Aromatic DNA Adducts in Adjacent Tissues of Breast Cancer Patients: Clues to Breast Cancer Etiology

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ABSTRACT

The etiology of the majority of human breast cancers is unknown. Environmental factors have long been suspected to play a role, but no specific causative agent has been identified. If the hypothesis that environmental carcinogen exposure contributes to human breast cancer is true, carcinogen-DNA adducts would be expected to be present in human breast tissues. To address this possibility, aromatic DNA adducts were measured in 87 surgical specimens of normal human breast tissues from 87 breast cancer patients undergoing mastectomy using the nuclease P1-enhanced version of the 32P postlabeling assay. Breast tissue samples from 29 noncancer patients undergoing reduction mammoplasty served as controls. Whereas aromatic DNA adducts were detected in all tissue samples examined, the total adduct levels in cancer patients were significantly higher than that in noncancer controls (mean ± SEM, 97.4 ± 23.4/10^5 nucleotides (range, 3.8–173.1) versus 18.1 ± 11.6/10^5 nucleotides (range, 5.6–56.7), respectively; P < 0.01, t test and Mann-Whitney test). This difference was not affected by the age distribution of the two groups. The typical smoking-related DNA adduct pattern (i.e., a diagonal radioactive zone) was observed in 29 of 87 tissues (17 of 17 current smokers, 5 of 8 former smokers, 4 of 52 nonsmokers, and 3 of 10 patients with unknown smoking status) and in 2 of 10 control tissues. It was of interest that a benzo(a)pyrene (BP)-like DNA adduct was observed in 36 normal adjacent breast tissues (41%), 27 of which were from nonsmokers. Levels of this BP-like adduct were extremely high (>100/10^5 nucleotides) in 5 patients (4 nonsmokers and 1 smoker) and moderately high (>10/10^5 nucleotides) in 13 other patients (8 nonsmokers and 5 smokers). One patient exhibited this adduct at a level of 1500/10^5 nucleotides, which is comparable to the highest level of total adducts reported in human tissues related to carcinogen exposure (e.g., cigarette smoking). In contrast, this adduct was absent (<1/10^5 nucleotides) in all of the control tissues. Cochromatography and rechromatography analysis of DNA samples from human breast tissues and from MCF-7 cells treated with BP revealed that this adduct could be generated by BP exposure but is not the major BP 7,8-diol-9,10-epoxide-deoxyguanine adduct detected previously in animal tissues and human mammary epithelial cells. These findings support the hypothesis that environmental carcinogen exposure, in addition to cigarette smoking, may be associated with the etiology of human breast cancer.

INTRODUCTION

Breast cancer is the second leading cause of cancer-related death in American women (1). Despite the advances in treatment and early detection of breast cancer, the mortality has not been significantly reduced. Prevention of this disease has been hampered by lack of knowledge of etiology of the majority of human breast cancers. Known risk factors (including a family history of breast cancer and reproductive factors related to lifetime exposure to endogenous estrogen) account for only about 30% of the cases (2). Environmental factors have long been suspected to contribute to human breast cancers, but no specific agents have been definitely implicated except radiation (3). Urban residence, dietary fat intake, cigarette smoking, alcohol consumption, and exposure to organochlorine compounds have been related to breast cancer risk, but the associations were either weak or inconclusive (2–6). It has been hypothesized that environmental carcinogen exposure may be an underlying link between some of the disparate risk factors and breast cancers (7). For example, increased risk of breast cancer in urban areas may be associated with increased hydrocarbon pollution, and alcohol consumption may compound this risk by increasing the activation of hydrocarbons through induction of cytochrome P-450 enzymes. A high-fat diet and hormonal stimulation to the breast may also act as promoters for cells already initiated by some carcinogens.

The hypothesis that environmental carcinogens are involved in human breast cancer is supported by several types of experimental evidence: (a) a number of compounds present in the human environment are potent mammary carcinogens in rodents (8); (b) the anatomical features of the breast make it a susceptible target organ for chemical carcinogens. Lipophilic aromatic compounds can be stored and concentrated in the breast fat pad (9), and human mammary epithelial cells have a high capacity to metabolize these compounds into DNA-binding species and, thus, can themselves become target cells for carcinogenesis (10–12); and (c) the spectrum of p53 gene mutations observed in breast tumors suggests the involvement of exogenous agents in inducing these mutations in a significant portion of cases (13, 14). If this hypothesis were correct, it should be possible to detect carcinogen-related DNA adducts in human breast tissues, and the levels would be expected to be highest in those women who have already developed breast cancer (i.e., individuals who have shown themselves to have 100% risk of breast cancer).

Using the 32P postlabeling method, DNA adducts in human breast tissues have been measured in three previous studies (15–17). The first report found aromatic DNA adducts in 3 of 10 reduction mammoplasty samples (15). The second study detected adducts in 5 of 24 autopsy breast samples (16). The third study examined normal adjacent tissues from 15 breast cancer patients and normal breast tissues from 4 reduction mammoplasty noncancer controls (17). Aromatic adducts were detected in all tissues examined, and the smoking-related DNA adduct patterns were seen in 30% of the cancer cases. These pilot studies demonstrated the feasibility of adduct measurement in human breast tissues but did not have proper control groups or adequate sample size for any comparison.

To address the possibility that environmental carcinogens such as PAHs may be etiological agents in human breast cancers, we have measured aromatic DNA adducts in normal adjacent tissues from 87 breast cancer patients and in normal tissues of 29 reduction mammoplasty noncancer controls. A significantly higher level of aromatic DNA adducts was found in adjacent breast tissues of cancer patients than in noncancer controls.

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2 To whom requests for reprints should be addressed, at Department of Clinical Investigation, Box 619, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030.

3 The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; BP, benzo(a)pyrene; BPDE-G, BP 7,8-diol-9,10-epoxide deoxyguanine; DRE, diagonal radioactive zone; RAL, relative adduct labeling.
DNA ADDUCTS IN HUMAN BREAST TISSUES

Data and Tissue Sample Collection. Breast tumors and their histologically normal adjacent tissues were obtained from 87 breast cancer patients undergoing mastectomy at The University of Texas M.D. Anderson Cancer Center between November 1993 and May 1995. The use of human surgical samples was approved by the Institutional Review Board. The majority of the cases (72 of 87) were newly diagnosed untreated breast cancers. Tumors and adjacent normal tissues were first examined and separated by the pathologists, then kept at −80°C until DNA extraction. Information regarding tobacco, alcohol, hormonal use, and other clinical parameters was obtained from the medical records of the patients. Normal breast tissues from noncancer patients undergoing reduction mammoplasty were obtained from a local hospital (19 samples) or provided by the National Cancer Institute Human Tissue Network (10 samples, Birmingham, AL).

In Vitro BP Treatment. Human mammary carcinoma cells MCF-7 were treated with BP to obtain a reference BP-DNA adduct pattern to compare with that found in the tissue samples. Cells were grown to 50–80% confluency in DMEM (GIBCO-BRL, Santa Clara, CA) with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA) and then treated with 4 μM BP (National Cancer Institute Chemical Carcinogen Repository) in 1% DMSO (Fisher, Pittsburgh, PA) in culture medium. Controls received the vehicle solvent alone in the medium. After 24 h, the cells were washed with PBS and then harvested by a brief treatment with trypsin:EDTA. Cells were pelleted by centrifugation and then stored at −80°C until DNA isolation.

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, which are needed to obtain 10 μg DNA, DNA was extracted from breast tissues after carefully dissecting out the surrounding fat. DNA was extracted by the conventional phenol/chloroform procedure as described previously (18), and adducts were detected by the nuclease P1-enhanced version of the 3²P postlabeling procedure (19). Briefly, 10 μg DNA were initially digested with micrococcal nuclease and spleen phosphodiesterase to 3’ mono-nucleotides. The unmodified normal nucleotides were then selectively dephosphorylated by nuclease P1 to nucleosides that are not substrates for subsequent polynucleotide kinase labeling. The modified nucleotides were then labeled with [γ-3²P]ATP and polynucleotide kinase to 3’,5’-bisphosphate and further analyzed by TLC. Chromatography conditions were as described previously (20) unless otherwise indicated in the figure legends. Adducts were detected by autoradiography, and adduct spots were excised from the chromatograms for scintillation counting. Adduct levels are expressed as a RAL X 10⁸ value, which is the cpm of adducted nucleotides/cpm of total nucleotides in the assay ratio (19). Total adduct level was calculated by summation of the amount of each individual adduct detected. To compare adducts detected in tissue DNA with those in BP-treated cells, the two DNA samples were mixed before enzymatic digestion, and the resulting chromatogram was compared with maps derived from the individual DNA samples. Adduct spots migrating to similar locations in the two chromatograms were excised, eluted with 6 N ammonium hydroxide:2-propanol (1:1, v/v), spotted on a fresh TLC plate, and rechromatographed in at least three other solvent systems for further separation. Adduct spots that could be resolved in any of these solvent systems are considered as structurally different; otherwise, they are considered as the same adducts.

Statistical Analysis. The average level of DNA adducts was expressed as mean ± SE, and comparisons between means were analyzed by Student’s t test, Mann-Whitney test, and Wilcoxon signed rank test. The average levels of adducts were compared between cancer patients versus controls, tumors versus normal adjacent tissues, premenopausal versus postmenopausal women, and patients with presurgical treatment versus those without. Regression analysis and the Spearman’s test was performed for determination of age dependency of adduct levels and dose-response relationship of smoking-related adducts.

RESULTS

Subjects. The 87 breast cancer patients studied in this project included 62 whites, 19 Hispanics, 5 African-Americans, and 1 Asian-American. The 29 controls include 14 whites, 13 African-Americans, and 2 Hispanics. The majority of the cases (70 of 87) were from the state of Texas, 12 from other states, and 5 from other countries.

Thirty-eight % (34 of 87) of these women were premenopausal. Twenty-three women had estrogen replacement, and nine had use of oral contraceptive history. There were 17 current smokers, 8 former smokers, and 52 nonsmokers. The occupational history was unknown for the majority of patients. The mean age of the cancer patients was 54 years (range, 25–86 years), whereas the mean age of control patients was much younger (mean, 32; range, 16–57; Fig. 1).

General Adduct Analysis. If the hypothesis that environmental carcinogen exposure may be involved in human breast cancer is true, one might expect a higher level of aromatic DNA adducts in women at increased risk for breast cancer. To address this issue, aromatic DNA adducts in normal breast tissues of cancer patients and noncancer controls were analyzed by the highly sensitive nuclease P1-enhanced version of 3²P postlabeling. Three types of DNA adducts were detected: (a) a single bulky adduct near the origin (Fig. 2, spot 1); (b) the typical smoking-related adduct pattern, known as DRZ (Fig. 3); and (c) various unidentified adducts (Fig. 2). Quantitative analysis of the chromatograms revealed that the level of total adducts (including all three types of adducts) in normal adjacent tissues of cancer patients was significantly higher than that found in breast tissues of noncancer controls (mean ± SEM (range) of RAL X 10⁸ values: 97.4 ± 23.4 (3.85–1737.1) versus 18.1 ± 11.6 (5.6–56.7), respectively (P < 0.01, Mann-Whitney test; Fig. 4).

DNA samples from 15 breast tumors were also analyzed, along with their corresponding normal adjacent tissues. The adduct patterns were comparable between the two types of tissues, as illustrated in Fig. 5. The majority of tumors showed relatively lower levels of

Fig. 1. Age distribution of cancer patients and controls.
adducts than their counterpart normal adjacent tissues (Table 1). However, the average total RAL $\times 10^9$ values were not significantly different between tumors and their adjacent normal breast tissues [mean $\pm$ SEM, 54.83 ± 8.0 versus 101.94 ± 27.4 ($P = 0.08$, t test); median, 50.0 versus 50.1 ($P = 0.07$, Wilcoxon signed rank test)].

**BP-like Adduct.** It was of interest that a bulky DNA adduct (Fig. 2, spot 1) was observed at significant levels in 36 of 87 (41%) normal adjacent tissues of breast cancer patients but was not observed in any of the controls. In some cases, this adduct was accompanied by a less intense satellite spot (Fig. 2, spot 2). The levels of spot 1 were extremely high (>100 adducts in $10^9$ nucleotides) in 5 patients (4 nonsmokers and 1 smoker) and moderately high (10–100 adducts in $10^9$ nucleotides) in 13 other patients (8 nonsmokers and 5 smokers). One patient exhibited this adduct at a level of 1500 adducts/$10^9$ nucleotides, which is comparable to the highest level of total adducts detected in human tissues related to carcinogen exposure (21). Smoking did not seem to be the main source of the BP-like adduct because the majority (27 of 36) of patients who showed this adduct were nonsmokers (Table 2).

The location of these two adduct spots was similar to that of adducts found previously in normal human mammary epithelial cells

**Fig. 2.** $^{32}$P-labeled DNA adduct profiles in adjacent normal tissues of 4 nonsmoking breast cancer patients (A, Cancer) and normal breast tissues of 4 noncancer controls (B, Control). Film exposure was at $-80^\circ$C for 16 h. Note the intense spot 1 and its satellite spot 2. P4, P29, P54, P73, N1, N2, N3, and N4, patient numbers.

**Fig. 3.** Typical smoking-related DNA adduct pattern (DRZ) in normal adjacent tissues of 2 breast cancer patients. Film exposure was at $-80^\circ$C for 16 h. Numbers at right bottom corner, patient numbers.

**Fig. 4.** Levels of total DNA adducts in normal adjacent tissues of cancer patients and normal breast tissues of noncancer controls. ---, median; --- ---, mean. Boxes, 25th and 75th percentiles of the observed values; bars, 10th and 90th percentiles.
DNA ADDUCTS IN HUMAN BREAST TISSUES

To determine whether spots 1 and 2 are BP-derived adducts, tissues DNA containing these adducts were analyzed by cochromatography with DNA of MCF-7 cells treated with BP. It was found that spot 1 migrated faster than the major BPDE-G adduct but comigrated with one of the minor adducts present in cells treated with BP (Fig. 6). Rechromatography of spot 1 and this minor BP adduct from cellular DNA using other solvents could not resolve them (Fig. 6). These observations suggest that spot 1 was possibly derived from BP exposure but was not the major BPDE-G adduct described previously.

Smoking-related DNA Adducts. The typical smoking-related DNA adduct pattern, known as DRZ (Fig. 3), which has been previously detected in many different types of human tissues (22), was observed in a total of 29 of 87 normal adjacent tissues, including tissue from 17 of 17 current smokers, 5 of 8 former smokers, 4 of 52 nonsmokers, and 3 of 10 patients with unknown smoking status. This type of DNA modification was also observed in 2 of 10 noncancer controls (the smoking status of those subjects was unknown). Quantitation of DRZ and total adduct levels in relation to smoking history in the subset of smokers are given in Table 3. It is noteworthy that one patient who had stopped smoking for 18 years still exhibited this adduct pattern. No dose-response relationship was found in either the level of DRZ or the total adducts according to the self-reported pack-year among the current smokers (data not shown).

Unidentified Adducts. In addition to the BP-like adduct and DRZ, numerous adducts that were detected in breast tissues remained unidentified. Although they were detected under the chromatography conditions usually used for aromatic adducts, some of these adducts may be derived from endogenous sources related to the metabolism of hormones and nutrients (21). However, it was found that the level of these adducts was not affected by history of hormonal use or menopausal status (data not shown). On the other hand, cancer patients displayed significantly higher levels of the unidentified adducts than did noncancer controls [37.9 ± 3.5 (range, 3.8–225.7) versus 18.65 ± 3.9 (range, 5.6–32.7); P < 0.001, Mann-Whitney test].

Table 1 DNA adducts in tumors (T) and normal tissues (N)

<table>
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<th>Patient</th>
<th>Tumor</th>
<th>Normal</th>
<th>T/N</th>
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<td>88</td>
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<td>95</td>
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<td>80</td>
<td>72.0</td>
<td>253.6</td>
<td>0.28</td>
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<td>48.2</td>
<td>0.49</td>
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<td>0.51</td>
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<td>93</td>
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<td>0.54</td>
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<td>90</td>
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<td>165.1</td>
<td>0.63</td>
</tr>
<tr>
<td>87</td>
<td>28.9</td>
<td>43.7</td>
<td>0.66</td>
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<td>97</td>
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<td>15.0</td>
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<td>77</td>
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<td>33.5</td>
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<td>98.6</td>
<td>1.15</td>
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<td>74.9</td>
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<td>94</td>
<td>60.0</td>
<td>29.6</td>
<td>2.03</td>
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<tr>
<td>78</td>
<td>38.5</td>
<td>16.7</td>
<td>2.28</td>
</tr>
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</table>

Mean ± SE 54.8 ± 8.0 101.9 ± 27.4 P = 0.08
Median 50.0 50.1 P = 0.07

Table 2 BP-like adduct and smoking status

<table>
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<tr>
<th>Smoking status</th>
<th>BP-like adduct status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smokers</td>
<td>8 (0.22)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>2 (0.06)</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>24 (0.67)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (0.06)</td>
</tr>
</tbody>
</table>

a P < 0.05 by χ² test.

b Number of cases (%).

Fig. 5. DNA adduct profiles in tumors (Tumor) and their corresponding normal adjacent tissues (Normal) from two breast cancer patients. Film exposure was at −80°C for 16 h. Note the similar patterns but variable intensities of major adducts in tumor and normal tissues. Numbers on left, patient numbers.

Fig. 6. Cochromatography (A) and rechromatography (B) analyses of DNA samples from human breast tissue and from MCF-7 cells treated with BP. A, Tissue, DNA from normal adjacent tissue of patient 88; MCF-7 cells, DNA from MCF-7 cells treated with BP; Mixture, mixture of the above two DNAs. B, rechromatography in three solvent systems of spot 1 eluted from 2-dimensional chromatograms derived from DNA samples of five individual human breast tissues (Lanes 1–5) and from MCF-7 cells (Lane 6). Solvents used for rechromatography are: B, 0.4 M Tris•HCl, 0.4 M H3BO3, 8 mM EDTA, 1.04 M NaCl and 6.4 M urea (pH 8.0); I/A, isopropyl alcohol:1 M NH4OH (1:1, v/v); PTU, 0.6 M NaH2PO4, 0.4 M Tris, and 6.8 M urea (pH 8.0).
DNA ADDUCTS IN HUMAN BREAST TISSUES

Among the 87 cancer cases, 15 patients received radiotherapy and chemotherapy (e.g., fluorouracil, Adriamycin, cyclophosphamide, and taxol) before surgical treatment. It is known that radiation and many chemotherapeutic drugs have DNA-damaging effect; however, only cyclophosphamide might be expected to induce DNA adducts. To determine whether these treatments interfere with the adduct analysis, the results from patients who received presurgical treatment were compared to those from the untreated patients. Overall, patients with presurgical treatment tended to have lower adduct levels (60.2 ± 16.6; n = 15) than those without such treatment (105.1 ± 28.0; n = 72), but the difference was not statistically significant (P = 0.47). The effect of each specific therapeutic agent on DNA was not investigated systematically due to the small sample size.

DISCUSSION

To test the hypothesis that environmental carcinogen exposure may be involved in human breast cancer etiology, this study using the 32P postlabeling method has confirmed the preliminary findings of others (15–17) and demonstrated that aromatic DNA adducts can be detected in breast tissue samples. Furthermore, it was found that the total adduct levels were significantly higher in normal adjacent tissues of breast cancer patients than those in normal breast tissues of noncancer controls. One putative BP-like adduct was detected at significant level in normal adjacent breast tissues of 41% of the breast cancer patients. In contrast, this adduct was absent in all noncancer controls. These observations support the hypothesis that aromatic hydrocarbons and other environmental carcinogens may be involved in human breast carcinogenesis. The measurement of DNA adducts may be a potentially powerful tool to demonstrate the possible association between carcinogen exposure and cancer risk.

In the present study, we have found an aromatic BP-like adduct at high levels in tissue DNA of over 40% of breast cancer patients. BP is an ubiquitous environmental pollutant derived from incomplete combustion of fossil fuel. Human exposure to BP and other PAHs is common, occurring mainly through food intake and by inhalation of
polluted air or cigarette smoking. BP-DNA adducts have been detected previously in tissue samples of smokers, workers exposed to high levels of PAH, or people who have consumed large amounts of barbecued food (23–25). Compared to the levels reported previously, this study demonstrates the highest level of a single aromatic adduct detected thus far in human target tissues.

Toxicology studies have suggested that the food chain (especially dairy products, beef, and produce) accounts for 97% of human exposure to BP, and ambient air exposure accounts for only 2% of the total exposure (26). The average smoker (an individual smoking 20 cigarettes/day) gets an additional 16% BP from smoking. Our finding that presence of the putative BP-related adduct is not associated with smoking history is consistent with the hypothesis that smoking is not the main source of exposure to BP in humans.

Dietary fat has long been suspected of playing a role in the etiology of breast cancer. On the basis of our observation and the toxicological data, it is tempting to postulate that diet may contribute to carcinogenesis as a source of environmental carcinogens. Such carcinogens include not only those produced during cooking, but also those enriched in food chain from contaminated environment. If this hypothesis were true, individuals consuming a high-fat diet, especially fatty foods from a more polluted area, may have more exposure to carcinogens and, consequently, a greater burden of DNA adducts. On the other hand, because breast fat can concentrate lipophilic compounds, the adduct levels in the breast tissue may be much higher than those in other non-fatty tissues. These possibilities require investigations in a more comprehensive epidemiological study.

The fact that the BP-like adduct did not comigrate with the major BP adduct but comigrated with one of the minor adducts detected in BP-exposed MCF-7 cells is intriguing. It is known that BP can be metabolized to approximately 20 oxidized metabolites, and the major BP adduct found in mammary epithelial cells is BPDE-G (27). Several possibilities may be invoked to explain why the adduct detected in breast tissue is not the major but a minor BP-associated adduct: (a) the high levels of carcinogen used to treat cells in vitro may result in a different adduct pattern than that seen in tissues that are exposed to a low level of BP over a prolonged period. This speculation is based on the previous observations that DNA adduct profiles and gene mutation spectra shift in a dose-dependent fashion in in vitro and animal systems (28, 29); (b) some adducts may be preferentially repaired such that the detected adducts in chronically exposed tissues might represent an unrepaired, persistent subset; and (c) because the chemical structure of this adduct remains unknown, it is possible that this adduct is derived from an indirect mechanism induced by BP; therefore, it does not contain BP moiety. Furthermore, the possibility that this adduct is derived from aromatic compounds other than BP has not been excluded. No matter what the source or chemical nature, the fact that this BP-associated adduct appeared in 41% of the tissue samples in breast cancer patients deserves further investigation with regard to its origin and biological significance.

This study demonstrated that smoking-related DNA adducts can be detected in breast tissues of all current smokers and in some former smokers, even 18 years after cessation of smoking. This observation suggests that smoking-induced DNA damage could persist for a long time. Whether smoking-induced DNA adducts contribute to breast cancer development is not clear from this study because of lack of information regarding the smoking history in controls. Epidemiological studies have generally not supported an association between smoking and risk of breast cancer (30). However, in a recent study (31), women with genotypes consistent with slow acetylation (a process involved in detoxification of some carcinogenic agents) were found to be at increased, dose-depen-
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REFERENCES

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