# Carcinogen-DNA Adducts in Human Breast Tissue[1]

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## Abstract

**Breast cancer is the second leading cause of cancer death among American women. Known risk factors account for only approximately one-third of the 182,000 new cases diagnosed each year in the United States. There is both concern and debate over the contribution of environmental exposures related to lifestyle, occupation, and ambient pollution, particularly in high risk areas such as Long Island, NY and the rest of the northeastern United States. Biomarkers such as carcinogen-DNA adducts can help to explore the role of environmental risk factors for breast cancer by documenting DNA damage from specific carcinogens directly in human tissue. In this pilot study, a total of 31 breast tissue samples were analyzed by the $^{32}$P-postlabeling method for carcinogen-DNA adducts characteristic of complex mixtures of aromatic compounds (such as polycyclic aromatic hydrocarbons) and tobacco smoke. The samples included tumor and tumor-adjacent tissues from 15 women with breast cancer and normal tissue samples from 4 women undergoing breast reduction. Among the breast cancer cases, the mean aromatic/hydrophobic-DNA adduct level in all tissues assayed was 5.3 ± 2.4 (SD) adducts/$10^8$ nucleotides compared to 2.3 ± 1.5 among the samples from the noncancer patients. Breast tissue (tumor and/or nontumor) from 30% (5 of 15) of women with breast cancer displayed a pattern of adducts (referred to as a diagonal zone of radioactivity) associated previously, in studies of other tissues, with exposure to tobacco smoke. The 5 positive samples were from current smokers; tissue samples from the 8 nonsmoking cases did not show this characteristic pattern ($P < 0.01$). While the nature of the study precludes an inference of causality, these results demonstrate the presence in human breast cells of DNA damage characteristic of environmental carcinogens (complex mixtures of polycyclic aromatic hydrocarbons and tobacco smoke) to which women are widely exposed. Although limited, our results indicate that biomarkers such as DNA adducts may be useful in investigating specific environmental exposures that may contribute to breast cancer causation as well as the populations and individuals most affected. If so, they might be helpful in suggesting new strategies for prevention of breast cancer.**

## Introduction

In the United States, breast cancer currently afflicts 1 of 9 women by the age of 85, accounting for an estimated 182,000 new cases and 46,000 deaths annually (1). The incidence of breast cancer has been increasing steadily at an annual rate of approximately 1% in the United States (2, 3). Populations at highest risk tend to be urban and to live in the northeastern and mid-Atlantic parts of the United States (4). Worldwide, 600,000 new cases are diagnosed every year. Despite its high prevalence, the etiology of breast cancer has not been well elucidated, and known risk factors (including family history of the disease and reproductive factors related to lifetime exposure to endogenous estrogen) account for only about 30% of the disease (4, 5). Women without any identifiable risk factors have approximately a 6% chance of developing breast cancer through the age of 80 years (2).

Concern has been growing that environmental exposures may be contributing to the increasing rates and geographic variation in breast cancer incidence (6, 7). However, thus far only ionizing radiation has been established as an "environmental" cause of breast cancer (8). Epidemiological studies have not shown a consistent relationship between exposure to organochlorines and the risk of breast cancer (9, 10), and alcohol has been implicated in some but not all studies (11). The spectrum of *p53* mutations observed in breast tumors suggests the involvement of exogenous agents in inducing these mutations in a significant proportion of cases (7). A number of chemicals to which there is widespread human exposure have been shown experimentally both to cause mammary tumors in laboratory animals and to bind to DNA in breast epithelial cells (12–15). These include PAHs,[3] heterocyclic amines, and other aromatic compounds that are ubiquitous pollutants found in cigarette smoke, ambient air, workplace environments, drinking water, and food (12, 16–21). In addition to cigarette smoke and consumption of charbroiled foods, incomplete combustion of fossil fuel is a common source of PAHs. Ecological data from Poland suggest that breast

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbons; DRZ, digital zone of radioactivity; CI, confidence interval.

cancer may be associated with industrial pollution, specifically PAH and other products of coal combustion (22).[4]

Tobacco smoke contains dozens of known carcinogens, including aromatic compounds (PAH and aromatic amines), nitrosamines, and oxygen free radicals (19). Although the prevalence of cigarette smoking has declined since the 1960s, it remains high. Overall, 25% of adults ages 18 years and over smoke; nearly 1 in 4 American women is a smoker; and there are an estimated 3000 new tobacco users each day in the United States, primarily teenagers (1, 23). Results of epidemiological studies based on self-reported smoking histories are conflicting regarding the effect of smoking on breast cancer risk (24). Most studies of active smoking have found either a small positive association (about 20–30%) or no association with breast cancer. However, few studies have considered age of onset of smoking. Recently, heavy smoking at an early age (before 16 years) has been associated with a greater risk of breast cancer (25). In a population-based case-control study of tobacco smoke and breast cancer, after adjusting for known risk factors, the odds ratios of breast cancer increased with amount of smoking from 2.5 (95% CI, 1.4–4.5) for an average lifetime consumption of 1–9 cigarettes/day to 3.3 (CI, 1.9–5.9) for 20 or more cigarettes/day. Passive smoking also significantly increased the risk of breast cancer (26). There have been several other reports of an increased risk of breast cancer from passive smoking. These results require confirmation (24). Multiple pathways have been suggested for the effects of smoking on breast cancer with hypothesized protective mechanisms including the antiestrogenic effect of smoking (25, 27). However, these potentially protective mechanisms appear to be balanced by other negative effects, including the direct exposure of breast tissue to the carcinogenic components of cigarette smoke (25, 27, 28). There is compelling evidence that constituents of cigarette smoke not only reach the breast but concentrate there (29, 30).

The uncertainty and resultant controversy over the role of environmental exposures, including tobacco smoke, in the etiology of breast cancer has provided an impetus to the development of biomarkers such as DNA adducts and their application in molecular epidemiology (31, 32). Adduct measurements have the potential as sensitive integrating dosimeters for potential mammary carcinogens. Extensive data indicate that most carcinogens are metabolically activated to electrophilic species capable of covalently binding to cellular macromolecules. If the adducts are not repaired prior to cell replication, gene mutation may result. DNA binding can result both in early stage initiation of the malignant process and in later stage progression of tumors (33, 34). In laboratory animals, the carcinogenic potency of a series of genotoxic carcinogens is correlated with their ability to form covalent adducts with DNA (35, 36).

It is recognized that carcinogen-DNA adducts detected in peripheral blood or breast tumor tissue are not reflective of long past exposures unless exposure has remained constant over the relevant portion of the lifetime of an individual (assuming that metabolic and repair processes have not been altered by age or disease). However, even when exposure has changed, an analysis of current adduct levels in conjunction with estimated current exposure can identify women who are/have been "high responders" to environmental exposures. Further, because the biological effect of adducts can be either to initiate cancer and/or, many years later, to trigger malignant conversion, adducts measured contemporaneously are biologically relevant to carcinogenic risk.

Prior research has demonstrated an association between PAH-DNA adducts, measured in peripheral leukocytes by immunoassay or $^{32}$P postlabeling, and a number of environmental exposures including workplace pollutants (17, 37, 38), ambient air pollution (39), cigarette smoke (40, 41), and dietary PAH (20, 42). PAH-DNA adducts have also been associated with risk of lung cancer (43). Aromatic DNA adducts and an adduct pattern characteristic of cigarette smoking (a DRZ) have been detected in respiratory tract and cervical tissues by the $^{32}$P-postlabeling method (44–49). However, such adducts have not been reported previously in human mammary tissue. Although some studies have suggested that adducts in tumor tissue may not be representative of those in normal tissue, roughly comparable levels of aromatic DNA and aflatoxin DNA adducts have been observed in the two types of tissue (including lung and breast) from the same individuals (48, 50).[5]

Because biomarkers such as adducts can reflect interindividual variation in biological response (hence potential risk) from environmental carcinogens, they may be helpful in identifying that subset of women for whom these exposures constitute risk factors. The goal of the present pilot study was to investigate whether adducts of the type formed by pervasive environmental carcinogens could be detected in breast tissue.

## Materials and Methods

Samples were collected at surgery from 15 women being treated for breast cancer at Columbia-Presbyterian Medical Center (New York, NY) (stages I through IV). The women ranged in age from 42–80 years. Seven of the patients were current smokers, 3 were former smokers (all quit ≥5 years before), and 5 had never smoked. For reference purposes only, 5 tissues were obtained from 4 women without cancer who were undergoing breast reduction surgery. These women were ages 20–40 years, and their smoking histories were unavailable. See Tables 1 and 2 for details regarding smoking history and tissue samples analyzed.

DNA from coded tissue samples was analyzed using the $^{32}$P-postlabeling method with the nuclease $P_1$ digestion procedure to enhance sensitivity. The method detects a range of aromatic/hydrophobic adducts, specifically PAH and to a lesser extent nitroaromatics and aromatic amines. There is recent evidence that DNA damage caused by oxygen free radicals may also be detected by this procedure (51, 52). In addition to providing data on aromatic/hydrophobic adducts, the postlabeling method can also document the presence of a DRZ in the two-dimensional thin layer chromatograms of the $^{32}$P-postlabeled DNA digests. Although the identities of the adducts have not been deter-

[4] M. Chorazy (Institute of Oncology, Gliwice, Poland), personal communication.

[5] D. Tang, R. M. Santella, A. Blackwood, T. L. Young, J. Mayer, A. Jaretzki, S. Grantham, D. Carbenry, K. M. Steinglass, W. Y. Tsai *et al.* A case control molecular epidemiologic study of lung cancer, accepted for publication.

*Table 1* Individual results of $^{32}$P-postlabeling analysis of DNA adducts in breast tissue from breast cancer patients and controls

| Subject | Smoking status | Tumor tissue | | Tumor adjacent/normal tissue | |
|---|---|---|---|---|---|
| | | Smoking-related adducts[a] | Total aromatic adducts/$10^8$ nucleotides[b] | Smoking-related adducts[a] | Total aromatic adducts/$10^8$ nucleotides |
| Cases | | | | | |
| 1 | NS[c] | – | 3.67 ± 0.76 | NA | NA |
| 2 | FS | – | 7.22 ± 1.28 | – | 1.86 ± 0.07 |
| 3 | NS | – | 5.62 ± 2.29 | – | 10.0 ± 1.37 |
| 4 | NS | – | 2.33 ± 0.25 | – | 1.88 ± 0.05 |
| 5 | CS | + | 1.58 ± 1.73 | + | 9.42 ± 1.24 |
| 6 | CS | + | 3.60 ± 0.54 | + | 6.91 ± 0.59 |
| 7 | FS | – | 1.68 ± 0.45 | NA | NA |
| 8 | CS | – | 5.68 ± 0.59 | NA | NA |
| 9 | FS | – | 5.95 ± 0.34 | – | 6.99 ± 1.17 |
| 10 | CS | + | 5.20 ± 4.09 | + | 5.76 ± 2.42 |
| 11 | CS | – | 8.32 ± 1.04 | NA | NA |
| 12 | CS | + | 5.13 ± 1.89 | – | 2.35 |
| 13 | CS | – | 3.68 | + | 7.43 |
| 14 | NS | – | 5.20 ± 1.51 | – | 8.32 |
| 15 | NS | – | 5.47 ± 0.36 | – | 6.50 |
| Total | | 4/15 | 4.7 ± 1.9 | 4/11 | 6.1 ± 2.9 |
| Controls | | | | | |
| 16 | NA | | | – | 1.76 ± 0.11 |
| 17 | NA | | | – | 4.41 ± 1.18 |
| 18 | NA | | | – | 2.18 ± 0.16 |
| 19 | NA | | | – | 0.91 ± 0.68 |
| Total | | | | 0/5 | 2.3 ± 1.5 |

[a] Detection of a diffusive diagonal zone of radioactivity (See Fig. 1). (+) Present, (−) Absent.
[b] Mean ± SD where multiple assays were run.
[c] NS, never smokers; NA, not available; CS, current smokers; FS, former smokers (all women who quit 5 yr ago).

*Table 2* Carcinogen-DNA adducts in tissue samples from breast cancer patients

| Subjects (*n*) | Tumor | | Tumor adjacent | | Tumor and tumor-adjacent combined | |
|---|---|---|---|---|---|---|
| | Smoking-related adducts[a] | Total aromatic adducts/$10^8$ nucleotides[b] | Smoking-related adducts[a] | Total aromatic adducts/$10^8$nucleotides[b] | Smoking-related adducts[a] | Total aromatic adducts/$10^8$ nucleotides[b] |
| Current smokers (*n* = 7) | 4/7 | 4.7 ± 2.1 | 4/5 | 6.4 ± 2.6 | 8/12 | 5.4 ± 2.4 |
| Nonsmokers (*n* = 8)[c] | 0/8 | 4.6 ± 1.9 | 0/6 | 5.9 ± 3.4 | 0/14 | 5.2 ± 2.6 |
| All (*n* = 15) | 4/15 | 4.7 ± 1.9 | 4/11 | 6.1 ± 2.9 | 8/26 | 5.3 ± 2.4 |

[a] Proportion of samples positive for DRZ characteristic of smoking exposure.
[b] Mean ± SD of all samples tested.
[c] Nonsmokers category includes 3 former smokers and 5 never smokers.

mined, the DRZ has been associated previously with exposure to cigarette smoke (44, 46, 47).

DNA was isolated by a solvent extraction procedure adapted for small tissue samples, as described by Schoket *et al.* (53). DNA samples (2–4 μg) were digested enzymatically to 3′ monophosphates with micrococcal nuclease and spleen phosphodiesterase. The digests were then treated with nuclease $P_1$ (54) and $^{32}$P labeled as described previously (55). Resolution of the labeled adducts was then carried out on polyethyleneimine-cellulose TLC sheets (56). Adducts were detected by autoradiography and quantitated by Cerenkov counting of the diagonal region of the chromatograms. Most samples (26 of 31) were analyzed two or three times on separate occasions and the means were used in analysis. The mean SD was 1.05. The remaining samples were analyzed once due to insufficient DNA. Because the efficiency with which uncharacterized adducts were labeled cannot be ascertained, it is possible that the adduct levels are underestimated. Therefore, the values reported should be regarded as minimum levels.

## Results

Table 1 provides results on the 19 subjects (31 specimens) studied. Aromatic adduct spots were detected in the chromatograms of all samples tested. Among the 26 tumor and tumor-adjacent specimens from the cases, adduct levels ranged from 1.6 to 10.0 adducts/$10^8$ nucleotides, with a mean of 4.7 ± 1.9 adducts/$10^8$ nucleotides in tumor tissue, 6.1 ± 2.9 adducts/$10^8$ nucleotides in tumor-adjacent tissue, and 5.3 ± 2.4 adducts/$10^8$ nucleotides in tumor and tumor-adjacent tissue combined. Among the 5 samples from the women without breast cancer aromatic adduct levels ranged from 0.43 to 4.41 adducts/$10^8$ nucleotides with a mean of 2.3 ± 1.5 adducts/$10^8$ nucleotides.

DNA samples from 5 of the 7 women (all cases) known to be current smokers (4 of 7 tumor and 4 of 5 tumor-adjacent samples) displayed the DRZ characteristic of smoking in contrast to 0 of 14 samples from the 8 nonsmokers ($P < 0.01$; Fisher's exact test, for the comparisons

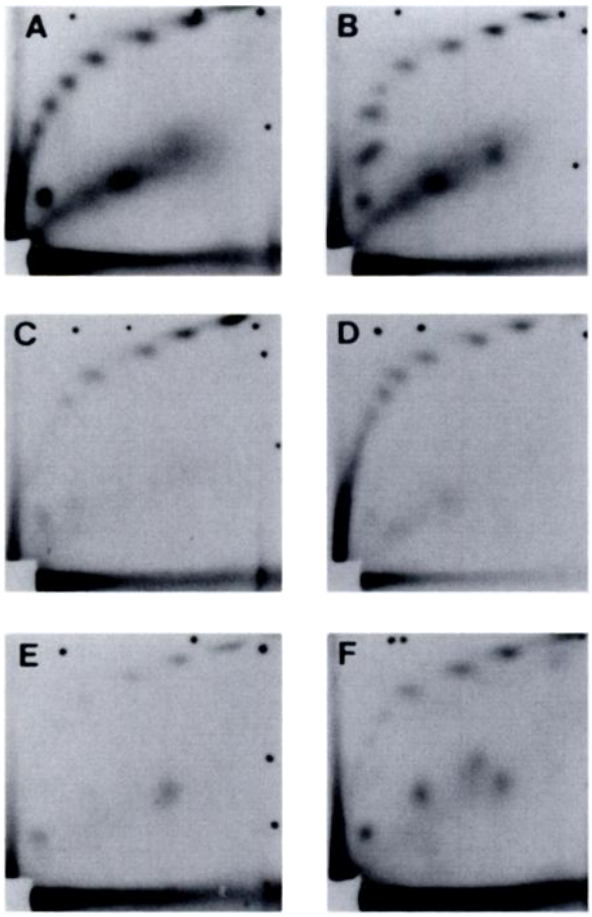


*Fig. 1.* Autorads obtained by exposing the TLC plates to film for 4 1/2 days at −75°C. *A*, smoker, tumor-adjacent tissue DNA; *B*, smoker, tumor-adjacent tissue DNA; *C*, non-smoker, tumor-adjacent tissue DNA; *D*, non-smoker, tumor-adjacent tissue DNA; *E*, smoking status unknown, control tissue from mammoplasty patient; and *F*, smoking status unknown, control tissue from mammoplasty patient.

between the number of positive cases and the number of positive samples; see Fig. 1). None of the 5 samples from the breast reduction mammoplasty patients was positive; however, smoking history was not known for these women.

## Discussion

In this pilot study we report that carcinogen-DNA adducts associated previously in other tissues with exposure to complex mixtures of PAH and with tobacco smoke have been detected in human breast tissue. The mean aromatic adduct value for cases (tumor and/or tumor-adjacent tissue) was 2.5-fold higher than that for the control samples (6.1 versus 2.3 nucleotides/$10^8$). The values for aromatic adducts were in the lower end of the range seen in lung tissue of smokers and nonsmokers but did not differ according to smoking status of the women.

The DRZ associated previously with smoking was detected only in tissue from known smokers, all of whom were breast cancer cases, and not in nonsmoking cases. A limitation of the study is that smoking status was not available for the controls; however, none of their tissues displayed the DRZ. The differing results for aromatic adducts and DRZ are consistent with the aromatic fraction being comprised of adducts related to multiple sources (diet, ambient air, and smoking), whereas the DRZ is empirically more directly related to smoking.

Although the difference between smokers and nonsmokers in terms of the DRZ was statistically significant ($P < 0.01$), the small number of subjects and the fact that the non-breast cancer patients are not comparable in terms of age (and perhaps other characteristics) to the cases preclude any inference as to causality. However, the results demonstrate the formation of adducts in the female breast that have been associated previously with human exposure to pervasive environmental carcinogens (including PAH and tobacco smoke). This finding is biologically plausible since aromatic compounds are highly lipophilic and can thus concentrate in the breast adipose tissue, resulting in exposure of the adjacent epithelial cells from which breast tumors arise. Because carcinogen-DNA adducts may act at both an early and a late stage in the carcinogenic process, their presence in breast tissue suggests a possible multifactorial role of these pollutants in breast cancer causation. Further studies are needed to test this hypothesis. It is perhaps relevant that the *p53* tumor suppressor gene is mutated in 35–50% of breast tumors (57–60) and that G to T transversions, a type of point mutation induced in various systems by a number of compounds including PAHs, heterocyclic amines, and cigarette smoke components (7, 61), account for about 20% of these mutations (7, 58). We are pursuing the role of these environmental agents in the etiology of breast cancer in further molecular epidemiological studies, which will analyze adducts in both peripheral leukocytes and in breast tissue.

Although limited, our results from this pilot study indicate that biomarkers such as DNA adducts may be useful in identifying specific environmental exposures that may contribute to breast cancer causation as well as the populations and individuals most affected. They may therefore be helpful in suggesting new strategies for prevention of breast cancer.

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