Lipid Peroxidation-induced Putative Malondialdehyde-DNA Adducts in Human Breast Tissues

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Abstract
The etiology of the majority of human breast cancers is unknown; however, oxidative stress and lipid peroxidation have been suggested to play a role in breast carcinogenesis. To address this possibility, DNA adducts induced by malondialdehyde (MDA), an end product of lipid peroxidation, were analyzed in surgical specimens of normal breast tissues of 51 breast cancer patients using the nuclease P1-enhanced version of the 32P-postlabeling assay. Normal breast tissue samples from 28 noncancer patients receiving reduction mammoplasty served as controls. Two previously characterized putative MDA-deoxyadenosine (dA) and one MDA-deoxyguanosine adduct were detected in all tissue samples examined. Normal breast tissues from cancer patients exhibited significantly higher levels of the putative MDA adducts [median (42.5) and range (2.2–202.8) of relative adduct labeling × 10^4 values] than those found in noncancer controls [median, 15.67; range, 2.4–382.1; P = 0.0001, Mann-Whitney U test]. Ten of the 51 cancer patients and 1 of the 28 controls were found to contain the putative MDA adducts at the level of >1/10^6 nucleotides, a frequency comparable to that found in human liver. Age and body mass did not significantly influence the levels of these adducts. However, the presence of a previously detected benzo(a)pyrene-like DNA adduct in the breast tissues was associated with higher levels of the putative MDA-dA adducts in cancer patients (P = 0.012). The level of the putative MDA-dA adducts was significantly lower in smokers and former smokers compared to nonsmokers among cases after adjusting for age, body mass index, and status of the benzo(a)pyrene-like adduct (P = 0.009). Tumor tissues (n = 11) displayed significantly lower levels of the putative MDA adducts (median, 10.2; range, 5.3–20.6) than their corresponding normal adjacent tissues (median, 25.5; range, 10.5–138; P < 0.01). These findings provide evidence that lipid peroxidation products can accumulate in human breast tissues and reach relatively high levels in the breast tissues of women with breast cancer. There seems to be an interaction between these endogenous DNA modifications and carcinogen exposure-induced DNA adducts. Detection and quantitation of the putative MDA-DNA adducts may potentially be a useful tool in the understanding of breast cancer etiology.

Introduction
Breast cancer is the second leading cause of cancer death among American women (1), and the incidence of breast cancer has been increasing in the United States (2). Despite advances in early detection and treatment, the mortality of breast cancer has not been significantly reduced over the past 20 years. At the same time, preventive strategies for breast cancer have been difficult to identify because the etiology of the majority of human breast cancers is unknown (3). There is accumulating evidence from animal and human systems implicating a role of oxidative stress and lipid peroxidation in the development of breast cancer. For example, in chemical carcinogen-induced mammary tumor animal models, high-fat diets are associated with increased tumor incidence, and this effect is diminished by antioxidants (4). In human studies, elevated levels of cholesterol peroxides, known to be direct-acting mutagens (5), were observed in the breast fluid of breast cancer patients (6). Increased levels of MDA, an end product of lipid peroxidation, were detected in the urine of women with mammographic dysplasia [a condition associated with increased risk for breast cancer (7, 8)] and in the serum of breast cancer patients compared with noncancer controls (9, 10). Finally, tamoxifen, an antiestrogen used in the treatment and chemoprevention of breast cancer, has been found to be an inhibitor of lipid peroxidation (11) and has also been shown to reduce serum levels of MDA in cancer patients (12).

One consequence of oxidative stress and lipid peroxidation is the formation of DNA adducts. Since DNA is believed to be the target molecule for carcinogens, endogenous DNA adducts derived from oxidative stress, lipid peroxidation, and other sources have been proposed to contribute to the etiology of human cancers (13, 14). With regard to breast cancer, a higher ratio of the carcinogenic hydroxy adduct (e.g., 8-hydroxyguanine) to the noncarcinogenic ring-opening products (e.g., 4,6-diamino-5-formamidopyrimidine) of DNA was detected in cancerous breast compared to normal breast tissue of noncancer controls (15). Furthermore, the levels of oxidative DNA dam-
age, e.g., 5-hydroxymethyluracil, in peripheral nucleated blood cells were significantly reduced by a low-fat diet intervention in women at high risk for breast cancer (16). These observations further support the hypothesis that oxidative stress may play a role in breast cancer etiology.

To investigate the possible role of environmental carcinogen exposure in breast cancer etiology, we assayed breast tissues of women with breast cancer for the presence of DNA adducts using a 32P-postlabeling technique. During the initial course of this study, we detected three types of bulky DNA adducts in adjacent normal tissues of breast cancer patients, i.e., a BP-like adduct, smoking-related adducts (i.e., DRZ), and some unidentified adducts (17). The levels of total DNA adducts were found to be significantly higher in normal breast tissues of cancer patients than in breast tissues of noncancer controls (women undergoing reduction mammoplasty). In the same population, to test the hypothesis that free radical and lipid peroxidation may be involved in breast cancer etiology as one common link between many suspected extrinsic and intrinsic risk factors and cancer development, we have now measured the more polar DNA adducts dominated by the lipid peroxidation-associated putative MDA adducts. If the working hypothesis was true, MDA adducts would be expected to be present in breast tissues of women at risk, and levels of these DNA adducts would be higher in breast tissues of cancer patients (i.e., women at 100% risk) compared to women without breast cancer.

To address this possibility, three putative MDA-DNA adducts were measured in adjacent normal tissues of 51 breast cancer patients and compared with those in normal breast tissues of 28 noncancer controls. The results presented here show that normal breast tissues of cancer patients contained significantly higher levels of MDA adducts than those of noncancer controls, and this difference was independent of age, smoking status, and body mass index. Interestingly, the level of the putative MDA adduct was positively correlated with the presence of a BP-related aromatic adduct, suggesting a possible interaction between carcinogen exposure and lipid peroxidation.

Materials and Methods

Tissue Sample Collection. Tumors and adjacent normal tissues were obtained from 51 breast cancer patients undergoing mastectomy at the University of Texas M. D. Anderson Cancer Center between November 1993 and May 1995. The use of surgical samples of human tissue was approved by the Institutional Review Board. All but eight of the breast cancer cases were newly diagnosed and untreated. Tumor and normal breast tissues were first examined and dissected by the pathologist and then kept at −80°C until DNA extraction. Information regarding tobacco, alcohol consumption, hormonal use, and other clinical parameters was obtained from the patients’ medical records. Normal breast tissues from 28 noncancer patients undergoing reduction mammoplasty were obtained from a local hospital or provided by the National Cancer Institute Human Tissue Network (Birmingham, AL).

DNA Adduct Analysis. Because the amount of tissue (usually 0.2–1 g) obtained from each patient was not sufficient for isolation of at least 106 epithelial cells (required to obtain 10 μg DNA for adduct analysis), DNA was extracted from breast tissues that were carefully dissected from the surrounding fat, and the lipid layer was removed after tissue homogenization and centrifugation. DNA extraction utilized the conventional phenol/chloroform procedure as described (18), and adducts were measured using the nuclease P1-enhanced version of 32P postlabeling (19). Briefly, 10 μg DNA were initially digested with micrococcal nuclease and spleen phosphodiesterase to 3′-mononucleotides. The unmodified normal nucleotides were dephosphorylated using nuclease P1 to nucleosides which are not substrates for polynucleotide kinase labeling. The modified nuclease P1-resistant nucleotides were then incubated with [γ-32P]ATP and polynucleotide kinase to generate 32P-labeled 3′,5′-bisphosphates and further analyzed using TLC. After the initial development with 2.3 M sodium phosphate (pH 5.75), the adducted nucleotides were separated according to their polarities. The most polar compounds, such as MDA-modified nucleotides, move to the upper portion of the chromatogram; the less polar compounds, e.g., 4-hydroxynonenal-induced DNA adducts, migrate to the central part of the map; and the nonpolar aromatic adducts remain in the lower part, including the origin area. The chromatogram was cut into three pieces as described previously (20), and adducts were transferred to fresh TLC plates. The final resolution of the most polar DNA adducts was achieved with two-dimensional chromatography using 2.1 M lithium formate/3.75 M urea (pH 3.75) and 0.14 M sodium phosphate/1.4 M urea (pH 6.4). DNA adducts were detected using autoradiography, and adduct spots were excised from the chromatogram for scintillation counting. Adduct levels are expressed as the RAL value, which is the ratio of cpm of adducted nucleotides: cpm of total nucleotides in the assay (19).

MDA-dA adduct profiles were obtained by reaction of 3′-dA with MDA, and a MDA-dG adduct standard was a generous gift from Dr. Lawrence Marnett (21). To compare adducts detected in tissue DNA with the MDA-modified dA or MDA-dG adduct standard, the two test DNA samples were mixed before enzymatic digestion and chromatographed on the same plate. The resulting chromatogram was then compared with maps derived from the individual DNA samples. Adduct spots migrating to similar locations on the two chromatograms were excised, eluted with 6 M ammonium hydroxide/2-propanol (1:1, v/v), spotted on a fresh TLC plate, and rechromatographed in another three solvent systems for further separation (see Fig. 2 legend). Adduct spots are considered structurally different if they can be separated by any of the solvents.

Statistical Analysis. The DNA adduct data were transformed into the square root of the RAL × 10^3 values to stabilize variance and normalize the distribution. The adduct level of a subgroup was expressed as median and range. Comparisons of adduct levels between cancer patients and controls, and between tumor and normal adjacent tissues were accomplished by using the Mann Whitney U rank test and Wilcoxon test, respectively. Multiple regression analysis and Spearman’s test were performed to determine the relationships between adduct levels and age, body mass index (weight/height^2), smoking status, and the simultaneous presence of the BP-like bulky aromatic adduct. The differences in adduct levels between cancer patients with regard to their smoking status and copresence of the BP-like adduct were analyzed using the Kruskal-Wallis test.

Results

Subjects. The 51 breast cancer patients included 37 white, 12 Hispanic, and 2 African Americans. The majority of cases (44/51) were from the State of Texas, six from other states, and one from another country. Forty-seven percent (24/51) of these women were premenopausal. The mean age of the cancer patients was 54 (range, 25–86) years. On the basis of their medical records, there were 15 current smokers, 4 former smokers, 24 nonsmokers, and 8 patients with unknown smoking histories. The occupational history was unknown for the majority of the patients. The 28 controls included 14 white, 1 Hispanic, and 13 African Americans.

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The mean age of the noncancer control subjects was much younger than that of the cancer cases (mean, 33 years; range, 16–57). Other information regarding smoking status and so forth of the controls was unavailable.

Identification of the Putative MDA-DNA Adducts in Breast Tissues. Using the 32P-postlabeling method, three major polar adducts were detected on the upper chromatograms in almost all breast tissue samples examined (Fig. 1, U1–U3). In addition, some unidentified adducts, e.g., spot 4 (Fig. 1B), were also observed on some of the chromatograms. Three major adducts were identified as putative MDA-DNA adducts, i.e., two MDA-dA adducts (Fig. 1, U1 and U2) and one MDA-dG adduct (Fig. 1, U3) by cochromatography comparison to adducts generated from MDA-treated DNA and mononucleotides in a previous study (21). Because of the polarity of these adducts, they move to the upper portion of the chromatogram along with the unmodified normal nucleotides during the initial development. To confirm that the adducts detected in human breast tissues are not the potentially labeled unmodified normal nucleotides, each adduct was excised and eluted from the chromatogram, spotted on a fresh TLC plate along with the four 32P-labeled major normal nucleotides, and developed in different solvents. As shown in Fig. 2, all three adducts were separated from normal nucleotides with different migration rates in 0.3 M borate, 0.3 M Tris-HCl, 6 mM EDTA, 4.8 M urea, and 0.78 M NaCl (pH 8.0). However, the chemical structures of the three putative MDA-DNA adducts are still unknown.

Comparison of DNA Adducts in Cancer Patients and Controls. To address the possibility that lipid peroxidation-induced DNA damage is related to breast cancer development, levels of the putative MDA adducts in normal breast tissues were compared between cancer patients and noncancer controls (Fig. 3). The median RAL x 10^10 values of MDA-dA (U1 + U2), MDA-dG (U3), and total MDA adducts (U1–U3) in normal adjacent tissues of cancer patients were 14.2, 26.6, and 42.5, respectively. These values were significantly higher (2–3-fold) than those found in normal breast tissues of noncancer controls (median of 5.2, 8.6, 15.7, respectively). Ten of the 51 cancer patients were found to contain the putative MDA adducts at the level of >1/10^7 nucleotides, a frequency comparable to that found in human livers (22). In contrast, only 1 of the 28 control samples from a 57-year-old woman showed a level of these adducts >1 x 10^7 (6.6 x 10^6), and this was the highest level seen in the total series studied. However, the differences in adduct levels between cancer cases and controls were not affected by this outlier (Fig. 3). The P values obtained using the Mann-Whitney U rank test in comparisons of the levels of each adduct between the two groups were all <0.001.

Factors Influencing the Levels of the Putative MDA Adducts. It is known that many factors can influence the extent of lipid peroxidation, e.g., smoking, dietary fat intake, alcohol consumption, and carcinogen exposure. In this pilot study, the limited information from the cancer patients' medical records...
Values for the DNA adduct levels are the median and range of RAL. The average age of each group is expressed as mean ± SD. To address this possibility, the adduct levels only allowed the examination of potential associations between DNA adduct levels and age, body mass index, and smoking status.

Since the majority of control subjects were younger than the cancer patients, it was possible that differences in DNA adduct levels were simply due to differences in age between cases and controls. To address this possibility, the adduct levels in cases and controls within the same age groups (i.e., ages 29–49 years only) were compared. A total of 22 cases and 17 controls within this age range were included. Even in this age-matched subgroup, the levels of the putative MDA adducts were significantly higher in cancer patients than those in controls (Table 1). In addition, a regression analysis showed no correlation between age and adduct levels in breast tissues of both cancer patients and controls. Although there appeared to be an age-dependent increase in adduct levels in the controls using the least square estimate regression analysis, this trend disappeared in the Robust regression analysis, which indicates that the trend was biased by the high level of adducts detected in a single 57-year-old control subject (data not shown).

Obesity as a risk factor may be related to breast cancer through its effect on estrogen metabolism or as a confounder of dietary fat (23). The relationship between body mass index and the level of the putative MDA adducts was examined. The body mass index was known for 34 of 51 cancer patients, and it was not significantly correlated with the adduct levels (data not shown).

The level of the MDA-dG adduct (U3) in cancer patients was not associated with smoking status. However, a multiple regression analysis showed that the level of the putative MDA-dA adducts (U1 and U2) was negatively associated with smoking (r = −0.36, P = 0.009) after adjusting for age, body mass index, and status of the BP-like adduct. On the other hand, a positive correlation (r = 0.34, P = 0.012) was found between the level of MDA-dA adducts (U1 and U2) and the presence of a BP-like bulky adduct in the same DNA sample; this adduct was previously detected in normal adjacent breast tissues in 24 of the 51 cancer patients and in 0 of the 28 controls (17). No correlation was found between the level of the MDA-dG (U3) adduct and the BP-like adduct. The influence of smoking and presence of the BP-like adduct on the levels of MDA-dA adducts was also confirmed using ANOVA (Kruskal-Wallis test, P = 0.016 and 0.015 for smoking and BP-like adduct, respectively; Fig. 4).

Comparison of DNA Adducts in Tumors and Their Adjacent Normal Tissues. Tumor tissues or rapid proliferating tissues are usually found to have lower levels of lipid peroxidation and DNA adducts than those of normal tissues; however, the converse has also been reported (24). When the levels of the putative MDA adducts were compared in 11 paired samples of tumors and corresponding normal adjacent tissues in the present study, significantly lower levels of all three adducts were observed in the tumors compared to the normal tissues in 8 of the 11 cases (Table 2).

Other DNA Adducts. In addition to the three putative MDA-DNA adducts, one intense spot (Fig. 1, spot 4) was detected on the upper chromatogram in 71% (36/51) of the cancer patients and 25% (7/28) of the controls. The chemical identity of this adduct is unclear. Among patients who demonstrated this adduct, the levels were similar between cancer patients (median, 15.35; range, 0.6–127.1; n = 36) and controls (median, 11.7; range, 3.5–242.8; n = 7, P = 0.79). The level of spot 4 was not found to be significantly associated with age, body mass index, smoking status, or presence of the BP-like adduct.

Since the putative MDA adducts represent 70% of the total adducts detected on the upper chromatogram, the level of total adducts, i.e., spots U1–U3 plus the unidentified adducts on the upper map, showed a similar trend to the spots U1–U3 alone (Fig. 5). It was significantly higher in cancer patients (median, 70.6; range, 2.7–324.6) than in noncancer controls (median, 25.53 range, 3.2–663.6, P = 0.0001). Age, body mass index, smoking, and presence of the BP-like adduct did not significantly influence the total adduct levels (data not shown).

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Table 1  MDA adducts in normal breast tissues of women between the age of 29 and 49 yr

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Cases (n = 22)</th>
<th>Control (n = 17)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>U1 + U2</td>
<td>14.4 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>0.005</td>
</tr>
<tr>
<td>U3</td>
<td>20.9 ± 1.0</td>
<td>8.3 ± 0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>U1-3</td>
<td>37.5 ± 1.8</td>
<td>15.1 ± 0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>68.4 ± 2.0</td>
<td>17.1 ± 0.6</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* P values were obtained using the Mann-Whitney U rank test.

Table 2  Comparison of MDA adduct levels in tumor and normal tissues (RAL × 10⁶)

<table>
<thead>
<tr>
<th>Patient</th>
<th>U1 + U2</th>
<th>U3</th>
<th>T</th>
<th>N</th>
<th>T</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2</td>
<td>16.6</td>
<td>5.7</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
<td>5.9</td>
<td>5.5</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.7</td>
<td>84.0</td>
<td>5.2</td>
<td>54.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.8</td>
<td>15.8</td>
<td>4.4</td>
<td>7.3</td>
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</tr>
<tr>
<td>5</td>
<td>4.3</td>
<td>41.0</td>
<td>2.6</td>
<td>22.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>3.7</td>
<td>5.7</td>
<td>6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9.0</td>
<td>60.0</td>
<td>11.6</td>
<td>71.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.0</td>
<td>64.0</td>
<td>5.6</td>
<td>3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3.6</td>
<td>10.8</td>
<td>5.3</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.2</td>
<td>7.8</td>
<td>2.2</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>4.4</td>
<td>7.2</td>
<td>5.8</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4.9</td>
<td>16.2</td>
<td>5.4</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* T: tumor; N: adjacent normal tissues.

P values were obtained using the Wilcoxon test.
The level of the MDA-dG adduct in liver tissues of 6 disease-occurs continuously in human breast tissues. The observation To address the hypothesis that lipid peroxidation may play a carcinogenic in vitro degradation of the polyunsaturated fatty acid constituents of higher than l/)0, which is close to the previously reported possible technical limitation, 10 of 51 cancer patients and 1 of observed levels of these adducts using this procedure might previously demonstrated that the MDA-dG adduct is suscepti- patients. The relatively lower levels observed here could be due to the average levels (median) of the putative MDA-dA, MDA- free individuals has been reported to be 5-11 adducts/107 bases (22, 29). The major adduct identified is the MDA-dG adduct. MDA is one of the major end products of peroxidative and in animal tissues using several methods (27, 28). Human tissues and urine samples have also been demonstrated to contain MDA-DNA adducts (22, 29). The major adduct identified is the MDA-dG adduct. The level of the MDA-dG adduct in liver tissues of 6 disease-free individuals has been reported to be 5—11 adducts/107 bases (22), which corresponds to 5400 adducts/cell. The average MDA-DNA adduct level in breast tissues of seven cancer-free women has been found to be three adducts per 107 bases (30).

In the current study, using the 32P-postlabeling method, the average levels (median) of the putative MDA-dA, MDA-dG, and total MDA adducts were 14.2, 26.6, and 42.5 adducts/109 nucleotides, respectively, in normal breast tissues of cancer patients. The relatively lower levels observed here could be due to tissue difference as well as technical difference. It has been previously demonstrated that the MDA-dG adduct is susceptible to nuclease P1 digestion (31). Therefore, some adducts could have been lost from detection during the analysis, and the observed levels of these adducts using this procedure might represent a minimum estimate of the actual levels. Despite this possible technical limitation, 10 of 51 cancer patients and 1 of 28 controls displayed a level of total putative MDA adducts higher than 1/107, which is close to the previously reported level in breast tissues (30). One limitation of the 32P-postlabeling assay, however, is that it does not provide information about the identity of the adducts. The identification of these putative MDA-DNA adducts reported here was performed using cochromatography analysis with DNA with mononucleotides reacted with MDA. The chemical structures of these putative MDA adducts still remain unknown.

Endogenous DNA damage has been implicated in spontaneous tumorigenesis and aging (32, 33). This study has demonstrated for the first time an association between endogenous DNA adducts and human cancer risk. Although the reduction mammaplasty samples may not be the ideal low-risk tissue control, the significantly higher levels of the putative MDA adducts found in cancer patients suggest that lipid peroxidation may contribute to human breast cancer. Although the design of the current study does not allow us to rule out the possibility that the increased adduct level in cancer patients is a consequence of the presence of cancer, previous studies have found that women with mammographic dysplasia (a condition associated with increased risk for breast cancer) exhibit higher levels of serum MDA (7, 8). Moreover, tumor tissues tended to have a lower level of lipid peroxidation (24). Thus, these findings suggest that the increased levels of these endogenous DNA adducts in cancer patients may not be due to the presence of cancer.

Comparison of the putative MDA adducts in normal and tumor tissue samples of the same individuals has shown that the majority (8/11) of breast tumors exhibited significantly lower levels than the normal adjacent tissues. Whether tumor tissues in general have lower levels of lipid peroxidation than normal tissues is controversial (24). Previous studies by other investigators have shown that breast tumors have higher phospholipid contents but similar levels of lipid peroxidation compared to normal adjacent tissues (34, 35). The lower level of MDA-related DNA adducts detected in tumors in our study could be a result of the “dilution” effect due to enhanced DNA turnover in tumors.

Diet, smoking, alcohol consumption, carcinogen exposure, certain disease status, and many other factors have been associated with the extent of lipid peroxidation in animal models and in other human cancers. Unfortunately, detailed information on many of these factors was not available in the current pilot study. Although the smoking history obtained from the medical records may not be completely accurate, we have analyzed the smoking-related DNA adducts (DRZ) in these patients and observed a good correlation between the presence of DRZ and the self-reported smoking history (17). Therefore, the classification of ever-smokers and never-smokers in the current study is reliable. Despite the limited information available, we attempted to explore the association between adduct levels and several known host factors. Age and body mass index did not show a significant influence on the levels of these adducts. Surprisingly, smoking history had an inverse relationship with the putative MDA adduct levels. The underlying explanation for this finding is not clear. It is possible that other important risk factors, e.g., diet (16, 36) and estrogen metabolism (37—39), may have a strong influence on the putative MDA adducts and may confound the smoking effect. It is noted that among all of the samples examined, one reduction mammaplasty sample had the highest level of the detected adducts. This sample was obtained from a 57-year-old woman. Unfortunately, we have not been able to obtain relevant information from the individuals in the control group, and we cannot explain the reason for this high value. Factors that influence the putative MDA adducts need to be identified in future studies which include more detailed epidemiological information.

The observation that patients with the BP-like adduct had

**Discussion**

To address the hypothesis that lipid peroxidation may play a role in human breast cancer etiology, the present study used the highly sensitive 32P-postlabeling technique to detect DNA adducts putatively derived from MDA, an end product of lipid peroxidation in human breast tissues. The results of this study clearly demonstrate that lipid peroxidation product-induced DNA adducts are detectable in human breast tissues from both cancer cases and controls, suggesting that lipid peroxidation occurs continuously in human breast tissues. The observation that cancer patients had significantly higher levels of the putative MDA-DNA adducts than did noncancer controls is consistent with previous findings that women with breast cancer or at high risk for breast cancer had elevated levels of MDA in their serum or urine (7—10).

MDA is one of the major end products of peroxidative degradation of the polyunsaturated fatty acid constituents of biological membranes and has been shown to be mutagenic and carcinogenic in *in vitro* systems and in experimental animals (25, 26). Lipid peroxidation can be induced by exposure to chemicals and ionizing radiation, but also occurs endogenously during normal metabolism. MDA-induced DNA adducts have been previously detected *in vitro* and in animal tissues using several methods (27, 28). Human tissues and urine samples have also been demonstrated to contain MDA-DNA adducts (22, 29). The major adduct identified is the MDA-dG adduct. The level of the MDA-dG adduct in liver tissues of 6 disease-free individuals has been reported to be 5—11 adducts/107 bases (22), which corresponds to 5400 adducts/cell. The average MDA-DNA adduct level in breast tissues of seven cancer-free women has been found to be three adducts per 107 bases (30).

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Endogenous DNA damage has been implicated in spontaneous tumorigenesis and aging (32, 33). This study has demonstrated for the first time an association between endogenous DNA adducts and human cancer risk. Although the reduction mammaplasty samples may not be the ideal low-risk tissue control, the significantly higher levels of the putative MDA adducts found in cancer patients suggest that lipid peroxidation may contribute to human breast cancer. Although the design of the current study does not allow us to rule out the possibility that the increased adduct level in cancer patients is a consequence of the presence of cancer, previous studies have found that women with mammographic dysplasia (a condition associated with increased risk for breast cancer) exhibit higher levels of serum MDA (7, 8). Moreover, tumor tissues tended to have a lower level of lipid peroxidation (24). Thus, these findings suggest that the increased levels of these endogenous DNA adducts in cancer patients may not be due to the presence of cancer.

Comparison of the putative MDA adducts in normal and tumor tissue samples of the same individuals has shown that the majority (8/11) of breast tumors exhibited significantly lower levels than the normal adjacent tissues. Whether tumor tissues in general have lower levels of lipid peroxidation than normal tissues is controversial (24). Previous studies by other investigators have shown that breast tumors have higher phospholipid contents but similar levels of lipid peroxidation compared to normal adjacent tissues (34, 35). The lower level of MDA-related DNA adducts detected in tumors in our study could be a result of the “dilution” effect due to enhanced DNA turnover in tumors.

Diet, smoking, alcohol consumption, carcinogen exposure, certain disease status, and many other factors have been associated with the extent of lipid peroxidation in animal models and in other human cancers. Unfortunately, detailed information on many of these factors was not available in the current pilot study. Although the smoking history obtained from the medical records may not be completely accurate, we have analyzed the smoking-related DNA adducts (DRZ) in these patients and observed a good correlation between the presence of DRZ and the self-reported smoking history (17). Therefore, the classification of ever-smokers and never-smokers in the current study is reliable. Despite the limited information available, we attempted to explore the association between adduct levels and several known host factors. Age and body mass index did not show a significant influence on the levels of these adducts. Surprisingly, smoking history had an inverse relationship with the putative MDA adduct levels. The underlying explanation for this finding is not clear. It is possible that other important risk factors, e.g., diet (16, 36) and estrogen metabolism (37—39), may have a strong influence on the putative MDA adducts and may confound the smoking effect. It is noted that among all of the samples examined, one reduction mammaplasty sample had the highest level of the detected adducts. This sample was obtained from a 57-year-old woman. Unfortunately, we have not been able to obtain relevant information from the individuals in the control group, and we cannot explain the reason for this high value. Factors that influence the putative MDA adducts need to be identified in future studies which include more detailed epidemiological information.

The observation that patients with the BP-like adduct had

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**Fig. 5.** Comparison of total polar adducts on the upper chromatogram in normal breast tissues of cancer patients and controls. See "Materials and Methods" for the statistical analysis.
significantly higher levels of MDA-related adducts than those without the BP-like adduct may suggest an interaction between that environmental carcinogen exposure and lipid peroxidation. Although the chemical identity and possible exposure source of this BP-like adduct is unknown (17), the correlation detected between the two types of adducts suggests that some common mechanisms may be involved in the accumulation of these DNA adducts in normal breast tissues. On one hand, carcinogens could induce lipid peroxidation during their metabolic activation; on the other hand, lipid peroxides could serve as cofactors in carcinogen activation (40). The possibility that a synergistic interaction between these two processes may significantly contribute to breast cancer development needs to be explored further.

This study has demonstrated that the 32P-postlabeling method is useful in the detection and quantification of the putative MDA-DNA adducts, especially in samples with limited material. Normal breast tissues of cancer patients contain significantly higher levels of lipid peroxidation-related DNA damage than noncancer controls. These preliminary findings suggest a role of lipid peroxidation-induced DNA damage in human breast cancer. The significance of endogenous DNA modifications in cancer development needs to be investigated further in detailed molecular epidemiological studies, and the putative MDA-DNA adducts reported here may serve as a biomarker in such studies.

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References
Lipid peroxidation-induced putative malondialdehyde-DNA adducts in human breast tissues.


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