Tissue Microarray Assessment of Prostate Cancer Tumor Proliferation in African-American and White Men

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Prostate cancer is a major health-care problem for African-American men. The age-adjusted incidence of prostate carcinoma in African-American men is approximately 50% greater than in white men. Furthermore, studies (1–3) have consistently demonstrated that the mortality rate from prostate cancer is significantly greater in African-American men than in white men. This fact remains true, even after adjustment for stage at presentation (2). Some researchers have speculated that variable access to health care may substantially contribute to the disparate prostate cancer survival between racial groups. Robbins et al. (3) recently reported on survival from prostate carcinoma in participants in a large health-care maintenance organization in which one would assume that prostate carcinoma was detected and treated similarly in all races. In that study, the death rate from prostate cancer was higher in African-American men than in white men. This fact remains true, even after adjustment for stage at presentation (2). Some researchers have speculated that variable access to health care may contribute to the disparate prostate cancer survival between racial groups. Robbins et al. (3) recently reported on survival from prostate carcinoma in participants in a large health-care maintenance organization in which one would assume that prostate carcinoma was detected and treated similarly in all races.

To investigate whether this difference is due to tumor biology or epigenetic factors, we have used high-density tissue microarray technology, developed by Kononen et al. (4). Tissue microarrays can contain up to 1000 tissue samples and can be used for multiple studies, thereby conserving tissue and assuring the uniformity of test conditions.

Our aim was to create a collection of clinically matched tumor samples from African-American and white men with prostate carcinoma and to investigate potential differences in tumor proliferation between the two groups. We matched African-American and white men before surgery by serum prostate-specific antigen (PSA) levels and fine-needle biopsy Gleason scores to identify men who presented with similar clinical features, so that any biologic differences identified could be attributed to race.

The biomarker selected for this study was the proliferation marker Ki-67. Ki-67 is a nuclear protein that is expressed in G1, S, G2, and M phases of the cell cycle but is not detected in cells in G0 phase (5). We defined the Ki-67 labeling index as the percent nuclear area stained with Ki-67 (6). Previous studies (7–9) of prostate cancer have described consistent associations between the Ki-67 labeling index and the Gleason grade (i.e., tumor grade).

Thirty matched clusters consisting of one African-American man and two white men were identified from 632 patients who underwent radical prostatectomy at our institution for clinically localized prostate carcinoma from 1994 through 1998. An institutional review board-approved consent for the molecular analysis of prostate cancer was obtained from all participants in this study. Matching was based on the Gleason score determined from a biopsy specimen and serum PSA levels. Tumors were staged by the tumor–node–metastasis system (10), and multiple samples of normal, high-grade prostate intraepithelial neoplasia (PIN) and prostate carcinoma were identified from each tumor for transfer into a tissue microarray block. A tissue microarray was assembled by use of a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). Three tissue samples from normal areas, three from high-grade PIN areas, and six from prostate cancer areas were taken from each patient. If a patient had multiple tumor foci, each tumor was sampled. This approach was taken because a priori we did not know which tumor would have the highest proliferation rate; previous studies using standard slides have assumed that the dominant lesion would best predict outcome.

The final tissue microarray consisted of 892 total arrayed samples in two blocks (Fig. 1, A–G). The identity of each 0.6-mm sample was tracked by their coordinate (X–Y) position and linked to a clinical database. Histologic review of the tissue microarray showed that, for each patient, the microarray contained, on average, 3.0, 0.9, and 4.6 samples of normal tissue, high-grade PIN, and prostate cancer, respectively. Immunostaining for Ki-67 (1:25 dilution; Coulter-Immunotech, Miami, FL) was performed, and results were quantified by use of an image analysis system (CAS2000 System; Bacus Labs, Lombard, IL). Examples of the prostate tissue samples from this study are shown in Fig. 1, E and F. Prostate xenographs with a high Ki-67 labeling index from the rapid autopsy program (11) were used as positive internal controls (Fig. 1, G and H). Six measurements of the Ki-67 labeling index were made for each tissue sample that contained 50–100 nuclei (total, 300–600 nuclei per tumor and 150–300 nuclei in normal tissues or high-grade PIN in the total tissue microarray).

Evaluation of preanalysis-matching criteria confirmed that biopsy Gleason scores within each cluster were identical and that there was no statistically significant difference in the mean preoperative PSA levels for African-American men (8.60 ng/mL) and for white men (8.70 ng/mL) (P = .682). However, the white men (mean age, 60.7 years) were older than the African-American men (mean age, 56.6 years) (P = .006). The preoperative matching produced two groups of patients with similar tumor grades and stage (data not shown).

For each person in the study, there was, on average, 3.0 normal samples (range, 2–4), 4.6 tumor samples (range,
In this brief communication, we investigated statistically significant differences in the Ki-67 labeling index between three tissue types as follows: The Ki-67 labeling index of high-grade PIN was 0.621 (95% confidence interval [CI] = 0.4–0.84) higher than normal, and the Ki-67 labeling index of prostate cancer was 0.244 (P = .030; 95% CI = 0.03–0.46) higher than high-grade PIN on a logarithmically transformed scale. These differences corresponded to high-grade PIN having a Ki-67 labeling index that was 1.9 times higher than normal and prostate cancer having a Ki-67 labeling index that was 1.3 times higher than high-grade PIN. However, some overlap in the Ki-67 labeling indexes between tissue types was seen (Fig. 2). These findings are similar to a recently concluded biomarker study using biopsy specimens and standard slides (our unpublished data).

Associations between the Ki-67 labeling index and pathology results for white patients (e.g., extraprostatic extension, seminal vesicle invasion, and Gleason score) revealed that the Ki-67 labeling index showed a statistically significant association for extraprostatic extension (P = .019) and seminal vesicle invasion (P = .011) but not Gleason score (P = .214). Associations between the Ki-67 labeling index and pathology results for African-American men revealed that the Ki-67 labeling index showed a trend toward association with worse pathology. However, these differences were not statistically significant (extraprostatic extension, P = .181; seminal vesicle invasion, P = .344; and Gleason score, P = .203). In a multivariate analysis adjusting for race, we found statistically significant associations between extraprostatic extension (P = .006) and seminal vesicle invasion (P = .012). There were no statistically significant differences in the multivariate analysis with respect to race and the four pathology variables.

In this brief communication, we have used a method involving high-throughput analyses of prostate cancer specimens by use of tissue microarray technology to investigate whether biologic differences in prostate cancer in African-American and white patients can be detected. The strategy of matching African-American and white patients resulted in two groups with final pathology results that were similar. Thus, this tissue microarray block...
should be useful in identifying biologic differences that may be associated with race.

We selected Ki-67 as the biomarker because its expression is associated with biochemical recurrence as measured by elevation in PSA levels after radical prostatectomy (8,12–14). Although this analysis failed to detect racial differences in the Ki-67 labeling indexes, data from longitudinal follow-up may.

REFERENCES


NOTES

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Fig. 2. Distribution of Ki-67 expression for African-American and white men for the three tissue types (i.e., normal tissue, high-grade prostatic intraepithelial neoplasia [PIN], and prostate cancer). In the boxplot, the lines connect the medians, the boxes cover the 25th to 75th percentiles, and the minimum and maximum values are shown by the ends of the bars. AA = African-American; PCA = prostate cancer; LI = labeling index.