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Analysis of the Abasic Sites in Breast Cancer Patients With 5 Year Postoperative Treatment Without Recurrence in Taiwan

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Abstract

Purpose: This prospective study aimed to investigate estrogen-induced carcinogenesis by assessing the background levels of abasic sites (apurinic/apyrimidinic sites, AP sites) in Taiwanese breast cancer patients following 5 years of postoperative treatment without recurrence (5-year survivors) (n = 70). The study also sought to compare the extent of these DNA lesions with those found in healthy controls and in breast cancer patients prior to treatment.

Methods: Abasic sites were measured using an aldehyde reactive probe and quantified as the total number of abasic sites per total nucleotides. Characterization of the abasic sites in subjects recruited for this study was conducted using the abasic site cleavage assay using putrescine or T7 exonuclease (T7 Exo) and/or exonuclease III (Exo III).

Results: The number of abasic sites detected in 5 year survivors (26.7 ± 10.2 per 10^6 total nucleotides, n = 70) was significantly reduced by 46.9% compared to those in breast cancer patients before treatment (50.3 ± 59.2 per 10^6 total nucleotides, $P < 0.001$), and was similar to the levels observed in healthy controls (23.3 ± 13.5 per 10^6 total nucleotides, $P > 0.05$). Further investigation into the specific types of abasic sites indicated that the number of abasic sites excisable by putrescine in controls, breast cancer patients, and 5-year survivors were 63.3%, 78.6%, and 67.7%, respectively. These findings suggest the involvement of oxidative stress rather than depurination/depyrimidination of DNA adducts in the formation of abasic sites. Further analyses were performed using exonuclease cleavage assay to characterize the specific types of abasic sites including 5′-cleaved, 3′-cleaved, intact, and residual abasic sites. Results demonstrated that the proportion of residual abasic sites detected in controls, breast cancer patients, and 5-year survivors were estimated to be 32.7%, 48.8%, and 34.0%, respectively.

Conclusion: Overall, these findings suggest clear evidence of treatment-related effects on the reduction of levels of abasic sites as well as on the profile of abasic sites in 5 year survivors.

Plain language summary

Purpose: This study aimed to analyze the impact of estrogen on cancer development by measuring the baseline levels of abasic sitesâ€"specific types of DNA damageâ€"in Taiwanese breast cancer patients who have survived for five years post-treatment

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without recurrence. The study compared these levels with those in healthy individuals and breast cancer patients prior to treatment.

Methods: The study measured abasic sites using an aldehyde reactive probe, assessing them per total nucleotides. The nature of these sites was further analyzed using assays that detect specific cleavages by putrescine, T7 exonuclease, or exonuclease III.

Results: The findings showed a significant reduction (approximately 47%) in abasic sites in patients five years post-treatment compared to those observed before treatment. The levels in long-term survivors were similar to those in healthy controls. Most of the abasic sites in the study were linked to oxidative stress rather than the breakdown of DNA itself. Additional detailed analysis of these sites was performed, identifying different types of abasic damages.

Conclusion: The study provides strong evidence that treatment significantly reduces the levels of abasic sites and alters their composition, which may be an important factor in the lack of recurrence in these patients.

Keywords
abasic sites, biomarker, DNA repair, DNA damage, oxidative stress

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Introduction

Apurinic/apyrimidinic (abasic/AP) sites are some of the most prevalent endogenous DNA lesions, arising from the loss of modified bases caused by chemical depurination/depyrimidination and oxidative stress.[1] Alkylating agents create a wide range of DNA adducts, leading to the formation of AP sites through spontaneous and/or enzymatic depurination/depyrimidination. Reactive oxygen species (ROS) can be generated as by-products of cellular respiration and in response to chemical exposures. Reactive oxygen species (ROS) can induce various oxidative lesions on the DNA molecule, including oxidized bases, DNA strand breaks, and AP sites.[2] Specifically, ROS induce AP sites by abstracting hydrogen from the deoxyribose moiety of DNA, where AP sites are formed after the excision of oxidized and alkylated bases by DNA glycosylases.[3] Without proper repair, AP sites could result in DNA replication blockade as well as mutation.[4-6] Some specific types of AP sites may lead to the formation of DNA-protein crosslink damage.[7-9]

Breast cancer is 1 of the leading causes of cancer death in Taiwan.[10] Genetic predisposition, personal habit, diets, and environmental exposure to carcinogens, may increase the risk of developing breast cancer. Elevation of estrogen quinone-derived protein adducts has been demonstrated in breast cancer patients when compared to those of healthy controls.[11,12] The formation of estrogen quinone-derived DNA adducts and the subsequent generation of intact AP sites have been linked to estrogen-induced carcinogenesis.[13] Genetic polymorphisms in DNA repair genes, such as PARP-1 and Apurinic/apyrimidinic endonuclease (APE1), may cause imbalances in DNA repair, leading to an increased risk of breast cancer.[14-16] Data on the direct assay of abasic sites in human leukocytes and its association with breast cancer risk have been reported in breast cancer patients[17] AP site levels in breast cancer patients were significantly increased, by 2.5-fold, compared to those in healthy controls. APE1 Asp148Glu (rs3136820) polymorphisms is associated with accumulation of AP sites in breast cancer patients[17] as well as risk of breast cancer.[18,19] In addition, some of clinical markers have been developed for the detection and evaluation of the prognosis of breast cancer including breast cancer imaging.[20]

In this study, we hypothesized that treatment–related effects contribute to the reduction of total number and the modulation of specific types of AP sites in 5-year survivors. We tested this hypothesis in a hospital-based study involving breast cancer patients with 5 years of postoperative treatment without recurrence (5-year survivors). AP sites were measured using an aldehyde reactive probe and quantified as the total number of AP sites per total nucleotides. Characterization of the AP sites in subjects recruited for this study was conducted by the AP sites cleavage assay using putrescine or T7 exonuclease (T7 Exo) and/or exonuclease III (Exo III) as described.[16,21]

Materials and Methods

Chemicals

Exo III and T7 Exo were purchased from Biolabs Inc (Ipswich, MA01938, USA). 2,2,6,6-Tetramethylpiperidin-1-yloxy, calf-thymus DNA, hydrogen peroxide solution, methyl methanesulfonate, putrescine and ribonuclease A were obtained from Sigma-Aldrich Inc (St. Louis, MO63178, USA). Aldehyde Reactive Probe (ARP) was purchased from Dojindo Inc (Rockville, MD20850, USA). Streptavidin-conjugated horseradish peroxidase was obtained from BioGenix software development Inc (Montreal, QCH1Y 3L1, Canada).

Subjects' Characteristics

Breast cancer patients with 5 years of postoperative treatment without recurrence (5-year survivors) (n = 78) were recruited

from a medical center in central Taiwan between June 2016 and December 2022. Eligibility criteria (Inclusion criteria): Participants were adult females aged 20 years and older. The sample size was determined through a website calculation based on a Type I error rate of 0.05, a power of 0.7, a control ratio, and varying P0/P1 estimates. Exclusion criteria: Participants who were smokers, heavy drinkers, victims of alcoholism, or had other female cancers such as uterine endometrial or ovarian cancers were excluded. Healthy control participants, breast cancer patients with less than 1 year of diagnosis/preoperative treatment (BCP), and breast cancer patients with more than 5 years of postoperative treatment without recurrence (5-year survivors) were recruited from a medical center and hospitals in central Taiwan between May 2009 and June 2012 (n = 78). The participants provided venous blood samples for AP site analyses and completed questionnaires regarding age, occupation, medical history, smoking habits, alcohol consumption, and other related factors.

We have providing median and range for participant ages to add clarity to the demographic description. The baseline data included demographic characteristics (eg, age), tumor characteristics (eg, tumor size, positivity of lymph node, metastasis, grade, pathologic stage, ER/PgR/HER2, information and histology). Patient with ductal carcinoma in situ only were excluded. Due to lack of availability of data, we excluded 8 patients from the 5-year survivors (n = 78) in Figure 1.

The study protocol was reviewed and approved by the Changhua Christian Hospital, Taiwan (CCH IRB No. 150802). Informed consent was obtained from all patients prior to blood collection. Blood samples were collected from the patients, and study subjects were enrolled retrospectively.

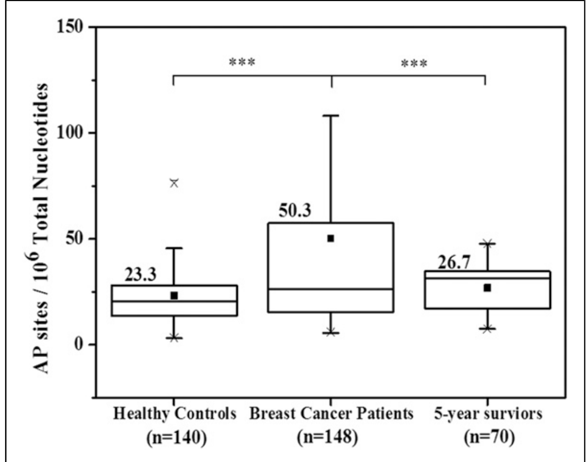

Figure 1. The background levels of abasic sites in leukocytes were measured in healthy controls, breast cancer patients, and 5-year survivors. (*), (**), and (***) indicate statistically significant differences compared to healthy controls at $P < 0.05$, $P < 0.005$, and $P < 0.001$, respectively. Note, data from healthy controls and breast cancer patients has been published.

Out of those recruited, seventy-eight 5-year survivors were ultimately enrolled in the study. Each participant provided informed consent after receiving a thorough explanation of the study and its potential implications. All participants completed questionnaires covering age, occupation, medical history, smoking habits, alcohol consumption, and other relevant factors. The process of collecting medical records involves reviewing a patient's medical history to understand its history and relevance to the research. Proposed items to be collected from medical records include: 1. Measurements of weight or sensory tests, MRI scans, CT scans, ultrasounds, etc; 2. Records of clinical standard treatments or diagnostics; 3. Studies of individual or group characteristics or behaviors ensuring that do not have the potential to cause discrimination against individuals or groups.

All participants provided sufficient blood samples for isolation of white blood cells and DNA analyses. All blood samples were stored at −80°C.None of the subjects had a history of alcohol consumption, cigarette smoking, or chemotherapy treatment, as these were exclusion criteria. We have de-identified all patient details. The reporting of this study conforms to remark guidelines.[22] Subjects' characteristics are summarized in Table 1. Women with breast cancer prior to treatment (n = 248) and healthy female subjects (n = 200) were recruited between May 2009 and June 2012, as previously described.[23,24] All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were reviewed and approved by the Changhua Christian Hospital, Taiwan (CCH IRB No. 081219; CCH IRB No.191119) and the Chung Shan Medical University Hospital, Taiwan (CSMUH IRB No. CS12106). We ensured that data were entered, stored, and audited accurately and confidentially. Entry procedures included double-checking entries for precision. Data were stored in secure, encrypted databases to prevent unauthorized access. Regular audits were conducted to verify data integrity and confidentiality. By signing the consent form, subjects agree that

Table 1. Subjects' Characteristics of Healthy Controls, Breast Cancer Patients, and 5-Year Survivors.

	Age (years)	BMI (kg/m^2)
Healthy controls (n = 140)		
Mean (SD)	42.0 (10.7)	23.0 (3.30)
Median	41.0	22.5
Range	23.0 ∼ 69.0	16.1 ∼ 32.9
Breast cancer patients (n = 240)		
Mean (SD)	50.8 (11.1)	24.3 (4.00)
Median	49.5	23.9
Range	16.0 ∼ 82.0	15.6 ∼ 40.9
5-year survivors (n = 78)		
Mean (SD)	56.5 (9.20)	24.5 (4.40)
Median	56.5	24.0
Range	37.0 ∼ 83.0	18.3 ∼ 43.0

original medical records may be directly monitored by inspectors, auditors, institutional review boards, and regulatory authorities to ensure that the clinical trial processes and data comply with relevant laws and regulations. Personal identities will remain confidential.

After DNA sample analysis, all data was double-checked by other co-worker and lab manager for quality assurance and quality control to ensure accuracy and confidentiality.

Reaction of calf-thymus DNA (CT-DNA) with Hydrogen Peroxide and Methyl Methanesulfonate

To determine the extent of AP sites induced by oxidative stress and methylation, CT-DNA was incubated with hydrogen peroxide (H_2O_2) and methyl methanesulfonate (MMS), under physiological conditions. The incubation medium (final volume, 800 μL) consisted of 150 mM phosphate-buffered saline (pH 7.4) and CT-DNA (800 μg). To these preparations, MMS (5 mM) or H_2O_2 (1 mM) was added and the reaction was carried out for 1 hour at 37°C. The reaction was terminated by chilling the medium over an ice bath. DNA was isolated by ethanol precipitation and assayed for the presence of AP sites by the ASB assay.

DNA Isolation

A leukocyte cell pellet was collected from the buffy coat by centrifuging whole blood. Genomic DNA was isolated from the white blood cells using the Pure Gene DNA extraction kit, followed by extraction and purification as described.[21]

Detection of AP Sites using the aldehyde reactive probe-slot blot (ASB) assay

AP site detection through the aldehyde reactive probe-slot blot (ASB) assay.[21]

Regular AP Site Assay

The number of total AP sites was measured by the ASB assay as described above [21].

AP sites Cleavage Assay by Putrescine

Eight μg DNA, 10 mM EDTA, and 100 mM putrescine were incubated in 135 μL of 10 mM Tris-HCl/KOH at 37°C for 30 minutes and then immediately analyzed using the ASB assay. The AP site cleavage assay with putrescine is a modification of the ASB assay, further characterizing the origin of AP sites.[21]

Excisable AP Sites Cleavage Assay by T7 Exo and Exonuclease III. The cleavage assay was performed using T7 Exo and Exo III to verify the identity of the specific types of AP sites that are not easy to remove in human leukocytes.

3′-Cleaved AP Sites

The 3′-cleaved AP sites were determined using Exo III cleavage assay as described by[25] and[16] with minor modifications as described.[26] 8 μg of DNA The sample was incubated with 1 mM aldehyde reactive probe (ARP) at 37°C for 30 minutes. After cold ethanol precipitation, the DNA was resuspended in NE buffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT). The DNA concentration was measured by a UV spectrophotometer, and the 0.275 μg of DNA solution was placed in ice bath then following by incubation by 30 U of Exonuclease III in 10 μL of 1x of NE Buffer 1 (NEB1) (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT) for 10 minutes. Addition of 1x TE buffer was added to terminate the enzyme reactivity. The further steps were same as the origin protocol of the ASB assay.[21] The amounts of AP sites cleaved by Exonuclease III was determined as 3′-cleavable of AP sites.

5′-Cleaved AP Sites

The 5′-cleaved AP sites were performed using T7 Exo cleavage assay as described by[25] and[16] with minor modifications by.[26] 8 μg of DNA was incubated with 1 mM of ARP at 37°C for 30 min. After cold ethanol precipitation, the DNA was resuspended in 1x NEB 4 buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT). The DNA concentration was measured by a UV spectrophotometer, and the 0.275 μg of DNA solution was placed on an ice bath then followed by incubation with 25 U of T7 Exo and 10 μL of 1x NEB 4 for 30 seconds. 210 μL 1x TE buffer was added to terminate the enzyme reactivity and the number of AP sites was determined by the ASB assay.[21] The amounts of AP sites excisable by T7 Exo was determined as 5′-cleaved AP sites.

Residual AP Sites

The number of residual AP sites were calculated followed Exo III and T7 Exo-excisable AP sites as described by[16] with minor modification as described by.[26] 8 μg of DNA was incubated with 1 mM of ARP at 37°C for 30 min. After cold ethanol precipitation, the DNA was resuspended in 1x NEB 4 buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT). The DNA concentration was measured by a UV spectrophotometer, and the 0.275 μg of DNA solution was placed in ice bath then followed by incubation by 30 U of EXO(III) and 10 μL of 1x NEB 1 (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT) for 30 sec, followed by addition of 25 U T7 Exo and 10 μL of 1x NEB 4 immediately for 30 seconds, and assayed using the ASB assay.[21] The remaining amounts of AP sites after cleavage by Exo III and T7 Exo were determined as residual AP Sites.

Intact AP Sites

After treated the DNA with Exo III and T7 Exo, the number of intact AP sites was determined as the total number of AP sites minus cleavable number of 3′-cleaved AP sites, 5′-cleavedAP sites, and residual AP sites.

Precision and Detection Limit of the Assay

The detection limit of the assay corresponds to 0.15 AP sites per 10^6 nucleotides. The precision, indicated by the estimated coefficient of variation, was 13.6% (n = 5).

Statistical Analysis

The evaluation of significance of differences was performed with one-way ANOVA, followed by Dunnett's multiple comparison test. (*), (**), and (***) indicate statistically significant difference from Healthy controls ($P < 0.05$), ($P < 0.005$), ($P < 0.001$). The relationship between levels of putrescine-excisable AP sites verse residual AP sites in healthy controls, breast cancer patients, and 5′-year survivors, linear discriminant analysis (LDA) classifier are performed by calculating multivariate normal distribution density function for each class. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), Advanced Statistics version 20.0.

Results

The Background Levels of AP Sites in Leukocytes Obtained from 5-Year Survivors

As shown in Figure 1, the total number of AP sites in 5-year survivors was estimated to be 27.0 ± 10.2 per 10^6 total nucleotides. AP site levels were reduced by 46.3% in 5-year survivors compared to breast cancer patients before treatment ($P < 0.001$), while the levels in 5-year survivors were comparable to those in healthy controls ($P > 0.05$). These findings suggest that treatment has a significant effect in reducing these pro-mutagenic DNA lesions in cancer patients.

As categorized by estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status, a significant reduction in AP site levels was observed in 5-year survivors diagnosed with luminal A breast cancer (ER (+)/PR (+)/HER2(−)) (28.0 ± 8.19 per 10̂6 total nucleotides) compared to breast cancer patients before treatment (60.8 ± 67.4 per 10̂6 total nucleotides) as shown in Figure 2 ($P < 0.001$).

Putrescine-Excisable AP Sites in Leukocytes

To identify the specific types of AP sites present in these subjects, nuclear DNA was incubated with putrescine, which induces mild β-elimination, tagged with ARP, and analyzed using the ASB assay to estimate the proportion of AP sites excised by putrescine. The proportion of excised AP sites in CT-DNA treated with MMS or H_2O_2, and in leukocytes derived from healthy controls, breast cancer patients, and 5-year survivors, is shown in Figure 3. Results indicated that the percentage of putrescine-excisable AP sites in healthy controls, breast cancer patients, and 5-year survivors was 63.3%, 78.6%, and 67.7%, respectively. These findings are comparable to those observed in CT-DNA treated with H_2O_2 (75.8%) but not with MMS (10.8%).

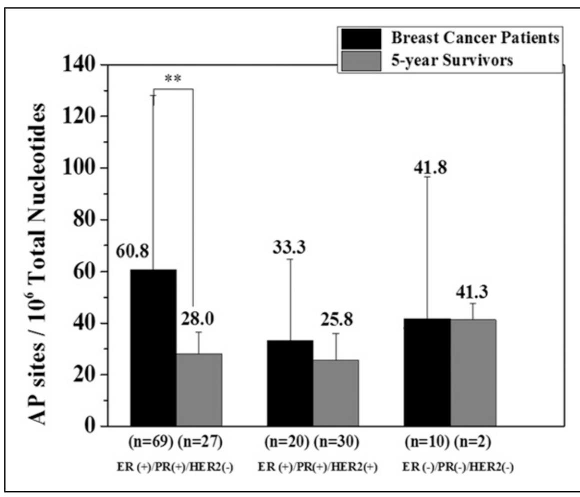

Figure 2. Abasic site levels in breast cancer patients and 5-year survivors, categorized by the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).

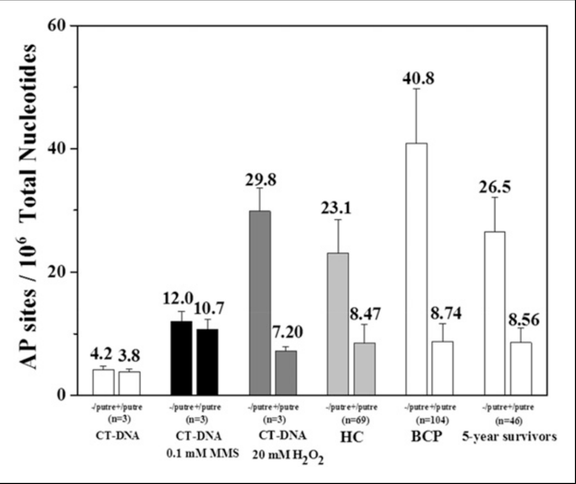

Figure 3. Putrescine-excisable abasic sites in CT-DNA treated with deionized water, 0.1 mM methyl methane sulfonate and 20 mM H_2O_2, and in leukocytes obtained from healthy controls, breast cancer patients, and 5-year survivors.

Characterization of AP Sites by the EXO III and T7 Exo Cleavage Assay in Leukocytes

To further explore the profile of the specific types of AP sites present in subjects, we performed the AP site cleavage assay using T7 Exo (5′->3′), Exo III (3′->5″), and combination of these 2 enzymes. Results were depicted in Figure 4. T7 Exo-excisable (5′-cleaved) AP sites account for 39.6%, 40.8%, and 41.7% in healthy controls, breast cancer patients, and 5-year survivor, respectively. Exo III-excisable (3′-cleaved) AP sites account for 24.5%, 14.4%, and 26.2% in healthy controls, breast cancer patients, and 5-year survivors, respectively. The residual AP sites were estimated to be 32.7% in healthy controls, 48.8% in breast cancer patients, and 34.0% in 5-year survivors.

To further explore the relationship between levels of putrescine-excisable AP sites verse residual AP sites in healthy controls, breast cancer patients, and 5′-year survivors, linear discriminant analysis (LDA) classifier are performed by calculating multivariate normal distribution density function for each class in Figure 5. The resulting combination was used as a linear classifier. Further investigation using LDA indicated that the 2 subject groups, ie, healthy controls and breast cancer patients, were separable using levels of putrescine-excisable AP sites verse residual AP sites as indicators. The quantitative evaluation of LDA classifier shows that the percentage of the true positive and true negative subjects are 94.7% (71/75) and 94.1% (48/51), respectively. It achieves 94.4% overall correct classification rate (overall accuracy) for both classes in test dataset with 99.3% sensitivity and 100% specificity. After treatment, levels of these specific types of AP sites were dramatically reduced and were comparable with those of healthy controls with 94.3% (33/35) of 5-year survivors being classified as negative subjects.

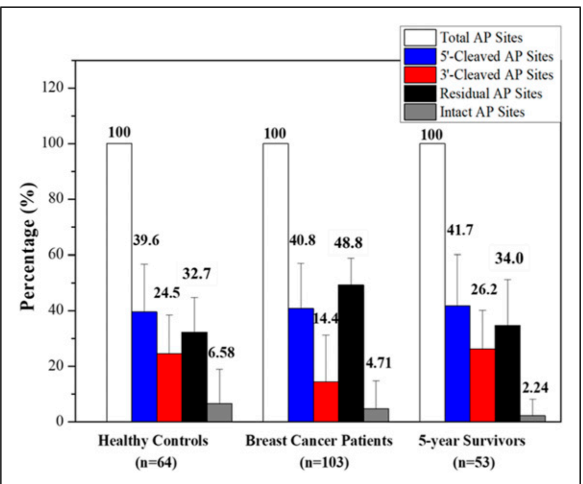

Figure 4. Results from the abasic sites cleavage assay using T7 exonuclease (T7 Exo) and/or exonuclease III (Exo III) in leucocytes derived from healthy controls, breast cancer patients and 5-year survivors. Data are normalized and expressed in percentage of total number of abasic sites.

Discussion

Evidence suggests treatment-related effects on the reduction of estrogen quinone-derived blood protein adducts in a hospital-based study of breast cancer patients who have undergone 5 years of postoperative treatment without recurrence (5-year survivors).[27] To investigate the potential differences in the formation of pro-mutagenic DNA lesions in breast cancer patients before and after medical treatment, we analyzed the number of AP sites in leukocytes derived from 5-year survivors and compared with those detected in healthy controls and breast cancer patients. Our investigation revealed that the total number of AP sites in 5-year survivors was significantly reduced by 46.9% compared to breast cancer patients ($P < 0.001$), while AP site levels in healthy controls were comparable to those in 5-year survivors ($P > 0.05$) (Table 1). This evidence suggests treatment-related effects in reducing the cumulative body burden of AP sites in 5-year survivors. Further analysis indicated that no significant difference in the levels of AP sites in 5-year survivors was observed as distinguished by age (Table 2) and by body mass index (BMI) (Table 3). Levels of AP sites in 5-year survivors with age greater 50 or with BMI greater 27 were comparable with those in healthy controls ($P > 0.05$) (Tables 2 and 3).

The Luminal A breast cancer is defined as estrogen-receptor (ER) positive, progesterone-receptor (PR) positive, and human epidermal growth factor receptor 2 (HER2) negative, and has low levels of the protein Ki-67, which helps control how fast cancer cells grow. Luminal A breast cancers tend to grow slowly and have the best prognosis.[28] As distinguished by status of ER, PR and HER2, significant reduction in the levels of AP sites in 5-year survivors diagnosed with luminal

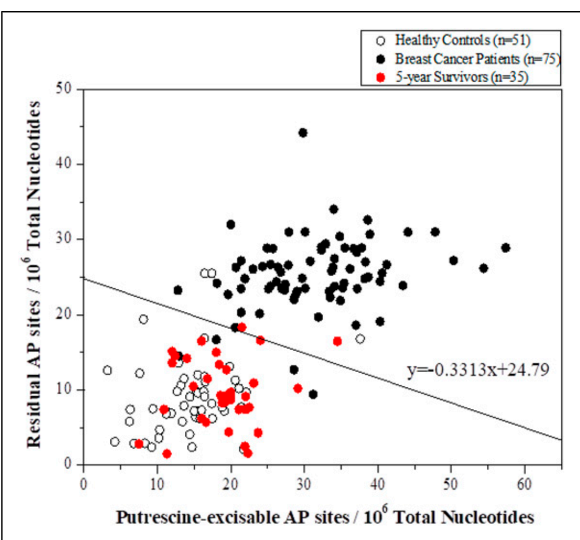

Figure 5. The relationship between levels of putrescine-excisable AP sites verse residual AP sites in healthy controls, breast cancer patients, and 5′-year survivors was performed using linear discriminant analysis (LDA) classifier by calculating multivariate normal distribution density function for each class.

Table 2. Total Number of Abasic Sites in Leukocytes, Categorized by Age, From Healthy Controls, Breast Cancer Patients, and 5-Year Survivors. (*), (**), (***) Indicates Statistically Significant Difference From Healthy Controls ($P < 0.05$), ($P < 0.005$), ($P < 0.001$).

	Age (years) < 50	Age (Years) ≥ 50
Healthy Controls (n = 140)	(n = 107)	(n = 33)
Mean (SD)	22.8 (14.3)	24.6 (9.91)
Median	19.2	22.0
Range	3.20 ~ 77.6	10.1 ~ 51.1
Breast cancer patients (n = 240)	(n = 122)	(n = 118)
Mean (SD)	56.2 (59.2)***	38.8 (31.7)*
Median	35.5	32.5
Range	5.60 ~ 265.7	5.60 ~ 202.4
5-year survivors (n = 78)	(n = 18)	(n = 60)
Mean (SD)	28.8 (8.92)**	25.2 (10.4)
Median	32.2	24.6
Range	8 ~ 39.9	8 ~ 47.8

Table 3. Total Number of Abasic Sites in Leukocytes, Categorized by Body Mass Index, From Healthy Controls, Breast Cancer Patients, and 5-Year Survivors. (*), (**), (***) Indicates Statistically Significant Difference From Healthy controls ($P < 0.05$), ($P < 0.005$), ($P < 0.001$).

	Body Mass Index (kg/m^2) < 27	Body Mass Index (kg/m^2) ≥ 27
Healthy Controls (n = 140)	(n = 123)	(n = 17)
Mean (SD)	23.0 (13.3)	24.9 (14.6)
Median	20.7	21.5
Range	3.20 ~ 77.6	10.2 ~ 76.4
Breast cancer patients (n = 240)	(n = 186)	(n = 54)
Mean (SD)	49.9 (52.2)***	36.0 (32.1)
Median	34,7	25.9
Range	5.60 ~ 265.7	6.50 ~ 191.1
5-year survivors (n = 78)	(n = 65)	(n = 13)
Mean (SD)	26.3 (10.3)*	24.0 (9.02)
Median	25.3	19.2
Range	8.0 ~ 47.8	12.6 ~ 35.5

A (ER (+)/PR (+)/HER2(−)) breast cancer (28.0 ± 8.19/ 10^6 total nucleotides) was observed when compared to those of breast cancer patients before treatment (60.8 ± 67.4/10^6 total nucleotides) ($P < 0.001$) (Figure 2). This translates to a 52.8% reduction in levels of AP sites in subjects with luminal A breast cancer. By contrast, this is not the case in patients with luminal B (ER (+)/PR (+)/HER2(+)) and triple-negative (ER (−)/PR (−)/HER2(−)) breast cancer ($P > 0.05$). The underlying mechanism(s) mediate the obvious reduction in the body burden of AP sites in patients with luminal A breast cancer after treatment is remain unknown and warrant further investigation.

ROS can induce sugar lesions on DNA by abstracting hydrogen from deoxyribose, leading to strand breaks and the formation of AP sites.[29] Oxidative stress-mediated AP sites, but not intact AP sites, are eliminated by putrescine.[30] It has been shown that estrogen-mediated ROS induced parallel formation of DNA single strand breaks and abasic sites in purified DNA.[16] In this study, we further characterized the types of AP sites in leukocytes using putrescine cleavage assay, demonstrating that the AP sites present in leukocytes contain 63.3%-78.6% putrescine-excisable AP sites in all subjects (Figure 3). This finding is in good agreement with that detected in H_2O_2-induced AP sites rather than MMS. It is likely that the AP sites identified in leukocytes from healthy controls, breast cancer patients, and 5-year survivors are primarily mediated by oxidative stress rather than by direct DNA alkylation, which forms intact AP sites.

Various carcinogens can modify DNA and trigger DNA damage. Without proper repair, this may lead to mutations and genomic deletions during tumorigenesis. Further characterization of the AP sites was conducted using the exonuclease cleavage assay (including Exo III and T7 Exo) to investigate specific types of AP sites in leukocytes, including total, 5′-cleaved, 3′-cleaved, intact, and residual AP sites. Results from the analyses of randomly selected individuals indicated that the proportion of residual AP sites detected in controls, breast cancer patients, and 5- year survivors were amount to 32.7%, 48.8%, and 34.0% respectively and no intact AP sites were

detected in all subjects (Figure 4). These findings suggest that there is clear evidence of treatment-related effects on the reduction of total number of AP sites in breast cancer patients, and the profiles of AP sites in 5-year survivors are quantitatively and qualitatively comparable with those of healthy controls after medical treatment. Overall, this evidence adds further support to the theme that a clinical relevance of these findings to the potential implications for personalized treatment in breast cancer patients, in particular in 5-year survivors. Further research is warranty by recruiting cancer patients before and after treatment and analyzing their respective levels of specific types of AP sites in leukocytes.

When analyzing the limitations of this study, several key factors must be considered. First, variations in data diversity and reliability among AP sites can lead to inconsistencies. Different AP sites may use varying data recording methods and standards, which can result in data inconsistencies and raise questions about the accuracy of the analysis results. Additionally, relying on a limited number of AP sites may lack representativeness. If the research relies only on data from a few AP sites, it may not yield broadly representative conclusions. Furthermore, the limitations of technical equipment and methodologies used in different studies can lead to variation in results. Finally, time and budget constraints can narrow the scope and depth of the research. However, with limited sample size, further research is warranty to reveal the treatment-related effects on the formation of specific types of AP sites in 5-year survivors.

Conclusion

In conclusion, we provide a direct assay for measuring the number of abasic sites in human leukocytes from 5-year survivors and present evidence of treatment-related effects on reducing the total number of AP sites in luminal A breast cancer patients, particularly in residual AP sites. Our investigation further supports the idea that the cumulative body burden of AP sites may serve as a significant predictor of breast cancer risk, with factors such as BMI, age, and specific breast cancer genotypes potentially modulating estrogen homeostasis and increasing the production of pro-mutagenic DNA lesions.

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Author Contributions

CL, CYF and PHL conceived and designed the study. CYF, CL, WCH, and DRC collected blood samples. GPB, SMH, CHW, QX, and TDD performed instrumental and statistical analyses and interpretation of data. PHL wrote the first draft of the manuscript, which was revised with contributions from CL and DRC. All authors read and approved the final manuscript. PHL supervised the overall study progress. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors alone are responsible for the content and writing of this article.

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Ethical Statement

The study protocol was reviewed by the Changhua Christian Hospital, Taiwan (CCH IRB No.150802). All experimental protocols were reviewed and approved by the Changhua Christian Hospital, Taiwan (CCH IRB No. 081219;CCH IRB No.191119) and the Chung Shan Medical University Hospital, Taiwan (CSMUH IRB No. CS12106). Informed consent was obtained from all patients prior to blood collection. Each participant provided informed consent after receiving a detailed explanation of the study and its potential implications. All participants completed questionnaires covering age, occupation, medical history, smoking habits, and alcohol consumption. All participants provided sufficient blood samples for isolation of white blood cells and DNA analyses. We have de-identified all patient details.

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Data Availability Statement

Materials and data generated in this study are available upon request from the corresponding authors.

Supplemental Material

Supplemental material for this article is available online.

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