DNA adducts of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 4-aminobiphenyl are infrequently detected in human mammary tissue by liquid chromatography/tandem mass spectrometry

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Introduction

Breast cancer is the most common cancer in women and the leading cause of cancer death among women 35–54 years of age in the USA, representing 27% of all cancers estimated to occur in this population in 2009 (1). Breast cancer rates also are rising in Asian–American women, at a rate that is four times higher than their USA white counterparts (2). Proposed risk factors for breast cancer are numerous and include reproductive characteristics associated with estrogen and other hormones, alcohol consumption and dietary fat intake (3). Environmental and dietary genotoxicants are also believed to be critical factors in women’s breast cancer risk. However, the Long Island Breast Cancer Study failed to firmly establish a connection between exposure to environmental and dietary pollutants such as pesticides, polychlorinated biphenyls or polycyclic aromatic hydrocarbons and breast cancer (3), and the role of other chemicals in the etiology of breast cancer requires study.

There are many compounds in the environment and diet that induce mammary tumors in experimental laboratory animals; some of these chemicals may contribute to the etiology of human breast cancer (4,5). Heterocyclic aromatic amines (HAAs) and aromatic amines are structurally related classes of chemicals that are formed in cooked meats (5) or arise in tobacco smoke (6,7). The principal source of exposure to most HAAs is from consumption of high-temperature cooked meats (5). 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most abundant carcinogenic HAA formed in well-done cooked meats: the concentrations of PhIP can range from less than 1 part per billion up to several hundred part per billion (8,9). PhIP induces tumors of the mammary gland in rodents (5). 4-Aminobiphenyl (4-ABP) is a prototypical aromatic amine: it is a constituent of tobacco smoke (7), occurs in some hair dyes (10) and also present in the atmosphere (11). 4-ABP is a well-established bladder and mammary carcinogen in rodents and a recognized human bladder carcinogen (7).

The roles of HAAs and aromatic amines in the development of cancer have been an area of extensive research. Some epidemiologic studies have reported that women who frequently consume well-done cooked meats containing PhIP are at an increased risk for breast cancer (reviewed in ref. 12). However, other epidemiological studies have not found an association between meat consumption and breast cancer risk (13–16), and the causal role of PhIP or other HAAs in breast cancer is unresolved (17). The contribution of genotoxicants in tobacco to the development of breast cancer is also unclear. The bulk of epidemiologic studies do not support an association between smoking and breast cancer risk (reviewed in ref. 18); however, this association was recently reported to be influenced by genotypes for N-acetyltransferase 2 and women with slow N-acetylator status were at an elevated risk for breast cancer (19). The decreased capacity of women with a slow N-acetyltransferase 2 status to detoxicate aromatic amines present in tobacco smoke, such as 4-ABP (7), may offer a biochemical mechanism for the elevated risk of breast cancer for women who smoke. On the basis of these epidemiological findings (7,19), the California Environmental Protection Agency in 2005 concluded that the evidence regarding secondhand smoke and breast cancer is ‘consistent with a causal association’ in younger women.

The supposition of dietary or environmental genotoxicant as a risk factor for human breast cancer would be greatly strengthened by the identification of specific biomarkers of exposure-induced DNA damage in breast tissue. Mutagens have been detected in human milk: a significant portion of the mutagenic activity is thought to be derived from aromatic amines (20–23). Higher levels of aromatic DNA adducts were detected in breast tissue samples of cancer patients than in samples from healthy subjects, when assayed by 32P-post-labeling (24,25). Some of these adducts may be derived from polycyclic aromatic hydrocarbons or aromatic amines present in tobacco smoke. More recently, putative DNA adducts of PhIP and 4-ABP were identified, by the 32P-post-labeling method, in exfoliated epithelial cells in milk of lactating mothers (26). Thirty samples from the 64 subjects contained presumed DNA adducts of PhIP and 18 subjects were positive for 4-ABP-DNA adducts. In an ensuing study, PhIP-DNA adducts were detected, by immunohistochemistry (IHC), in mammary tissue of 82% of the subjects with breast cancer (N = 106) and also found in 71% of the tissue samples of healthy control patients.
(N = 49) (27). 4-ABP-DNA adducts were also detected at high frequency in human mammary tissue of smokers and non-smokers by IHC, and the smoking status was correlated with the levels of 4-ABP-DNA in non-tumor-adjacent normal tissue (28).

The frequent detection of PhIP- and 4-ABP-DNA adducts in mammmary tissue at elevated levels strengthens the biological plausibility of PhIP and 4-ABP as causal agents of breast cancer and reinforces the paradigms of well-done meat consumption and tobacco smoking as lifestyle risk factors for breast cancer. These findings are an important public health issue; however, the biomarker data are controversial because both IHC and 32P-post-labeling are non-specific screening methods and fail to provide spectral data to corroborate the identity of the lesion. Furthermore, there is a plethora of genotoxins, and the potential to incorrectly characterize DNA adducts, by IHC and 32P-post-labeling screening methods, is large. Imprecise biomarker data could lead us astray in identifying the genotoxins involved in the pathogenesis of human breast cancer.

To our knowledge, liquid chromatography/tandem mass spectrometry, a specific and quantitative assay, has not been employed to measure PhIP or 4-ABP-DNA adducts in human mammary tissue. In the present study, we employed a validated, highly sensitive and specific liquid chromatography-electrospray ionization multistage mass spectrometry (LC-ESI/MS/MS) technique (LC-ESI/MS/MS9 technique (29,30)) to measure DNA adducts of PhIP and 4-ABP in tumor-adjacent normal tissue of 70 newly diagnosed breast cancer patients from Minneapolis, St Paul, MN.

Materials and methods

Study subjects and tissue samples
Tumor-adjacent normal breast tissue biopsy samples were provided by the Masonic Cancer Center, University of Minnesota Tissue Procurement Facility, Minneapolis, MN. Ninety-nine samples were collected from consecutive cases (as feasible as possible) so that there was no bias in sample selection. The specimens were from women with newly diagnosed breast cancer. We established 20 μg of DNA as the minimum amount required for quantification at three adducts per 10⁹ nucleotides (vide infra). DNA (>20 μg) was recovered from 70 of the specimens. The age of these 70 subjects ranged from 31 to 79 years and the median was 50 years. Specimens were from patients with no documented prior neoadjuvant or radiation therapy. Race–ethnicity was recorded on the charts for 68 of these subjects: 96% were non-Hispanic whites (65 subjects), 1 subject was African American and 2 subjects were Asian. There was no systematic inquiry on the smoking status of these patients. Nonetheless, 54 of the medical charts noted some aspects of the smoking status of the respective patients: 37 were never smokers, 13 were former smokers and 4 were current smokers. The usage of these human biospecimens was approved by the Institutional Review Boards at the Masonic Cancer Center, University of Minnesota and Wadsworth Center, New York State Department of Health.

Synthesis of dG-C8-PhIP and dG-C8-4-ABP DNA adducts and PhIP- and 4-ABP-modified calf thymus DNA
N-(deoxyguanosin-8-yl)-PhIP (6G-C8-PhIP) and N-(deoxyguanosin-8-yl)-4-ABP (6G-C8-4-ABP) and their isotopically labeled [13C10]-dG-C8-PhIP and [13C10]-dG-C8-4-ABP adducts were added to hepatocyte DNA (2–10 μM) was added to hepatocytes by chloroform/phenol extraction (33). The internal standards [13C10]-dG-C8-PhIP and [13C10]-dG-C8-4-ABP were added to hepatocyte DNA at a level of five adducts per 10⁶ nucleotides and added to hepatocyte DNA (2–10 μg) at a level of one adduct per 10⁶ nucleotides, prior to enzymatic digestion. The enzymatic hydrolysis of DNA was performed with DNase I, nuclease P1, alkaline phosphatase and phosphodiesterase, under conditions shown to be highly efficient in the recovery of the dG-C8 adducts of PhIP and 4-ABP (29).

Assessment of the efficacy of enzymatic digestion of DNA
The DNA hydrolysate (2 μg) was assayed with an Agilent 1100 high-performance liquid chromatography system (Palo Alto, CA), monitoring ultra violet absorbance at 260 nm. A Phenomenex Synergi 4u Fusion-RP 80A (150 × 4.6 mm) (Torrance, CA) was employed for chromatography of the deoxynucleosides with a linear gradient starting at 95% 20 mM ammonium acetate (pH 4.5) and 5% acetonitrile and reaching 40% acetonitrile at 20 min. The flow rate was 1 ml/min.

Measurements of DNA adducts
Assessments of DNA adducts were performed with a NanoAcquity™ UPLC system (Waters Corporation, Milford, MA) interfaced with a linear quadrupole ion trap mass spectrometer (LTQ MS; Thermo Fisher, San Jose, CA). A Waters Symmetry trap column (180 μm × 20 mm, 5 μm particle size) was employed for online solid phase enrichment of the DNA adducts. The analytical column was a C18 AQ (3.0 × 150 mm, 3 μm particle size) from Michrom Bioresources, Auburn, CA. The DNA digestions were injected onto the trap column and washed with 0.2% formic acid in 10% acetonitrile at a flow rate of 12 μl/min for 5 min. Thereafter, the DNA adducts were back-flushed onto the C18 AQ column. A linear gradient was employed to resolve the DNA adducts, starting at 0.01% formic acid containing 10% acetonitrile and arriving at 0.01% formic acid in 95% acetonitrile at 20 min. The flow rate was set at 5 μl/min. Adducts were measured at the MS1 scan stage in the positive ionization mode with an Advance CaptiveSpray™ source from Michrom Bioresource. The mass spectral parameters were optimized as previously reported (29).

Calibration curves
Calibration curves were constructed with [13C10]-dG-C8-PhIP and [13C10]-dG-C8-4-ABP set at 50 adducts per 10⁶ deoxynucleotides (3.7 pg adduct) with unlabeled DNA adducts added at a level of 0, 1–100 adducts per 10⁹ nucleotides (0, 0.074–74 pg adduct) in calf thymus DNA digest (50 μg). The calibration curves were done in triplicate at each level, and the data were fitted to a straight line (area of response of the adduct/internal standard versus the level of the adduct per 10⁹ nucleotides) using ordinary least squares with equal weightings. The coefficient of determination (r²) values of the slopes exceeded 0.998.}

Results

Performance of the analytical method to measure PhIP- and 4-ABP-DNA adducts
PhIP and 4-ABP undergo metabolic activation by cytochrome P450s or peroxidases to produce electrophiles that react with DNA (29,32). The structures of the carcinogens and their principal DNA adducts are shown in Figure 1. The performance of the analytical method is summarized in Table I. The intraday and interday precisions and the estimates of the limit of detection and limit of quantification (LOQ), defined as the signal to noise ratio >3 and 10, respectively, were conducted with calf thymus DNA modified with [3H]-PhIP and [3H]-4-ABP that contained known levels of dG-C8-PhIP (31) and dG-C8-4-ABP (32). The level of PhIP-DNA modification was established at 0, 3, 10 or 50 adducts per 10⁹ DNA bases, and the level of 4-ABP-DNA modification was established at 0 or 19 adducts per 10⁹ DNA bases, by dilution of the carcinogen-modified DNA with untreated calf thymus DNA. The DNA was digested with the cocktail of enzymes and assay by LC-ESI/MS/MS. The calibration curves for dG-C8-PhIP and dG-C8-4-ABP are presented in Supplementary Figure S1A and B (available at Carcinogenesis Online). The LOQ values for both adducts was less than three adducts per 10⁹ nucleotides, when 13 μg of DNA digest was assayed by MS. The levels of dG-C8-PhIP were within 14% of the target values for all levels of spiking, and the measured level of dG-C8-4-ABP was within 12% of the target value. These LOQ values for dG-C8-PhIP and dG-C8-4-ABP are ~100-fold lower than the LOQ values reported for IHC (27,28,34,35).

A DNA matrix from adjacent non-tumor mammary tissue of women newly diagnosed with breast cancer (these subjects did not have detectable levels of adducts, vide infra, less than one to two adducts per 10⁶ bases) was employed to further validate the analytical
method. Calf thymus DNA modified with [3H]-PhIP and [3H]-4-ABP was diluted by 10- to 330-fold with DNA isolated from mammary tissue, followed by enzymatic digestion of DNA and analysis by LC-ESI/MS/MS. The estimated levels of DNA adducts were in good agreement to the target values (Table II). Similar levels of accuracy and precision were obtained with two other breast tissue DNA samples spiked with PhIP- and 4-ABP-modified calf thymus DNA (data not shown). Thus, constituents that may have been co-purified with human mammary DNA samples did not impair the enzymatic hydrolysis of DNA or the subsequent analysis of PhIP- or 4-ABP-DNA adducts.

PhIP- and 4-ABP-DNA adduct formation in human hepatocytes
PhIP- and 4-ABP-DNA adduct formation were measured in human hepatocytes incubated with the procarcinogens (10 μM) for 8 h (33). Both dG-C8-PhIP and dG-C8-4-ABP were easily identified and quantitated, by LC-ESI/MS/MS, with as little as 0.4 μg of DNA assayed by MS. The LC-ESI/MS/MS traces of PhIP- and 4-ABP-DNA adducts from untreated hepatocytes and hepatocytes treated with carcinogens are shown in Figure 2, along with the product ion spectra of the aglycone adducts $\left[\text{BH}_2\right]^+\text{,}$ which provide rich structural information about the structures of the adducts and corroborate their identities (29).

The level of dG-C8-PhIP was estimated at 1.3 ± 0.1 adducts per 10$^6$ nucleotides and the level of dG-C8-4-ABP was estimated at 5.0 ± 0.3 adducts per 10$^6$ nucleotides (mean ± SD, $N = 3$).

PhIP- and 4-ABP-DNA adduct formation in breast tissue
Adjacent non-tumor mammary tissue biopsy samples from 70 subjects were screened for dG-C8-PhIP and dG-C8-4-ABP adducts. Only one biospecimen contained the dG-C8-PhIP adduct at a level above the LOQ value: the adduct was estimated at three adducts per 10$^9$ nucleotides. The reconstructed ion chromatograms of dG-C8-PhIP, depicted in Figure 3A, were from a subject who did not harbor DNA adducts of either carcinogen. The reconstructed ion chromatograms of the biopsy sample that were positive for dG-C8-PhIP are shown in Figure 3B. The identity of dG-C8-PhIP was confirmed by its product ion spectrum at the MS$^3$ scan stage, which was in excellent agreement to the spectrum of the synthetic dG-C8-PhIP.
standard (Figure 3C). dG-C8-4-ABP was not detected in any of the biopsy samples assayed. The efficacy of DNA hydrolysis by the cocktail of enzymes was confirmed by high-performance liquid chromatography of the non-modified nucleosides: the digestion was complete for all DNA samples (Supplementary Figure S2 is available at Carcinogenesis Online).

Discussion

The findings of some epidemiological studies (reviewed in ref. 12) have revealed that frequent consumption of well-done cooked meat, a surrogate for exposure to PhIP (8,9), increases the risk of developing breast cancer in women. A case–control study in Uruguay showed a strong relationship between grilled meat consumption and breast cancer risk (36). A nested case–control study among 41 836 cohort members of the Iowa Women's Health Study reported a statistically significant dose–response relationship between doneness levels of meat consumed and increased risk of breast cancer (37). On the basis of these epidemiological studies and the laboratory research conducted on mammary carcinogenesis of PhIP, including metabolism and DNA adduct formation in experimental animals (5,38), a paradigm has been put forth for a causal role of consumption of well-done cooked meats containing PhIP in the etiology of human breast cancer. However, other epidemiological investigations have failed to find a correlation between frequent meat consumption and breast cancer risk (13–16), although higher red meat intake during adolescence may increase the risk of premenopausal breast cancer (39). The findings on the effect of tobacco smoking and breast cancer risk have also been inconsistent. Much of the epidemiological data do not support a role of tobacco smoking in breast cancer risk (18), although recent data do suggest that slow N-acetyltransferase 2 acetylator status augments the risk of breast cancer for women who smoke (19).

The identification of carcinogen-DNA adducts in mammary tissue may help us to identify specific environmental or dietary genotoxicants as risk factors for breast cancer. The analyses of DNA adducts are usually conducted on biopsy samples of patients that are obtained during clinical diagnosis of cancer (24,25). The DNA adducts detected in these clinical samples are likely due to recent exposures, whereas the presence of DNA adducts should be more relevant when measured earlier in time, when the multistage process of malignant tumor formation and progression has begun, and not many years later when the cancer has been diagnosed. Hence, the assumption made here is that current adduct levels are correlated with levels that existed during the extended time period of tumor formation and progression. Tobacco smoking and diet are mostly long-term habitual exposures; thus, this assumption is likely to be valid in most subjects. The high prevalence of PhIP- and 4-ABP-DNA adducts detected by IHC in mammary biopsy samples (27,28) and by $^{32}$P-post-labeling in human milk (26) would appear to reinforce the supposition of a causal role of PhIP and 4-ABP in the pathogenesis of breast cancer.

IHC and $^{32}$P-post-labeling DNA adduct screening methods have strengths and limitations. The major advantage of the IHC method

Fig. 2. LC-ESI/MS/MS3 ion chromatograms of dG-C8-PhIP and dG-4-ABP adducts formed during incubation of human hepatocytes with the procarcinogens: (A) untreated hepatocytes, (B) hepatocytes treated with procarcinogens (10 μM) for 8 h and (C) Product ion spectra at the MS3 scan stage of dG-C8-PhIP, dG-C8-4-ABP and the $[^{13}C_{10}]$-dG-C8-PhIP and $[^{13}C_{10}]$-dG-C8-4-ABP internal standards. The internal standards were added prior to digestion of DNA at a level of one adduct per 10^6 nucleotides.
ABP, respectively (34,35); however, the specificities of the antibodies and their potential to cross-react with DNA adducts of structurally related carcinogens were not comprehensively characterized for either PhIP- or 4-ABP-raised antibodies (34,35). As a result, the identities of the DNA adducts detected in human tissues by IHC are uncertain. The primary advantage of the \(^{32}\)P-post-labeing method over other screening methods is the intrinsic sensitivity of the assay (41); however, this technique also fails to provide spectral data to corroborate the identity of the lesion. In contrast to IHC and \(^{32}\)P-post-labeling screening methods, LC-ESI/MS/MS\(^n\) provides both structural information on the adducts and quantitative measurements (29). The spectral data acquired by the linear ion trap MS instrument allow for complete mass spectral characterization and near unequivocical identification of the DNA adduct (29).

Results of the present study are fundamentally at odds with published results derived from the IHC or \(^{32}\)P-post-labeling assays. In the Little Rock, Arkansas study (26), 30/64 milk samples from lactating women were positive for dG-C8-PhIP adduct detected by \(^{32}\)P-post-labeling. In the Houston, Texas study (27), 82% of the paraffin-embedded sections obtained from tumor-adjacent normal breast tissue of cancer patients were positive for dG-C8-PhIP by IHC. Notably, the rate of positivity of DNA adducts detected by IHC or 32P-post-labeling analysis (42). There was a statistically significant correlation between dietary intake of HAA and level of DNA adducts in breast tissue among the study women. But significantly, via comparison with external standards, the authors failed to identify any of the \(^{32}\)P-post-labeling-detected DNA adducts as a HAA-derived adduct. Thus, our results are in complete agreement with the German findings. Given the positive association between dietary HAA and the as yet unknown DNA adducts detected by the \(^{32}\)P-post-labeling analysis, one can speculate that these adducts were formed either from non-HAA chemicals present in fried meats or from other lifestyle-related exposures that are positively associated with consumption of fried meats.

Apparent DNA adducts of 4-ABP were detected by IHC at very high frequency (100%) in 55 adjacent non-tumor tissues of breast cancer cases from women participating in the Long Island Breast Cancer Project (28). 4-ABP adducts were also frequently detected (28%) by \(^{32}\)P-post-labeling in human milk samples from women residing in Little Rock, AR (26). In contrast, our subjects harbored no adducts of 4-ABP, when assayed by LC-ESI/MS/MS\(^n\). Our observed rate of 0/70 is statistically significantly different from the rate of 28% (18/64) and 100% (55/55) noted in the two published studies, respectively. The upper 95% confidence limit of our observed rate is <4%. It is of note that the cases in the present study are comparable in age and ethnicity distributions to those in the Houston (27) and Long Island studies (28,43). Our subjects were 96% non-Hispanic whites with a median age of 50 years. In the Houston study, 78% of the subjects were non-Hispanic whites with a median age of 43 years (27) and 94% of the subjects in the Long Island Breast Cancer Project were non-Hispanic whites with a median age of 59 years (28,43). Age and ethnicity were not reported in the Little Rock study (26).

What would be the possible reasons for the inconsistent findings of our study from those of the three studies described above? Although the actual exposures to PhIP and 4-ABP were unknown for women in these four different localities, given the recognized relative uniformity and ubiquitous presence of PhIP in the USA diet (9,44), the ubiquitous over other screening methods is its ability to detect DNA adducts in specific cell types within a tissue and its applicability to screen paraffin-embedded tissue sections (40). The major disadvantage of the IHC method is that the specificity of the antibodies, even monoclonal antibodies, is uncertain. The antibodies can potentially cross-react with a number of DNA lesions, leading to errors in the identification and quantification of DNA adducts. The IHC studies employed polyclonal or monoclonal antibodies raised against DNA modified with PhIP or 4-ABP, respectively (34,35); however, the specificities of the antibodies and their potential to cross-react with DNA adducts of structurally related carcinogens were not comprehensively characterized for either PhIP- or 4-ABP-raised antibodies (34,35). As a result, the identities of the DNA adducts detected in human tissues by IHC are uncertain. The primary advantage of the \(^{32}\)P-post-labeing method over other screening methods is the intrinsic sensitivity of the assay (41); however, this technique also fails to provide spectral data to corroborate the identity of the lesion. In contrast to IHC and \(^{32}\)P-post-labeling screening methods, LC-ESI/MS/MS\(^n\) provides both structural information on the adducts and quantitative measurements (29). The spectral data acquired by the linear ion trap MS instrument allow for complete mass spectral characterization and near unequivocical identification of the DNA adduct (29).

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adducts were measured in breast tissue of female cancer patients and tumor surrounding DNA. In one human pilot study, PhIP-DNA dG-C8-4-ABP and 12 of the 27 (44%) bladder cancer patients had dG-C8-4-ABP was detected in pancreas and bladder tissues and 4-ABP by LC-ESI/MS/MS at variable levels in human tissues. The potential to misidentify DNA adducts is large and could be one of the reasons for the high detection rates of putative PhIP- and 4-ABP-DNA adducts in the mammary tissue, suggesting that exposure to 4-ABP does not necessarily result in the formation of dG-C8-4-ABP DNA adducts in the mammary tissue. Due to the poor selectivity of the IHC or \(^{32}\)P-post-labeling methods, the potential to misidentify DNA adducts is large and could be one of the reasons for the high detection rates of putative PhIP- and 4-ABP-DNA adducts in previous studies.

There are several reports on the detection of DNA adducts of PhIP and 4-ABP by LC-ESI/MS/MS at variable levels in human tissues. We previously identified dG-C8-PhIP and LC-ESI/MS/MS in salivary DNA of healthy subjects on unrestricted diets. (29) Fifteen of 37 subjects harbored dG-C8-PhIP at levels ranging from 1.8 to 9.7 adducts per 10\(^6\) nucleotides; the median value was 2.7 adducts per 10\(^8\) nucleotides. dG-C8-4-ABP was detected in pancreas and bladder tissues of cancer patients (49). Six of the 12 (50%) pancreas tissues contained dG-C8-4-ABP and 12 of the 27 (44%) bladder cancer patients had quantifiable levels of dG-C8-4-ABP adducts in either tumor or non-tumor surrounding DNA. In one human pilot study, PhIP-DNA adducts were measured in breast tissue of female cancer patients administered with \(^{[1]}\)C-PhIP (20 µg/70 kg body wt) by accelerator mass spectrometry (MS) (50). The estimates of PhIP-DNA adduct formation ranged from 26 to 480 adducts per 10\(^{12}\) nucleotides; these levels of PhIP adducts are ~1000- and 12 000-fold lower than the levels of PhIP-DNA adducts reported in human mammary tissue by IHC or \(^{32}\)P-post-labeling methods.

The data obtained by accelerator MS suggest that the IHC and \(^{32}\)P-post-labeling methods significantly overestimate the levels of PhIP-DNA adducts in breast tissue.

In summary, the findings of our study on specific identification of PhIP- and 4-ABP-DNA adducts using an LC-ESI/MS/MS method differ from those of the previous reports on the ubiquitous presence of PhIP- and 4-ABP-DNA adducts detected by non-selective IHC and \(^{32}\)P-post-labeling screening methods. Our findings call for further studies where DNA adduct levels detected by bioanalytical and MS methods are compared directly for the same tissue samples. We expect that such direct comparisons will further support the necessity to employ specific MS methods to measure DNA adducts in human populations and more clearly determine the potential role of PhIP and 4-ABP as causative agents in the etiology of human breast cancer. The lack of detectable levels of dG-C8-PhIP and dG-C8-4-ABP adducts in the mammary tissues of breast cancer patients in the present study, if confirmed, suggests that PhIP and 4-ABP may play a minor role or a different mechanism from the dG-C8 pathway involved in mammary carcinogenesis in humans.

Supplementary material

Supplementary Figures S1A and S1B and S2A and S2B can be found at http://carcin.oxfordjournals.org/

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