Age-related Radical-induced DNA Damage Is Linked to Prostate Cancer

Donald C. Malins, Paul M. Johnson, Thomas M. Wheeler, Edward A. Barker, Nayak L. Polissar, and Mark A. Vinson


Abstract

We measured concentrations and ratios of mutagenic (8-OH) lesions to putatively nonmutagenic formamidopyrimidine (Fapy) lesions of adenine (Ade) and guanine (Gua) to elucidate radical (+OH)-induced changes in DNA of normal, normal from cancer, and cancer tissues of the prostate. The relationship between the lesions was expressed using the mathematical model $\log_{10}[(8-OH-Ade + 8-OH-Gua)/(FapyAde + FapyGua)]$. Logistic regression analysis of the log ratios for DNA of normal and cancer tissues discriminated between the two tissue groups with high sensitivity and specificity. Correlation analysis of log ratios for normal prostates revealed a highly significant increase in the proportion of mutagenic base lesions with age. Data from correlation analysis of the log ratios for normal tissues from cancer were consistent with an age-dependent, dose-response relationship. The slopes for both correlations intersected at $-61$ years, an age when prostate cancer incidence is known to rise sharply. The age-related increase in the proportion of $\log_{10}$-induced mutagenic base lesions is likely a significant factor in prostate cancer development.

Introduction

OH$^2$-induced nucleotide base lesions have been shown to be associated with cancer; however, an etiological relationship has remained elusive (1–3). The OH$^2$ modification of purines to mutagenic base lesions (1, 3–6) and cancer development (1, 3, 7) are dynamic processes (Fig. 1). The OH, arising from the metal (e.g., Fe$^{2+}$)-catalyzed decomposition of H$_2$O$_2$ (a product of the redox cycling of hormones; Ref. 7), reacts with Ade and Gua to form redox ambivalent 8-oxyl derivatives (5, 6). These are either converted oxidatively to mutagenic 8-OH purines or reductively to putatively nonmutagenic (8), ring-opened products, formamidopyrimidines (Fapy), both of which are subject to enzymatic repair (e.g., by glycosylases; Ref. 9). A fraction of the 8-OH purines is miscoded (with a 1–2% level of misrepair for 8-OH-Gua; Ref. 3) and Fapy structures have been shown to block DNA synthesis (8, 10). Thus, although the driving force in the synthesis of both base lesions is the OH, the apparent blocking effect of Fapy lesions on DNA synthesis likely modulates the mutagenic potential of the 8-OH derivatives and their proposed role in the etiology of cancer (1, 3, 7). In the present report, changes in the DNA of normal and transformed prostate tissues were studied with respect to age, 8-OH and Fapy lesion concentrations, and the ratio of each lesion type represented by the mathematical model, $\log_{10}[(8-OH-Ade + 8-OH-Gua)/(FapyAde + FapyGua)]$. This log model was used in our previous study of breast cancer (1). We chose prostate cancer for this study because the disease incidence is highly age-dependent (11, 12).

Materials and Methods

Tissue Acquisition and DNA Isolation. With Institutional Review Board approval, we obtained prostate tissues from the peripheral zone where 70% of tumors occur (11). We analyzed 57 blinded samples from the following three groups: (a) histologically normal tissue from the non-cancerous prostate; (b) histologically normal tissue from the cancerous prostate (NC); and (c) prostate tumors. We obtained 48 samples from the Baylor College of Medicine Specialized Program of Research Excellence tissue bank project: 7 normal, 31 NC (Gleason scores of the tumors elsewhere in the gland ranged from 3 to 9), and 10 cancer (all with Gleason scores of 7; Ref. 13). We obtained eight normal autopsies from the Northwest Tissue Center, Seattle, WA, One cancer sample (Gleason score of 9) was also obtained from the Cooperative Human Tissue Network, Pittsburgh, PA, for a total of 15 normal, 31 NC, and 11 cancer samples. Tumor purity was >80% for all cancer samples. DNA was isolated as reported from each tissue and lyophilized completely to dryness (1).

GC-MS: Sample Preparation and Analysis. Approximately 20 μg of DNA was hydrolyzed with 150 μl of 60% formic acid in Reacti-Vials (Pierce, Rockford, IL). The formic acid solution was sparged for 1 h with ultrahigh-purity nitrogen before it was added to the vial. The vial was then purged with nitrogen, sealed with Teflon-coated septa and heated at 140°C for 30 min. As reported (14), this procedure does not produce significant differences with respect to the levels of 8-OH-Gua obtained by enzymatic hydrolysis. Hydrolysates were lyophilized for 16 h and then derivatized with 50 μl of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane and acetonitrile (4:1, v/v; Refs. 1 and 15). The derivatization solution was sparged for 15 min with nitrogen before being added to the vial. The vial was then purged with nitrogen, sealed with Teflon-coated septa, and heated at 60°C for 2 h. In a study of the chemical oxidation of Gu, no evidence was found for a significant increase in 8-OH-Gua by GC-MS under these conditions. Gua and 8-OH-Gua standards were solubilized in 5.6 m NH$_3$OH at 60°C for 30 min. The more easily solubilized Ade, 8-OH-Ade, FapyAde, and FapyGua standards were dissolved in 0.56 m NH$_3$OH at room temperature. All stock solutions were 1.00 mg/ml.

The samples were analyzed by GC-MS using a modification of a previously reported procedure (15). The gas chromatograph was an HP model 6890 with an HP mass 5973 mass spectrometer (Hewlett-Packard, Palo Alto, CA). The column was an HP-5 MS (12 m × 0.2 mm inside diameter) with a constant flow rate of 0.6 ml/min. The injection port was set to splitless, and the injection volume was 1.0 μl. Modified bases were analyzed by selected ion monitoring (1, 15); unmodified bases were analyzed using the scan mode. The quantity of DNA injected was calculated from the concentrations of Ade and Gua, as determined from the response curves for external standards.

Statistical Analyses. One outlier (>3 SDs from the mean) was removed from the normal tissue group and was not included in statistical analyses. (Removal of this data point was conservative and did not lower the P for comparisons of means.) An F-test was used to test for significant differences...
in SDs for concentrations of base lesions (lesion/10⁵ unmodified base) and the log ratio; t tests were performed to test for significant differences between concentrations of the base lesions and log ratio values. Equal variance was assumed unless the F-test showed significant differences in variance. Logistic regression analysis of log ratios was used to test the hypothesis that we could predict the source of a DNA sample (i.e., normal versus cancer) based on the log ratio. The relationship between the log ratio and age and base lesions (nm/mg DNA) and age were evaluated using Pearson correlation analysis.

Results and Discussion

With the exception of FapyGua, a number of significant differences (P ≤ 0.05) were found between the means of the base lesion concentrations for normal and cancerous prostate tissues (Table 1). A great diversity of base lesion concentrations has been reported between different tissues (16). However, perhaps the most relevant comparison of our data are with data from GC-MS analyses of base lesions from the DNA of human benign prostatic hyperplasia (2). In each case, our values for the four base lesions were generally higher (a maximum of 2.5 times those reported for benign prostatic hyperplasia).

The base lesion data were used to construct the model, \( \log_{10}(8\text{-OH-Ade} + 8\text{-OH-Gua})/(\text{FapyAde} + \text{FapyGua}) \), which reflects the proportion of mutagenic 8-OH to Fapy lesions (1). The mean log ratios effectively discriminated between the DNA from normal and cancer, and NC and cancer tissues (both at P ≤ 0.001); however, no difference was found between the means for the normal and the NC. The very different mean log ratios (Table 1; normal = −0.03 versus cancer = 0.31) are reflective of the high degree of discrimination achieved between the normal and cancer DNA (Fig. 2), which may be a function of the redox (5) and/or the hypoxic status (17) of the tumors versus the normal tissues. Logistic regression analysis of the log ratios for normal and cancer DNA also showed very little overlap and, hence, a high degree of discrimination between these tissue groups.

The sensitivity (percentage of cancer samples correctly classified) was 82% and the specificity (percentage of normal prostate samples correctly classified) was 93% (based on a cutoff point of 0.54, selected as described previously; Ref. 1). The probability, from logistic regression, that the DNA comes from a prostate cancer rises very rapidly for log ratios >0.0.

We found a striking similarity between the present log ratios for prostate cancer and the log ratios from comparable data on normal and cancer DNA reported for the female breast (1). That is, the mean log ratios for prostate cancer (0.31 ± 0.06) and breast cancer (0.38 ± 0.13) were not significantly different (P = 0.6). Moreover, the sensitivity and specificity values for the normal and tumor tissues of the prostate were within 10% of those obtained from a comparable logistic regression analysis of normal and tumor tissues from the breast (1). Together, these findings suggest that the OH-induced base damage profiles in different estrogen-responsive tissues may have broadly similar features in relation to cancer development.

Pearson correlation analysis revealed a positive correlation (0.82; P < 0.001) between the log ratio and age for normal prostate DNA, and, as shown in Fig. 3A, the proportion of mutagenic lesions increases significantly with age. The 8-OH-Ade and FapyGua concentrations were also correlated with age (0.79; P < 0.001 and −0.57; P = 0.02). The increase in mutagenic 8-OH-Ade (18) and the loss of FapyGua, which likely blocks DNA synthesis (8, 10), may act in concert to increase prostate cancer risk in older men. This age-related trend toward high proportions of mutagenic base lesions in DNA is consistent with the known epidemiology of prostate cancer, i.e., risk increases sharply with age for men >60 years of age (11). Illustrative of the age-related differences, the mean log ratio for normal prostates from men <30 years of age was −0.26 ± 0.02, whereas men >60 years of age had a mean of 0.12 ± 0.05 (P = 0.001). The difference in the log ratio reflects a higher proportion of mutagenic base lesions in the samples from older men. This resulted from a significantly higher concentration (nm/mg DNA) ± SE of 8-OH-Ade (0.09 ± 0.03 and 0.36 ± 0.06; P = 0.01) and a lower concentration of FapyGua (3.5 ± 0.43 and 1.7 ± 0.38; P = 0.02) in prostate DNA from older men. There were no significant differences in the 8-OH-Gua and FapyAde concentrations. The age-related linear increase in the proportion of mutagenic base lesions in the normal tissue DNA, reflected in the log ratio, may well be a significant etiological factor in prostate cancer development in men >60 years of age.

We found a negative correlation (−0.34; P = 0.03) between the log ratio and age for the NC (Fig. 3B). This is consistent with a dose-

Table 1 DNA base lesions and log ratios with Ps from a t test for differences between prostate tissues

<table>
<thead>
<tr>
<th>Base lesions and log₁₀ ratio</th>
<th>Normal</th>
<th>NC</th>
<th>Cancer</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 14</td>
<td>n = 31</td>
<td>n = 11</td>
<td>Normal vs. NC</td>
</tr>
<tr>
<td>FapyAde/10⁵ Ade</td>
<td>65.5</td>
<td>44.6</td>
<td>35.3</td>
<td>0.02</td>
</tr>
<tr>
<td>FapyGua/10⁵ Gua</td>
<td>266.5</td>
<td>433.7</td>
<td>265.5</td>
<td>0.07</td>
</tr>
<tr>
<td>8-OH-Ade/10⁵ Ade</td>
<td>36.6</td>
<td>77.10</td>
<td>107.19</td>
<td>0.001</td>
</tr>
<tr>
<td>8-OH-Gua/10⁵ Gua</td>
<td>218.24</td>
<td>370.46</td>
<td>456.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Log₁₀[(8-OH-Ade + 8-OH-Gua)/(FapyAde + FapyGua)]</td>
<td>-0.03</td>
<td>0.04</td>
<td>0.31</td>
<td>0.26</td>
</tr>
</tbody>
</table>
response relationship (in which individuals with higher log ratios would be expected to develop cancer earlier in life). The more negative log ratio with increasing age was primarily attributable to increases in the concentrations of FapyGua (concentrations of 8-OH-Gua and FapyAde showed virtually no age correlation). FapyGua was positively correlated with age (0.31; \( P = 0.05 \)), whereas the correlation between the 8-OH-Ade and age was only marginally significant (−0.28; \( P = 0.07 \)). Consistent with the negative trend of the log ratio with age for the NC, there was also a negative, though nonsignificant (\( P = 0.4 \)), trend among the tumor samples (not shown).

Although we do not know when the tumors actually appeared in the patients, it is of particular interest that the intersection of the positive age correlation slope for normal tissue (Fig. 3A) and the negative age correlation slope for the NC (Fig. 3B) occurs at \( \approx 61 \) years. This is about the age at which prostate cancer incidence rises exponentially (11). The implication is that men \( > 60 \) years of age with a log ratio \( > 0.03 \) are likely to be at especially high risk for developing prostate cancer. Thus, our base lesion data are consistent with epidemiological findings (12) and the proposed role of the \( \cdot \text{OH} \) in cancer development (Refs. 1 and 3; Fig. 1).

In interpreting our findings, we recognize that in histologically normal tissue the log ratio may well “teeter-totter” on the brink of positive and negative values over time, likely portending a fluctuating cancer risk. These fluctuations are potentially regulated by variable antioxidant levels that modulate the redox status of DNA as well as by the glycosylases that repair both the 8-OH and Fapy lesions (Refs. 9 and 19; Fig. 1). Other factors may relate to actions of hormones and xenobiotics that produce the precursor of the \( \cdot \text{OH} \) (H\(_2\)O\(_2\)) via redox cycling (20).

Subtle structural changes in DNA have also been attributed to the \( \cdot \text{OH} \) (21, 22). For example, the introduction of a single 8-oxo lesion into either Ade or Gua of a 25-base DNA strand produces conformational changes that may well alter the fidelity of replication (21). Additionally, Fapy residues are believed to block DNA synthesis (8, 10) and transcriptional activity was shown to be mediated by AP-1 binding factors that may be regulated by the redox status of DNA (23).

In turn, the changes in redox status would be expected to influence the ratio of 8-OH to Fapy lesions, thus influencing the likelihood of mutations as well as the rate of DNA synthesis (8, 10).

The log ratio was shown to be a potentially effective biomarker for assessing prostate and breast cancer risk (1), higher values suggesting an elevated risk for cancer development. It is noteworthy that the mean log ratio (0.16) for men \( \geq 70 \) years of age with normal prostates is somewhat higher than the mean log ratio for the NC (0.04; Table 1). This is consistent with the known high cancer risk for this older group (11). On the basis of our findings, a reasonable goal for cancer prevention might be to reach or maintain a mean log ratio of \( \approx -0.3 \), similar to that for men \(< 30 \) years of age (Fig. 3A). Modulation of the redox-sensitive log ratio might be achieved by dietary antioxidants (24–26) such as lycopene (found in tomatoes) or by intervention with potent radical-trapping agents such as N-acetyl-cysteine (27). It is also conceivable that the viability of tumors in estrogen-responsive tissues might be threatened if the log ratios are not maintained in the characteristic positive state (mean, 0.31; Table 1). Thus, it may be possible to specifically target tumor cells through intervention to shift the log ratio to a less positive status, thereby curtailing cancer progression and tumor survival.

Acknowledgments

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References


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