Polycyclic Aromatic Hydrocarbon-DNA Adducts in Prostate Cancer

Benjamin A. Rybicki,1 Andrew Rundle,3 Adnan T. Saveria,2 Steadman S. Sankey,1 and Deliang Tang4

Departments of 1Biostatistics and Research Epidemiology and 2Surgical Pathology, Henry Ford Health System, Detroit, Michigan; and Departments of 3Epidemiology and 4Environmental Health Sciences, Columbia University Mailman School of Public Health, New York, New York

ABSTRACT

The formation of DNA adducts can lead to DNA replication errors and the potential for carcinogenesis. DNA adducts have been detected in prostate cells, but the distribution of adducts with respect to prostate cancer risk factors and histology is unknown. In a study of 130 Caucasian (n = 61) and African-American (n = 69) men with prostate cancer who underwent radical prostatectomy, we quantified polycyclic aromatic hydrocarbon (PAH)-DNA adducts in prostate tumor and adjacent nontumor cells by immunohistochemistry. A strong correlation between paired adduct levels in the two cell types was observed (r = 0.56; P < 0.0001); however, nontumor cells had a significantly higher level of adducts compared with tumor (0.30 absorbance units ± 0.05 versus 0.17 absorbance units ± 0.04; P < 0.0001). Variables significantly associated with PAH-DNA adduct levels in tumor cells included primary Gleason grade, tumor volume, and log-transformed prostate-specific antigen (PSA) at time of diagnosis. Tumors with a primary Gleason grade of 5 had significantly lower PAH-DNA adduct levels than tumor cells with a primary Gleason grade of 3 or 4 (P < 0.0001 for both). Tumors that involved 10% or less of the prostate gland had significantly higher PAH-DNA adduct levels than tumors that involved 15 to 20% of the prostate gland (P = 0.004). PSA levels were inversely associated with PAH-DNA adduct levels in tumor cells (P = 0.009). A similar, albeit less significant, inverse association was observed between PSA and PAH-DNA adduct levels in nontumor cells (P = 0.07). Interestingly, increasing primary Gleason grade was associated with increasing PAH-DNA adduct levels in adjacent nontumor cells (P = 0.008). Our results show that PAH-DNA adducts are present in the prostate but vary with regard to cellular histology. In prostate tumor cells, decreased cellular differentiation and increased tumor proliferation may reduce PAH-DNA adduct levels.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH), which result from incomplete combustion processes, are ubiquitous environmental contaminants and known carcinogens (1). PAH are thought to derive their carcinogenic properties through their ability to form PAH-DNA adducts (2–4). PAH is metabolized in the liver and other tissues by the cytochrome P-450 enzyme system into reactive electrophiles (2, 5), which can then interact with DNA (3, 4, 7). Both stable and unstable adducts are thought to form DNA adducts can be formed via P450-mediated, one-electron oxidation; these adducts quickly depurinate leading to apurinic sites in DNA (3, 4, 7). Both stable and unstable adducts are thought to contribute to carcinogenesis (2, 8–11).

Epidemiologic literature is supportive of a link between occupational PAH exposures and prostate cancer risk. Both case-control (12) and cohort (13) studies have found that most jobs associated with prostate cancer have the potential for occupational PAH exposure. In three separate studies that examined specific occupational exposures, two found modest associations with selected PAH sources (14, 15), whereas the third found no association with PAH-related exposures (16). PAH exposures that occur years before disease onset may have a greater impact on prostate carcinogenesis. The highest elevated standard mortality ratio for prostate cancer in Swedish chimney sweeps, who are occupationally exposed to high levels of PAH, was with at least 10 years of occupational exposure (the standard mortality ratio remained elevated for 20 to 29 and 30+ years of exposure) and at least 20 years since the first exposure (17). Experimental evidence supports a link between exposure and the formation of DNA adducts in prostate. In studies done in rat prostate, a correlation between in vivo exposure and DNA adduct formation was shown (18). Two separate in vitro experiments were able to detect DNA adducts in prostate after exposing human prostate tissue to adduct-forming compounds (19, 20).

Despite evidence that supports DNA adduct formation in prostate carcinogenesis, unlike some other cancers where DNA adduct formation is thought to play a role, notably lung (21, 22) and breast (23, 24), population level studies of DNA adducts in the prostate do not exist. In the present study, we quantified PAH-DNA adducts in tumor and adjacent nontumor prostate cells of men who underwent radical prostatectomy. Our study represents an initial step toward understanding this potential biomarker of DNA damage in prostate carcinogenesis on the population level.

MATERIALS AND METHODS

Study Sample. The study population consisted of men who were part of the Henry Ford Health System. Henry Ford Health System comprises an 800-bed hospital in the city of Detroit, three smaller hospitals in surrounding suburbs, and 31 medical clinics located throughout the metropolitan Detroit area. Eligible cases were men who used the Henry Ford Health System as their primary source of health care, lived in the study area at time of recruitment, had no other serious medical problems that would preclude participation, and had no previous history of prostate cancer. Potential cases were identified by Henry Ford Health System pathology reports of primary adenocarcinoma of the prostate. Cases recruited for study were sent a letter introducing the study protocol followed by a phone call from a study interviewer. Those who agreed to participate were asked to complete a two-part interviewer-administered risk factor questionnaire (the first part was conducted over the phone, and the second part was done in person) and donate a blood sample for DNA analysis. All study protocols were approved by the Henry Ford Hospital Institutional Review Board.

Between July 1, 2001 and May 20, 2002 we attempted to enroll 260 men who had been diagnosed with prostate cancer within the last two years. Of the 201 men contacted and found eligible for study, 190 agreed to participate and completed the study protocol (95%). Of these 190 cases, 130 underwent radical prostatectomy and had sufficient tumor tissue for PAH-DNA adduct immunohistochemistry analyses. The demographic and clinical characteristics of the study population are shown in Table 1.

Pathology. H&E stained slides of study cases were reviewed by the study pathologist (Adnan T. Saveria) to confirm the diagnosis and to identify a paraffin block with sufficient tumor and nontumor prostatic tissue for staining. For each patient sample, we used a microtome to cut five consecutive sections (5-μm-thick) from the tissue block. One slide was H&E stained and examined by the study pathologist who circled two separate areas of tumor and nontumor cell populations to be used for adduct scoring. Tumors were given a tumor-node-metastasis staging score and characterized according to type, lymph node involvement, primary grade, secondary grade (i.e., Gleason score),...
within experimental batches through the use of generalized estimating equations. Both univariable and multivariable, were adjusted for correlations that can occur as some variables exhibited deviations from normality. All models, both DNA adduct levels were calculated with the nonparametric Spearman statistic. Correlations between all of the explanatory variables and each of the PAH-DNA adduct levels between tumor and nontumor cells deviated significantly from zero.

The immunohistochemical assay for PAH-DNA adducts was carried out as described previously (23, 25). This assay uses the monoclonal 5D11 antibody, which in cell culture studies has been shown to produce staining levels strongly correlated (r = 0.99; P < 0.001) with treatment dose of benzo(a)pyrene-diolepoxide (BPDE; refs. 26, 27). Studies of antibody specificity found that preabsorption of the antibody with BPDE-DNA, or pretreating the cells with DNase, or running the assay without the primary antibody all reduced staining to background levels (25, 28). Although the 5D11 antibody was produced in response to the adduct of the carcinogenic intermediate of benzo(a)pyrene (BPDE-DNA), it cross reacts with various affinities with other structurally related PAH; hence the terminology "PAH-DNA" is commonly used (25, 29, 30). Previous results from similar studies with immunohistochemical assays to measure PAH-DNA adducts have been reported in absorbance units (23, 25, 31, 32), which provide a scale reflecting the relative intensity of staining. We likewise reported our results in absorbance units.

Staining was quantified by absorbance image analysis with a Cell Analysis System 200 microscope (Becton-Dickinson, San Jose, CA) running the Cell Measurement Program. Absorbance of light at a wavelength of 500 nmol/L was measured because methyl green does not absorb light at this wavelength, whereas diaminobenzidine does. For each prostate specimen, two technicians independently scored 50 epithelial cells (five fields with 10 cells per field scored) in the two areas (tumor and nontumor) circumscribed by the study pathologist. The mean of the two technicians' scores was used as the final score. Scored cells were selected to be representative, in terms of intensity, of the cells in the field. Samples were run on different days in 11 separate batches.

A reliability study was done to evaluate the reproducibility of the scoring procedures for prostate cells. The reliability study used a test-retest design (34), in which 59 prostate sections from the entire series of prostate sections analyzed in this study were done randomly selected for rescoring. The rescoring of the 59 samples was done in the same manner as the initial scoring. Comparisons of the scoring and rescoring (both tumor and nontumor) were done with intraclass correlation analyses, which provides an estimate of test-retest reliability (35).
adducts in tumor cells were similar for primary Gleason grades 3 and 4 (0.177 absorbance units ± 0.008 vs 0.180 absorbance units ± 0.009), but the mean level of adducts in tumors of primary Gleason grade 5 (0.157 absorbance units ± 0.007) was significantly less (P < 0.0001) than that of primary Gleason grades 3 and 4. Prostate-specific antigen (PSA) at time of diagnosis was modeled two different ways. As a continuous log-transformed variable it had a significant (P = 0.009) inverse association with PAH-DNA adduct levels in tumor cells; as a three-level categorical variable with cut points at 4 and 10 ng/mL, it was borderline significant (P = 0.07; Table 2). In the extreme tertiles of PSA levels <4 ng/mL and >10 ng/mL, an inverse relationship between PSA and adduct levels was observed. Overall, those with PSA >10 ng/mL had lower mean PAH-DNA adduct levels (0.163 absorbance units ± 0.011) compared with those with PSA levels between 4 and 10 ng/mL (0.180 absorbance units ± 0.007) or <4 ng/mL (0.182 absorbance units ± 0.010). Opposite from what was observed in tumor cells, an increasing level of PAH-DNA adducts in nontumor cells was associated with increasing primary Gleason grade in adjacent tumor cells (P = 0.008). Nontumor prostate cells that were adjacent to tumor cells with primary Gleason grades 4 or 5 had slightly higher PAH-DNA adduct levels than nontumor cells adjacent to tumor cells with a primary Gleason grade of 3. Similar to tumor cells, PAH-DNA adduct levels in nontumor cells were inversely related to PSA levels, but the association was weak (P = 0.27 for PSA as a three-level categorical variable; P = 0.07 for PSA a log-transformed continuous variable). We also examined possible predictors of the difference in adduct levels between nontumor and tumor cells (last 2 columns of Table 2). The variable with the strongest association was primary Gleason grade (P < 0.0001) with higher primary Gleason grades having larger mean differences between nontumor and tumor cells. Total Gleason score was also associated with differences in adduct levels between nontumor and tumor cells (P = 0.05) with the same pattern of association as was observed for primary Gleason grade. Tumor stage was the other variable significantly associated with a difference in adduct levels between nontumor and tumor cells (P = 0.02), but no clear pattern emerged between mean differences and tumor stage.

![Fig. 2. Correlation between absorbance scores in tumor and nontumor prostate cells.](image)

### Table 2: Adjusted mean levels of PAH-DNA adducts in prostate expressed in absorbance units based on general estimating equations modeling (n = 130)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tumor cells (1)</th>
<th>Normal cells (2)</th>
<th>(2)–(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean absorbance ± SEM</td>
<td>P *</td>
<td>Mean absorbance ± SEM</td>
</tr>
<tr>
<td>Age in years at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60 (n = 54)</td>
<td>0.175 ± 0.008</td>
<td>0.45</td>
<td>0.296 ± 0.007</td>
</tr>
<tr>
<td>≥60 (n = 76)</td>
<td>0.179 ± 0.008</td>
<td></td>
<td>0.303 ± 0.007</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (C, n = 61)</td>
<td>0.176 ± 0.009</td>
<td>0.70</td>
<td>0.299 ± 0.007</td>
</tr>
<tr>
<td>African American (AA, n = 69)</td>
<td>0.179 ± 0.008</td>
<td></td>
<td>0.301 ± 0.008</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A (n = 14)</td>
<td>0.183 ± 0.011</td>
<td>0.76</td>
<td>0.295 ± 0.016</td>
</tr>
<tr>
<td>2B (n = 9)</td>
<td>0.176 ± 0.008</td>
<td></td>
<td>0.301 ± 0.007</td>
</tr>
<tr>
<td>3A (n = 12)</td>
<td>0.182 ± 0.012</td>
<td></td>
<td>0.289 ± 0.016</td>
</tr>
<tr>
<td>3B (n = 5)</td>
<td>0.178 ± 0.011</td>
<td></td>
<td>0.311 ± 0.017</td>
</tr>
<tr>
<td>1&quot; Gleason grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (n = 105)</td>
<td>0.177 ± 0.008</td>
<td>&lt;0.0001</td>
<td>0.296 ± 0.007</td>
</tr>
<tr>
<td>4 (n = 22)</td>
<td>0.180 ± 0.009</td>
<td></td>
<td>0.320 ± 0.010</td>
</tr>
<tr>
<td>5 (n = 3)</td>
<td>0.157 ± 0.007</td>
<td></td>
<td>0.316 ± 0.016</td>
</tr>
<tr>
<td>2&quot; Gleason grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (n = 85)</td>
<td>0.179 ± 0.008</td>
<td>0.32</td>
<td>0.304 ± 0.007</td>
</tr>
<tr>
<td>4 (n = 40)</td>
<td>0.174 ± 0.008</td>
<td></td>
<td>0.288 ± 0.009</td>
</tr>
<tr>
<td>5 (n = 5)</td>
<td>0.173 ± 0.018</td>
<td></td>
<td>0.335 ± 0.038</td>
</tr>
<tr>
<td>Total Gleason grade</td>
<td></td>
<td></td>
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<tr>
<td>6 (n = 72)</td>
<td>0.179 ± 0.008</td>
<td>0.22</td>
<td>0.298 ± 0.007</td>
</tr>
<tr>
<td>7 (n = 43)</td>
<td>0.179 ± 0.008</td>
<td></td>
<td>0.305 ± 0.009</td>
</tr>
<tr>
<td>8–10 (n = 15)</td>
<td>0.166 ± 0.012</td>
<td></td>
<td>0.295 ± 0.011</td>
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<tr>
<td>PSA at diagnosis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;4 ng/mL (n = 21)</td>
<td>0.182 ± 0.010</td>
<td>0.07</td>
<td>0.305 ± 0.010</td>
</tr>
<tr>
<td>4–10 ng/mL (n = 85)</td>
<td>0.180 ± 0.007</td>
<td></td>
<td>0.302 ± 0.007</td>
</tr>
<tr>
<td>&gt;10 ng/mL (n = 24)</td>
<td>0.163 ± 0.011</td>
<td></td>
<td>0.287 ± 0.009</td>
</tr>
<tr>
<td>Tumor volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10% (n = 57)</td>
<td>0.184 ± 0.008</td>
<td>0.01</td>
<td>0.305 ± 0.010</td>
</tr>
<tr>
<td>15 to 20% (n = 35)</td>
<td>0.171 ± 0.009</td>
<td></td>
<td>0.290 ± 0.008</td>
</tr>
<tr>
<td>&gt;20% (n = 38)</td>
<td>0.174 ± 0.008</td>
<td></td>
<td>0.301 ± 0.006</td>
</tr>
<tr>
<td>Resected at margins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 41)</td>
<td>0.177 ± 0.010</td>
<td>0.95</td>
<td>0.302 ± 0.007</td>
</tr>
<tr>
<td>No (n = 89)</td>
<td>0.178 ± 0.007</td>
<td></td>
<td>0.299 ± 0.008</td>
</tr>
<tr>
<td>Perineural invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 75)</td>
<td>0.180 ± 0.007</td>
<td>0.35</td>
<td>0.301 ± 0.005</td>
</tr>
<tr>
<td>No (n = 55)</td>
<td>0.176 ± 0.009</td>
<td></td>
<td>0.299 ± 0.010</td>
</tr>
</tbody>
</table>

Abbreviations: C, Caucasian; AA, African American.

* P for test of difference between means.

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intensity wanes for with increasing cellular differentiation as depicted for tumor cells of primary Gleason grades 4 (Fig. 3B) and 5 (Fig. 3C). Also, note the greater contrast between nontumor and tumor cells in staining intensity with increasing primary Gleason grade.

Next, we tested three separate multivariable models for PAH-DNA adduct levels in tumor cells, nontumor cells, and the difference between nontumor and tumor cells (Table 3). For each cell type, we tested variables that were associated with PAH-DNA adduct levels with a \( P \) of 0.1 or lower in univariable analyses. In tumor cells, the univariable inverse relationships between PAH-DNA adducts and log PSA, primary Gleason grade, and tumor volume remained significant in a multivariable model that included all three. Individual variables with the strongest associations with PAH-DNA adduct levels included log PSA (\( P = 0.002 \)), primary Gleason grade of 3 (\( P < 0.0001 \)), primary Gleason grade of 4 (\( P = 0.001 \)), and tumor volume <10% of the prostate gland (\( P = 0.02 \)). In nontumor prostate cells, the two variables associated with PAH-DNA adducts in a univariable model (log PSA and primary Gleason grade) remained highly significant in the multivariable model. The last model examined independent variables associated with adduct level differences between nontumor and adjacent tumor cells. Of the three variables tested, primary Gleason grade, total Gleason grade, and tumor stage, only primary Gleason grade was significant (\( P = 0.0005 \)) in the multivariable model.

**DISCUSSION**

In the present study, we showed that PAH-DNA adducts are detectable in human prostate cells and vary according to histology. Whereas epidemiologic studies of PAH-DNA adducts in the prostate do not exist, comparable studies in breast have been done. The breast and prostate may share similar mechanisms of DNA adduct formation.

![Image](cancerres.aacrjournals.org)
because both lack direct contact with the environment and have cellular growth and function mediated by exogenous hormones. Several studies have shown higher DNA adduct levels in breast cells of cases compared with controls (23, 36–38). In regards to cell type, Rundle et al. (23) showed that tumors of breast cancer cases had slightly higher PAH-DNA adduct levels compared with adjacent nontumor cells.

Cells under oxidative stress are highly vulnerable to DNA adduct formation, and oxidative stress likely occurs early in prostate carcinogenesis (39, 40), which could explain the higher level of PAH-DNA adducts we observed in nontumor prostate cells adjacent to tumor cells. If PAH-DNA adduct formation as a result of oxidative stress is a precursor to cell transformation in the prostate, then one would expect to observe PAH-DNA adducts in nontumor cells. What is not clear, however, is why PAH-DNA adduct levels are lower in prostate tumor cells compared with adjacent nontumor cells. Consistent with this observation was our finding that PAH-DNA adduct levels in prostate tumor cells were inversely proportional to primary Gleason grade, tumor volume, and PSA at diagnosis. Whereas our results cannot show a temporal relationship between PAH-DNA adduct formation and prostate carcinogenesis, assuming that PAH exposures and genetic susceptibilities did not vary with tumor histology, our results suggest that PAH-DNA adducts in prostate cells start to diminish as cells transform. Our results also suggest that PAH-DNA adducts in prostate tumor cells continue to diminish as cancer foci grow and become more poorly differentiated. One possible explanation could be the lower activity levels of metabolic enzymes in tumor cells, such as CYP1A1 and CYP1A2, necessary for activation of adduct-forming compounds such as benzo(a)pyrene. For example, it seems that benzo(a)pyrene does not induce CYP1A1 and CYP1A2 expression in androgen-independent prostate tumor cell lines (41). Another explanation for the lower PAH-DNA adduct levels in prostate tumor cells and in less differentiated tumors may relate to patterns of estrogen receptor expression. In breast tumor tissue, there was a strong, positive association between adduct levels and expression of estrogen receptors, suggesting that adduct levels are influenced by hormonal pathways (23). Recent work in prostate has shown that loss of estrogen receptor β expression because of methylation occurs during prostate carcinogenesis and tumor progression (42, 43). Furthermore, there seems to be cross-talk between the estrogen receptor and P450 enzymes (44). The lower levels of adducts we observed in prostate tumor cells and the association between loss of differentiation and lower adduct levels may relate to alterations in hormone signaling pathways that parallel carcinogenesis.

In our prior work with breast cells, we found the reproductibility of the same immunohistochemical assay used in the present study was high (24). In the work with breast cancer, the score from a lone technician was used as the final absorbance score; whereas in the present study of prostate, the average of two technicians’ independent scores were used as the final absorbance score. The averaging of two independent measurements produces a higher reliability than a lone measurement (in the present study, a single reviewer had correlations of 53 to 58% for tumor and 73 to 74% for nontumor). Yet, the reliability of a lone scorer in the analyses of breast cells was as high or higher (82% for tumor and 93% for nontumor) than the reliability estimate with the average of two independent scores in the prostate analyses (83% for both tumor and nontumor). This suggests that immunohistochemical staining intensity is more difficult to consistently score in prostate than in breast. This difficulty probably reflects the more complex histology of prostate in comparison with breast, and it was the reason behind our more stringent protocol for scoring prostate cells.

Whereas our study was not designed to directly test whether PAH-DNA adducts increase risk for prostate cancer, our results suggest that formation of PAH-DNA adducts precede prostate carcinogenesis and diminish in prostate tumor cells as they become less differentiated. Because prostate cancer is a multifocal disease, our results may have some utility in making inferences about changes in PAH-DNA adduct levels throughout the different stages of prostate carcinogenesis. Future studies should directly test whether PAH-DNA adduct formation predates prostate cancer in healthy individuals. Studies that examine environmental and genetic determinants of PAH-DNA adducts in the prostate are also needed to better understand the complete DNA damage and repair pathway in prostate carcinogenesis.

REFERENCES

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