td>2.686 (0.314–20.048)</td>
<td>0.798</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc-positive</td>
<td>25</td>
<td>2.112 (0.500–8.071)</td>
<td></td>
<td></td>
<td>31</td>
<td>2.286 (0.571–20.048)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc-negative</td>
<td>39</td>
<td>3.536 (0.163–21.200)</td>
<td>0.187</td>
<td>31</td>
<td>3.543 (0.114–11.143)</td>
<td>0.838</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53-positive</td>
<td>11</td>
<td>4.694 (1.964–21.200)</td>
<td></td>
<td></td>
<td>15</td>
<td>2.686 (0.980–20.048)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53-negative</td>
<td>49</td>
<td>2.571 (0.163–13.321)</td>
<td>0.0134</td>
<td>45</td>
<td>3.238 (0.154–15.540)</td>
<td>0.162</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AIa</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole group</td>
<td>49</td>
<td>6.11 (0.60–40.31)</td>
<td></td>
<td></td>
<td>57</td>
<td>8.23 (0.15–50.90)</td>
<td></td>
<td>0.166</td>
</tr>
<tr>
<td>Caveolin-1-positive</td>
<td>9</td>
<td>3.030 (0.642–12.066)</td>
<td></td>
<td></td>
<td>23</td>
<td>9.091 (1.931–42.105)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caveolin-1-negative</td>
<td>40</td>
<td>6.246 (0.606–40.316)</td>
<td>0.075</td>
<td>34</td>
<td>7.922 (0.152–50.909)</td>
<td>0.961</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2-negative</td>
<td>41</td>
<td>6.294 (0.606–40.316)</td>
<td>0.954</td>
<td>47</td>
<td>6.818 (0.152–50.909)</td>
<td>0.191</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc-positive</td>
<td>20</td>
<td>8.849 (1.116–40.316)</td>
<td></td>
<td></td>
<td>31</td>
<td>6.932 (1.161–50.909)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc-negative</td>
<td>29</td>
<td>5.708 (0.606–30.682)</td>
<td>0.064</td>
<td>26</td>
<td>9.126 (0.152–42.105)</td>
<td>0.466</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53-negative</td>
<td>36</td>
<td>6.089 (0.606–40.316)</td>
<td>0.394</td>
<td>41</td>
<td>7.611 (0.152–45.455)</td>
<td>0.076</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes:

- na or N, the number of patients tested; +, total n varies depending on the number of specimens eliminated from the analysis because of failure to demonstrate any positive staining after several repeated attempts.
- Derived from the Fisher’s exact test.
- Derived from the Mann-Whitney test.
- KiI, percentage of Ki67-positive tumor cells.
- AI, number of apoptotic bodies among 1000 tumor cells.

Quantitative Immunostaining Analyses. Monoclonal antibodies to Bcl-2 (124), p53 (DO-7; Dako Corp., Carpinteria, CA), Ki-67 (MIB-1; Immunotech, Westbrook, ME), c-myc (NCL-c-MYC; Novocastra Laboratory, Ltd., Newcastle-upon-Tyne, United Kingdom), and a polyclonal antibody against caveolin-1 (Santa Cruz Biotechnology, Santa Cruz, CA) were then used to immunostain the adjacent sections with the avidin-biotin-peroxidase technique. For verifying the specificity of the reactions, some sections were incubated in normal mouse (for monoclonal primary antibodies) or rabbit serum (for caveolin-1) replacing the primary antibodies. The terminal deoxynucleotidyl transferase-mediated nick end labeling technique developed by Gavrieli et al. (15) was used to label the cancer cells undergoing apoptosis. All stainings were evaluated by an investigator (G. Y.) blinded to the clinical information as well as the ethnic/racial status of the patients. Positive expression of both Bcl-2 and caveolin-1 were defined as >50% of the cancer cells immunoreactive for corresponding antibodies in any microscopic measuring field (with a real area of 0.0625 mm²). Positive c-myc expression was assigned if >5% of cancer cells were labeled by the c-myc antibody; positive p53 expression was defined as described previously (16). The KiI refers to the percentage of Ki-67-positive cancer cells in a cancer. The AI was defined as the number of apoptotic bodies among 1000 cancer cells. Comparisons in the incidences of positive expression of the biomarkers and in Ki-67 labeling rate and apoptotic index in relation to the biomarkers were made between the white and AA patients.

Results

The incidence of positivity for each biomarker is shown in Table 1. Caveolin-1 immunoreactivity was detected in the cytoplasm of cancer cells in a granular pattern (Fig. 1), and the proportion of caveolin-1-positive cells varied within individual specimens. In addition to stromal and endothelial cells, caveolin-1 was also expressed within prostate cancer cells in 40 of 142 total (white and AA) specimens tested (28.17%). The inci-
Ethnic/Racial Differences in Biomarkers in Prostate Cancer

of the total cancer specimens (61 of 142), there were significant \((P = 0.396, \text{Fisher’s exact test})\). There were no significant differences in the KiI between the two ethnic groups \((P = 0.815, \text{Mann-Whitney test})\) as a whole. In a comparison of subgroups stratified according to biomarker expression p53-positive cancer in whites but not in AA had a significantly higher KiI relative to p53-negative cancers \((0.0134, \text{Mann Whitney test})\). There was no significant difference in KiI between the two groups \((P = 0.1228)\) or in any biomarker expression subgroup (Table 1).

Because caveolin-1 positivity discriminated white patients from AA patients in that AA patients had significant higher rates of positivity, we further explored this marker in regard to the percentage of positive cancer cells and compared these values to Gleason score in White versus AA patients. The results indicated that White patients demonstrated increasing expression of caveolin-1 proceeding from moderately differentiated to poorly differentiated prostate cancer, a result that was consistent with our previous reports of a larger patient population after radical prostatectomy (11). In AA patients, a reversal of that trend was suggested with a significantly higher percentage of caveolin-1-positive cancer cells in AA versus white patients in moderately differentiated prostate cancer (see Fig. 1B).

**Discussion**

Our studies have revealed significantly higher levels of caveolin-1 expression per case in AA versus white prostate cancer when measured in immunostaining. These differences are in contrast to other prominent biomarkers including bcl-2, p53, and c-myc that have been associated previously with prostate cancer, yet failed to show any significant differences between these two ethnic/racial groups. We further demonstrated a difference in AA versus white prostate cancer in regard to the relationship of caveolin-1 positivity as a percentage of cancer cells with histological differentiation as assessed by Gleason score. Interestingly, in white patients, the frequency of caveolin-1-positive prostate cancer cells increased during the transition from moderately differentiated to poorly differentiated disease, whereas in AA a reversal of that trend was suggested. Importantly, AA prostate cancer demonstrated a significantly higher percentage of caveolin-1-positive cells in moderately differentiated disease relative to whites. We have previously associated caveolin-1 with metastatic prostate cancer (10) and have demonstrated recently that caveolin-1 labeling has independent prognostic significance for recurrence after radical prostatectomy (11). Experimental studies have further demonstrated that caveolin-1 may contribute to malignant progression in part through facilitation of survival under adverse conditions, such as depletion of androgens both in vitro and in vivo (12). We and others have observed previously that high levels of caveolin-1 expression can lead to growth suppression under some conditions (17–19), and some have suggested that caveolin-1 is a tumor suppressor gene (19). However, mutation analysis of the caveolin-1 gene has thus far not revealed alterations consistent
with classic tumor suppressor activities (20). In general, conditional growth-suppressive activities are not inconsistent with antiapoptotic functions that can be selected for during malignant progression. A well-known additional example of these coexisting functions is the bcl-2 gene, which can exhibit growth suppression and/or antiapoptotic activities, depending on the context and/or levels of expression (21, 22). Overall, in consideration of past studies and this current report, one could speculate that seeding of prostate cancer cells into the lymphatic or hematogenous circulation at a relatively early stage of progression may yield more productive metastases in AA versus white patients, in part through overexpression of caveolin-1. Testing this hypothesis will require additional experimental and clinical studies. However, these new data provide insight into the biological aggressiveness of AA versus white prostate cancer, and notwithstanding the complexity of prostate cancer and remarkable heterogeneity of this disease may, together with Gleason grade, provide additional prognostic information and inform therapeutic decisions specifically in moderately differentiated AA prostate cancer.

Acknowledgments

We thank Drs. Dov Kadmon, Armin Weinberg, and Larry Kaufman for helpful discussions regarding these studies.

References


C. Ren and T. C. Thompson, unpublished data.
Elevated Caveolin-1 Levels in African-American versus White-American Prostate Cancer

Guang Yang, Josephine Addai, Michael Ittmann, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/9/3430

Cited articles
This article cites 19 articles, 8 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/9/3430.full.html#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/6/9/3430.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.