A Similar Pattern of Chromosomal Alterations in Prostate Cancers from African-Americans and Caucasian Americans

Michael L. Cher, Paul E. Lewis, Mousumi Banerjee, Patrick M. Hurley, Wael Sakr, David J. Grignon, and Isaac J. Powell

Departments of Urology [M. L. C., P. E. L., P. M. H., I. J. P.], Pathology [M. L. C., P. E. L., W. S., D. J. G.], and Biostatistics [M. B.], Wayne State University School of Medicine, Detroit, Michigan 48201, and Genitourinary Oncology Program of the Karmanos Cancer Institute, Detroit, Michigan [M. L. C., M. B., P. M. H., W. S., D. J. G., I. J. P.]

ABSTRACT

A combination of genetic and epigenetic factors may explain the disproportionate incidence and mortality of prostate cancer among African-American males (AAMs) as compared with Caucasian American males (CAMs). We wished to determine whether primary prostate cancers from AAMs and CAMs harbor different patterns or frequencies of chromosomal alterations.

Comparative genomic hybridization (CGH) was performed on clinically localized, untreated primary prostate cancers from 16 AAMs and 16 CAMs. Detailed statistical analysis was used to define gains and deletions with high sensitivity and specificity and to compare the frequency and pattern of alterations between the two groups of tumors.

The two groups of patients had indistinguishable preoperative serum prostate-specific antigen levels, and the two groups of tumors had similar pathological stages and grades. Chromosomal gains and deletions occurred in regions known to be frequently altered in prostate cancer. Specifically, the most frequent alterations were deletions of regions on chromosomes 13q, 5q, 16q, and 8p and gains of regions on 8q and 5q. When tumors from AAMs and CAMs were compared, the frequencies of alteration (deletion, gain, or no alteration) were similar across 98.9% of the length of the genome. The patterns of alterations of the most frequently altered chromosomes were also similar between tumors from AAMs and CAMs.

We concluded that primary prostate cancers from AAMs and CAMs harbor a similar pattern and frequency of chromosomal alterations. These data support the notion that sporadic prostate cancers from AAMs and CAMs develop by similar chromosomal mechanisms. Biological differences, if present, do not occur on the chromosomal level.

INTRODUCTION

Many studies have shown that AAMs with prostate cancer have significantly poorer survival than CAMs (1–5). This difference in survival may be explained in part by the fact that AAMs consistently present with higher stage tumors (6–12). Indeed, we have shown that high-grade prostatic intraepithelial neoplasia is more common among AAMs, and the diffuse form appears earlier (13, 14).

Socioeconomic or education factors have been cited as the reasons for the disparity in disease outcome. However, disproportionate mortality occurs even when access to medical care is equal (6). Furthermore, among several different types of human cancers, prostate cancer consistently shows a great disparity in incidence between AAMs and CAMs, and this difference may persist after adjusting for socioeconomic differences (15, 16). Others have also shown that socioeconomic factors cannot completely explain racial differences in prostate cancer incidence and mortality (17, 18).

Even on a stage-by-stage basis, some studies have shown that AAMs may have worse survival than CAMs (19, 20), although this issue has not been resolved (6). Thus, differences in survival may be explained not only by increased stage at presentation (which may itself be due to biological factors) but by biological differences within stages. Studies to date thus lead to the hypothesis that there may be “racially” determined differences in prostate cancer tumor biology, and that AAMs may have biologically more aggressive tumors.

Several hypotheses have been advanced to try to explain ethnic differences in tumor biology. These hypotheses can be categorized in two ways: (a) an inherited predisposition toward more aggressive disease may be more common among AAMs. For example, AAMs tend to have shorter CAG microsatellite alleles in exon 1 of the androgen receptor gene, which could impose a predisposition to the development or progression of prostate cancer (21); and (b) epigenetic (environmental) factors may facilitate disease development or progression among AAMs compared with CAMs. Epigenetic influences on normal or cancerous prostate cells may be further categorized into those that alter metabolic processes (e.g., gene expression patterns) and those that cause permanent changes in the genome (e.g., carcinogenic agents). Of course, epigenetic processes and inher-
Table 1  Preoperative PSA, pathological stage, Gleason score, and overall fraction of genome found to be either deleted or gained

<table>
<thead>
<tr>
<th>Serum PSA</th>
<th>Pathological stage</th>
<th>Gleason score</th>
<th>Fraction of genome imbalanced</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAMs</td>
<td>CAMs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.4</td>
<td>pT2</td>
<td>7</td>
<td>0.21</td>
</tr>
<tr>
<td>4.8</td>
<td>pT1</td>
<td>6</td>
<td>0.05</td>
</tr>
<tr>
<td>9.7</td>
<td>pT2</td>
<td>7</td>
<td>0.12</td>
</tr>
<tr>
<td>5.7</td>
<td>pT1</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>4.3</td>
<td>pT2</td>
<td>7</td>
<td>0.22</td>
</tr>
<tr>
<td>5.5</td>
<td>pT2</td>
<td>7</td>
<td>0.26</td>
</tr>
<tr>
<td>46.0</td>
<td>pT2</td>
<td>6</td>
<td>0.10</td>
</tr>
<tr>
<td>6.7</td>
<td>pT1</td>
<td>7</td>
<td>0.12</td>
</tr>
<tr>
<td>6.8</td>
<td>pT1</td>
<td>7</td>
<td>0.26</td>
</tr>
<tr>
<td>4.3</td>
<td>pT1</td>
<td>7</td>
<td>0.10</td>
</tr>
<tr>
<td>5.5</td>
<td>pT2</td>
<td>7</td>
<td>0.12</td>
</tr>
<tr>
<td>3.0</td>
<td>pT1</td>
<td>8</td>
<td>0.23</td>
</tr>
<tr>
<td>6.4</td>
<td>pT2</td>
<td>7</td>
<td>0.06</td>
</tr>
<tr>
<td>8.2</td>
<td>pT2</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>6.0</td>
<td>pT1</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>9.1</td>
<td>pT1</td>
<td>7</td>
<td>0.22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum PSA</th>
<th>Pathological stage</th>
<th>Gleason score</th>
<th>Fraction of genome imbalanced</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAMs</td>
<td>CAMs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.9</td>
<td>pT1</td>
<td>7</td>
<td>0.16</td>
</tr>
<tr>
<td>5.0</td>
<td>pT2</td>
<td>7</td>
<td>0.36</td>
</tr>
<tr>
<td>10.1</td>
<td>pT2</td>
<td>7</td>
<td>0.17</td>
</tr>
<tr>
<td>12.0</td>
<td>pT1</td>
<td>7</td>
<td>0.16</td>
</tr>
<tr>
<td>5.9</td>
<td>pT1</td>
<td>8</td>
<td>0.15</td>
</tr>
<tr>
<td>6.4</td>
<td>pT1</td>
<td>7</td>
<td>0.23</td>
</tr>
<tr>
<td>9.9</td>
<td>pT2</td>
<td>7</td>
<td>0.28</td>
</tr>
<tr>
<td>16.0</td>
<td>pT1</td>
<td>8</td>
<td>0.41</td>
</tr>
<tr>
<td>8.8</td>
<td>pT1</td>
<td>7</td>
<td>0.15</td>
</tr>
<tr>
<td>16.4</td>
<td>pT2</td>
<td>7</td>
<td>0.17</td>
</tr>
<tr>
<td>3.2</td>
<td>pT1</td>
<td>7</td>
<td>0.09</td>
</tr>
<tr>
<td>17.4</td>
<td>pT1</td>
<td>9</td>
<td>0.14</td>
</tr>
<tr>
<td>7.2</td>
<td>pT2</td>
<td>7</td>
<td>0.12</td>
</tr>
<tr>
<td>6.5</td>
<td>pT1</td>
<td>7</td>
<td>0.09</td>
</tr>
<tr>
<td>10.1</td>
<td>pT2</td>
<td>7</td>
<td>0.20</td>
</tr>
<tr>
<td>12.0</td>
<td>pT1</td>
<td>9</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 2  Regions of the genome in which >30% of tumors showed gain or deletion in at least five contiguous data channels

<table>
<thead>
<tr>
<th>Deletions</th>
<th>Gains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>%</td>
</tr>
<tr>
<td>5q13.1-1q4</td>
<td>50</td>
</tr>
<tr>
<td>5q21</td>
<td>34</td>
</tr>
<tr>
<td>6q16.1-1q21</td>
<td>41</td>
</tr>
<tr>
<td>10cen-1q21.1</td>
<td>41</td>
</tr>
<tr>
<td>16cen-q21</td>
<td>50</td>
</tr>
</tbody>
</table>

* The maximum percentage within the region is shown.

CGH is a molecular cytogenetic method that assays tumor tissue for somatic chromosomal gains and deletions. An advantage of this technique for a comparative ethnic study of tumor tissue is that the entire genome is examined; there are no preset biases regarding which parts of the genome may be differentially altered among the races. In a previous CGH study of advanced and metastatic prostate cancers, we found a region on chromosome 4q that was gained at a higher frequency in AAMs compared with CAMs (22). The significance of this finding was unclear because of the small number of tumors from AAM patients. In an effort to document ethnic biological differences at the chromosomal level in prostate cancer, we present another series of prostate cancer tissues from AAMs and CAMs analyzed for somatic chromosomal alterations using CGH.

MATERIALS AND METHODS

Tumor Tissue. Surgical specimens from patients undergoing radical prostatectomy for clinically localized prostate cancer were processed in the Pathology Department immediately following surgery. Areas thought to contain tumor in the peripheral zone were identified by gross examination, dissected into individual pieces, and frozen in blocks. Sections were cut from these blocks and stained by H&E. Microscopic examination confirmed the presence of adenocarcinoma and guided further dissection of the block to isolate areas containing at least 70% malignant nuclei. Further frozen sections were cut and placed into tubes for DNA extraction. Final pathological stage and Gleason score were determined from the permanently embedded, formalin-fixed specimen as described previously (23, 24). Tumors were classified as pT1 if pathological examination showed the tumor to be confined to the prostate with no extension to the inked surgical margin and no extraprostatic extension. Tumors with extraprostatic extension or seminal vesicle invasion were classified as pT2.

Tumor Hybridizations. CGH was performed as described previously (22). Briefly, FITC-dUTP (NEN) was incorporated into 2 μg of high molecular weight tumor DNA by nick translation. This green-labeled tumor DNA was cohybridized with red-labeled normal DNA and cot-1 DNA to metaphase preparations. After 2 days, slides were washed and counterstained.

Control Hybridizations. Aliquots of normal DNA extracted from lymphocytes from healthy men were labeled green and red and hybridized against each other as described above. The quality of these hybridizations was monitored by direct visual examination through the fluorescence microscope. Hybridizations were eliminated if the fluorescence intensity of either color was judged to be weak, inhomogeneous, or granular. Hybridizations were also eliminated if the morphology of the target chromosomes was compromised by overdenaturation. Finally, hybridizations were eliminated if the "hybridization artifact" (chromosome regions 1p, 19, 22, or other regions showing green:red ratios different from 1:0; Refs. 22 and 25) appeared severe. This last criterion was based on either visual examination through the microscope or examination of fluorescence ratio tracings. Based on these criteria, 13 normal-normal hybridizations were chosen to be included into a set of controls.
A clinical cancer research 1275

Fluorescence Intensity Ratio Distributions. Tumor hybridizations were included in the study only if they met the same criteria as the control hybridizations as described above. The QUIPS Image Analysis System (Vysis, Inc., Downers Grove, IL) was used to capture 10 or more metaphase images from each hybridization. Fluorescence intensity green:red ratio distributions (means and SDs) were generated from 7–20 images of each chromosome type from 5–10 of the best metaphase images from each hybridization. These ratio distributions were organized into 1747 data channels along the genome (beginning with chromosome 1p telomere and ending with chromosome Yq telomere). In this system, each channel corresponds to roughly 1.9 Mb. Green:red fluorescence ratio mean and SD values for all of the data channels were exported from the commercial software package into a spreadsheet for calculation of t-statistics.

Standardized t-Statistical Analysis of CGH Ratios. Channel by channel t-statistics for each tumor-normal hybridization were calculated by comparison of tumor-normal fluorescence ratio distributions to the fluorescence ratio distributions from the entire set of 13 normal-normal control hybridizations (22, 25). In other words, the tumor-normal fluorescence ratios were not compared with the theoretical normal value of 1.0; instead, they were compared with the series of control hybridizations with fluorescence ratio means that differ from 1.0 by various amounts along all data channels of the genome. Thus, positive values of t indicated gains; negative values indicated deletions. The magnitude of the absolute value of t gave an indication of the relative confidence of the true presence of a gain or deletion.

Previously, we demonstrated that this analysis has several advantages over standard interpretation of fluorescence ratio tracings (22, 25). This method allows a higher sensitivity in detection of chromosomal imbalances with little loss of specificity. In addition, subjectivity of analysis is greatly reduced by standardizing the analysis across the genome both within individual hybridizations and across different tumor hybridizations. Finally, the method compensates for the hybridization artifact, whereby certain regions of target chromosomes show nonrandom deviations from expected fluorescence ratios. In other words, chromosome regions 1p, 19, 22, and telomeres can be evaluated with the same degree of confidence as other regions of the genome.

Determination of Gains and Deletions. The normal-normal control hybridizations were used to help pick a t-statistic threshold for the delineation of gains and deletions. A set of 13 t-statistics (each consisting of one hybridization analysis of 1747 t values) was generated by comparing each individual normal-normal control hybridization to the entire set of 13 control hybridizations. Theoretically, perfect control hybridizations would result in t = 0 in each data channel. In our set of 13

Downloaded from clincancerres.aacrjournals.org on July 15, 2017. © 1998 American Association for Cancer Research.
control hybridizations, we found that only 234 of 20,904 (1.1%) 
\( t \) values exceeded \( t = 1.21 \) (data channels corresponding to 
centromeres and heterochromatic regions excluded from analysis). Therefore, a threshold of \( t > 1.21 \) was determined to have 
a high sensitivity for the detection of gains and deletions with a 
relatively low false-positive detection rate.

RESULTS

Patient Data. Clinical data are presented in Table 1. The 
pathological stage groupings were comparable between the two 
groups. AAMs had eight pT2 and 8 pT3 tumors; CAMs had 
seven pT2 and nine pT3 tumors. The most common Gleason 
score among both groups of tumors was 7, present in 13 AAM 
tumors and 12 CAM tumors. There was no detectable difference 
in the mean preoperative serum PSA levels between the two 
groups of patients. Two patients in each group had one first-
degree family member with prostate cancer. No patient had 
familial prostate cancer (three or more affected first-degree 
relatives) as defined by Smith et al. (26).

Gains and Deletions. Among all 32 tumors, the pattern 
of gains and deletions detected was similar to that found in 
previous analyses of prostate cancers by CGH (22, 27, 28). 
Specifically, deletions of regions on chromosomes 13q, 5q, 16q, 
and 8p predominated. Chromosome 8q and 5q gains were found 
as well (Table 2). No high-level amplifications were detected in 
any tumor. Compared with our previous CGH study of meta-
static prostatic cancer tissue, the locations of the most frequent 
changes were the same, but the frequencies of alteration were 
lower (22). This suggests a process of ongoing nonrandom 
chromosomal alterations as prostate cancers progress in stage 
from localized to metastatic. Examples of the use of the \( t \)-
statistic technique to delineate deletions and gains are shown in 
Fig. 1.

The fraction of genome imbalanced for each tumor was 
defined as the number of data channels with \( |t| > 1 \) greater than 
2.0. This measurement provides an estimate of the overall 
length of chromosomal regions present at copy numbers 
deviating from the average DNA content of the total genome. 
This value ranged from 0.05 to 0.41 (mean, 0.17; Table 1). 
We found no relationship between fraction of genome imbal-
anced and Gleason score due to the preponderance of Gleason 
7 tumors. We also found no relationship between fraction of
contingency table was set up with the number of tumors from AAMs and CAMs. At each data channel, a 2 × 3 contingency table was set up with the number of tumors from AAMs and CAMs that showed no change, t > 2.0 (gain), or t < −2.0 (deletion). For each contingency table, we tested for significant differences in frequencies in both the χ² test and Fisher’s exact test. Based on either of these tests, significant differences were found in only 19 of 1747 (1.1%) of data channels. Of the 19 channels with significant differences in frequencies, there were only three regions consisting of 3 contiguous channels (12q21, 15q21, and 17p13-p12); the other 10 channels were noncontiguous and dispersed widely. Therefore, we concluded that the region-specific frequencies of chromosomal alterations between the two groups of tumors were very similar across the vast majority of the genome. An example of how the pattern of alterations was similar between the two groups is shown in Fig. 2, where deletions and gains are depicted for two of the most frequently affected chromosomes. Finally, we tested for differences in mean fraction of genome imbalanced and found no differences between tumors from AAMs and CAMs (0.14 versus 0.19; P = 0.13).

DISCUSSION
In this study, we explored the possibility that biological differences between tumors from CAMs and AAMs could be detected at the level of whole or partial chromosome gains and deletions. Theoretically, any such somatic differences could result from the influence of epigenetic factors (such as differences in diet or other exposure to carcinogens). Also, an inherited predisposition to prostate cancer development or progression (e.g., differential frequency of inheritance of an altered gene) could impinge upon genomic integrity and result in differences in the pattern or frequency of somatic chromosomal alterations.

Overall, we found a similar pattern and frequency of alterations across all chromosome arms in tumors from CAMs and AAMs. Indeed, a few data channels (1.1% of the length of the genome) had significant differences in frequencies of alterations between tumors from AAMs and CAMs. However, because we performed a large number of tests (each at α = 0.05), the differences we found could be due to chance alone. Three of the regions with differences in frequency consisted of three contiguous data channels (approximately 6 Mb each). Differences extending across multiple contiguous data channels should be given more weight; however, the lower limit for the detection of differences in frequencies between subgroups of tumors is unknown. Because of the imperfect optical characteristics of the microscope and camera, fluorescence ratio distributions (and thus t-statistics) in a given channel are not completely independent of the surrounding channels. Therefore, we believe that the differences in these regions are probably not significant. In any case, the vast majority (98.9%) of the length of the genome exhibited no differences in frequencies of deletion or gain. In addition, the patterns of alterations appeared to be similar (Fig. 2). These data support the notion that the patterns and frequencies of chromosomal alterations in sporadic tumors from AAMs and CAMs are identical.

Although it is still possible that differences in somatic chromosomal alterations may exist between tumors from CAMs and AAMs, we feel that any such differences are so subtle that an extremely large number of tumors would need to be studied to detect them. Alternatively, significant somatic genomic differences may be so small that they are beyond the resolution of CGH (e.g., single bp changes).

We designed this study to include newly diagnosed, sporadic prostate cancers with similar clinicopathological features when categorized by race. Nonetheless, it is possible that the disease outcome will be worse for the AAMs included in our study, although the pattern of somatic chromosomal alterations was similar. Other studies have suggested that disease outcome may be worse for AAMs even within a given stage of disease at diagnosis (19, 20). If one assumes that the chromosomal alterations were similar among the tumors from AAMs and CAMs in those studies, alternative explanations for worse outcomes must be postulated.

We conclude that sporadic prostate cancers develop and progress by similar chromosomal pathways, regardless of race. This study lends weight to the notion that biological differences must be explained by factors other than permanent genomic changes at the chromosomal level. Such factors may include subtle changes due to differences in inherited predispositions or ongoing epigenetic exposures leading to differences in gene expression.

REFERENCES
A similar pattern of chromosomal alterations in prostate cancers from African-Americans and Caucasian Americans.

M L Cher, P E Lewis, M Banerjee, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/4/5/1273

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.