DNA Adducts in Human Pancreatic Tissues and Their Potential Role in Carcinogenesis

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Abstract

Pancreatic cancer is the fourth and fifth leading cause of cancer death for men and women, respectively, in the United States. Although the etiology of this cancer is poorly understood, smoking and dietary fat have been implicated by epidemiological studies. To test the hypothesis that DNA damage derived from carcinogen exposure and diet is involved in pancreatic carcinogenesis, aromatic and lipid peroxidation-related DNA adducts in 13 normal tissues adjacent to tumor and 20 tumors from pancreatic cancer patients were analyzed by \( ^{32} \)P-postlabeling. Normal pancreatic tissues from 5 nonpancreatic cancer patients and 19 healthy organ donors served as controls. To correlate the DNA adduct level with patients’ characteristics, information on age, sex, body mass index, and smoking status of pancreatic cancer patients were collected from medical records. A significantly higher level of total DNA adducts was detected in pancreatic cancer patients as compared with controls. The mean level of adducts/10\(^6\) nucleotides in adjacent normal pancreatic tissues from pancreatic cancer patients (A tissues) was 102 ± 21 compared with 39 ± 6 and 13 ± 1 in pancreatic tumor tissues (T tissues) and normal pancreatic tissues from controls (C tissues), respectively. Among the adducts observed, one single aromatic adduct (spot 1) was present in 100, 90, and 0% of the A, T, and C tissues, respectively. Two novel clusters of adducts (spots 2 and 3) were observed in 11 of 13, 12 of 20, and 2 of 24 of A, T, and C tissues, respectively, and the presence of these adducts was positively correlated with smoking status. In addition, the previously defined smoking-related diagonal radioactive zone was detected in three A samples only, although 50% (10 of 20) of the patients with pancreatic cancers in this study were ever smokers. Putative lipid peroxidation-related adducts were detected in all samples examined and were significantly higher in A than in T and C samples. Multiple regression analyses showed that body mass index was positively correlated to the levels of spot 1 and the lipid peroxidation-related adducts in A tissues and the total aromatic adducts in tumors. Smoking was also positively correlated to the level of total adducts. These observations are consistent with previous epidemiological findings and support the hypothesis that DNA damage related to carcinogen exposure and lipid peroxidation is involved in human pancreatic carcinogenesis.

Introduction

Pancreatic cancer is the fourth and fifth leading cause of cancer death in men and women, respectively, in the United States (1). Because diagnosis usually occurs late in the natural history of the tumor, the mortality rate of pancreatic cancer is approximately equal to the incidence rate; about 86% of patients die in the first year following diagnosis, and virtually all patients die by 5 years after diagnosis (2). This deadly disease merits an intensive search for etiological clues to identify high-risk individuals for primary prevention. Unfortunately, relatively little is known about environmental or genetic factors that cause this disease. Hereditable genetic factors account for only 5% of the total pancreatic cancer burden (3). Pancreatitis and other medical conditions, e.g., partial gastrectomy, dietary fat, and alcohol and coffee consumption have been suggested to be related to pancreatic cancer, but no conclusive causative relationship has been reported (4, 5). The most consistently identified epidemiological risk factor for pancreatic cancer is cigarette smoking, and a 2–10-fold risk of developing pancreatic carcinoma has been demonstrated for heavy smokers (6, 7). In addition, there appears to be a potential relationship between pancreatic cancer and environmental carcinogens, including dietary carcinogens, industrial carcinogens, and radiation, although the associations are not as strong as for other cancers (8–10).

In addition to epidemiological data, several lines of experimental evidence support a role of carcinogen exposure in human pancreatic cancer: (a) more than 80% of pancreatic cancers harbor activating point mutations in codon 12 of the K-ras oncogene (11, 12) and higher mutation frequency has been associated with smoking or drinking status of the patients (13); (b) most carcinogens need to be activated by drug-metabolizing enzymes into reactive species to exert their carcinogenic effect, and human pancreatic tissues have been shown to contain these carcinogen-activating enzymes (14–16); (c) nitrosamine compounds, which are present in both human diet and cigarette smoke, induce pancreatic tumors in laboratory animals (17).

Many chemical compounds exert their carcinogenicity not only through direct covalent binding to DNA but also through indirect mechanisms, e.g., the induction of genotoxic reactive oxygen species and lipid peroxidation products. To address the hypothesis that DNA damage derived from carcinogen exposure and endogenous processes is involved in human pancreatic carcinogenesis, aromatic and lipid peroxidation-related DNA adducts were measured in pancreatic tissues of cancer and noncancer patients by \( ^{32} \)P-postlabeling. A significantly higher level of aromatic DNA adducts and lipid peroxidation-related DNA adducts was observed in A tissues of pancreatic cancer patients compared to that found in C tissues of noncancer controls. A significant correlation between smoking or BMI and the level of DNA adducts in pancreatic cancer patients was also observed. These findings support previous clinical epidemiological studies and provide further evidence for a potential etiological role for smoking- and lipid peroxidation-induced DNA damage in human pancreatic cancer.

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3 The abbreviations used are: A, adjacent normal pancreatic tissues from pancreatic cancer patients; C, normal pancreatic tissues from controls; T, pancreatic tumor tissues; BMI, body mass index; MDA, malondialdehyde.
Materials and Methods

**Data and Tissue Sample Collection.** Twenty T tissues and 13 histologically normal A tissues were obtained from 20 pancreatic cancer patients undergoing surgical resection at The University of Texas M. D. Anderson Cancer Center. The 20 patients include 10 males and 10 females (median age, 58 years; range, 26–83 years): 16 white, 2 black, and 2 Hispanic Americans. Ten patients reported a smoking history (8 males and 2 females), and ten denied such a history (8 females and 2 males). Fourteen of the 20 patients received chemotherapy before surgery. For controls, pancreatic tissues were obtained from 19 previously healthy individuals (8 females and 11 males; median age, 41 years; range, 8–56 years) through an organ donor program at the University of Bern, Switzerland. The controls also included normal pancreatic tissues obtained from five nonpancreatic cancer patients who underwent surgical treatment for other gastrointestinal cancers at The University of Texas M. D. Anderson Cancer Center. Among the 24 controls, 2 had a known smoking history and 1 was an alcoholic. The use of human surgical samples was approved by the Institutional Review Board. All tissue samples were kept at −80°C before DNA extraction. Information regarding tobacco and alcohol use, previous medical history, and other characteristics of the cancer patients was collected from their medical records.

**DNA Isolation and Adduct Analysis.** DNA was isolated by a procedure involving enzymatic digestion of protein and RNA followed by solvent extraction (18). DNA adducts were analyzed by the nuclease P1-enhanced version of the 32P-postlabeling assay (18). For detection of DNA adducts with different polarities, each chromatogram was cut into three portions after the first development, and adducts on each portion of the map were separated as described previously (19). The smoking-related adducts and aromatic bulky adducts were retained on the lower map; the lipid peroxidation-related, more polar adducts migrated to the central and upper maps. The total adduct level was the sum of the adducts on maps that were derived from all three cut-outs.

**Statistical Analysis.** The average level of DNA adducts was expressed as the mean ± SE, and comparisons between means were analyzed by Student’s t test or the Mann-Whitney test. Multiple regression analyses were performed for determination of the relationship between adduct levels and age, sex, smoking status, and BMI (weight, kg/height, M2).

**Results**

**Patterns of DNA Adducts in Pancreatic Tissues.** The typical chromatograms demonstrating the patterns of DNA adducts detected in A tissues of pancreatic cancer patients are shown in Fig. 1. Four major nonpolar adducts (L1–L4) were consistently present on the lower maps of tissue samples in a majority of pancreatic cancer patients. Specifically, spot L1 was detected in 100 and 90%, spot L2 and L3 in 77 and 65%, and spot 4 in 92 and 65% of normal adjacent tissues and tumors, respectively. In contrast, these adducts were detectable in only 33% (L1), 8% (L2 and L3), and 13% (L4) of C tissues. In addition, some more polar DNA adducts, putatively endogenous DNA modifications related to oxidative stress and lipid peroxidation processes, were detected on the central and upper maps. Three major adducts, i.e., C1, C2, and C3 on the central maps were present in 77, 69, and 92% of A tissues and 75, 75, and 80% of tumors, respectively. The three previously (20) characterized putative MDA-DNA adducts (U1–U3) were the major adducts detected on the upper maps. Spots U1 and U2 were found in 100% of adjacent tissues and 95% of tumors; and U3 was found in 100% of A tissues and 80% of tumors. Spots on the central and upper maps were also detectable in control samples, but the intensities were distinctly low compared to those in cancer patients.

**Levels of DNA Adducts in Pancreatic Tissues.** The total level of DNA adducts (relative adduct labeling × 108, mean ± SE, range) in A tissues (102 ± 21, 39–305) was significantly higher than that found in tumors (39 ± 6, 4–84) and control tissues (13 ± 1, 4–26; Fig. 2). Five of 13 (39%) of the A tissues contained total DNA adducts at a level greater than 100 adducts in 108 nucleotides and another 39% (5/13) at the levels between 50 and 90 adducts in 108 nucleotides. The rest of the A tissue samples (3 of 13) had adduct levels of 40–50 adducts in 108 nucleotides. In contrast, none of the C tissues showed adduct levels above 6 adducts in 108 nucleotides. The nonpolar adducts on lower maps accounted for more than 70% of the total adducts in all three types of tissues. Notably, the more polar adducts on the central and upper maps showed a similar trend as the nonpolar adducts, i.e., the A tissues contained a higher level of adducts than tumor and C tissues (Table 1).

**Relationship between DNA Adduct Levels and Age, Smoking, and Body Mass Index.** To explore possible epidemiological factors that might be associated with the levels of DNA adducts, multiple regression analysis was performed to examine the relationship between adduct levels and several characteristics of the pancreas cancer patients, such as age, sex, smoking, and BMI. Age was not correlated with adduct levels in any of the tissue samples analyzed (data not shown). Male patients tended to have higher levels of adducts than females, but this trend was associated with the smoking history of the patients. Eight of the 10 smokers in this study were males, and smoking was significantly correlated to the presence of adducts L2 and L3. Seven of 10 smokers and 3 of 10 nonsmokers displayed spots L2 and L3 compared to 3 of 10 smokers and 7 of 10 nonsmokers who did not have these adducts (P < 0.05 by χ2 test). The level of total adducts in smokers tended to be higher than that in nonsmokers (P = 0.09, t test). Interestingly, BMI was consistently correlated to the levels of total aromatic adducts and total adducts in tumors (Fig. 3) when other factors i.e., age, sex, and smoking, were controlled in the regression analysis.

**Discussion**

Carcinogen exposure from smoking and dietary and industrial sources has been incriminated in human pancreatic cancer (8–10). The significantly greater burden of DNA adducts in pancreatic cancer patients than in controls reported in this pilot study has provided direct evidence for the involvement of carcinogen exposure in this disease.
Pancreatic cancer is considered to be one of the human cancers associated with cigarette smoking. Based on the association between DNA adducts and smoking status observed in the current study, it is possible that some of the adducts are derived from carcinogens present in cigarette smoke. A recent study has detected a cigarette carcinogen, 4-aminobiphenyl, that induced DNA adducts in human pancreatic tissues (17). The present observations that spots 2 and 3 were predominantly detected in smokers, and that smokers tended to have a higher level of DNA adducts than nonsmokers, strongly support the role of smoking in human pancreatic cancers. Despite the limited epidemiological information and the small number of study subjects, this study has demonstrated the feasibility of DNA adduct measurement in human pancreatic tissues and the association between DNA damage and cancer development in this disease.

The elevated level of DNA adducts in patients with pancreatic cancer could be a consequence of excess exposure, increased activation, or decreased detoxification during carcinogen metabolism, as well as deficient DNA repair. Few studies have explored these mechanisms and their relationship to human pancreatic carcinogenesis. One study has shown that patients with pancreatitis and pancreatic cancers had significantly higher levels of cytochrome P-450 enzymes than those in noncancer controls, whereas for the detoxification enzyme, i.e., glutathione S-transferase, levels were comparable (14). These findings may partially explain the significantly higher level of DNA adducts detected in cancer patients than those in controls. However, another study using a human pancreatic cell line found that cytochrome P-450 1A1 was not inducible by dioxin (15). This result suggests that activation of procarcinogens derived from cigarette smoke may be different in pancreas as compared to lung. Further study to determine the metabolic differences in these target tissues of cigarette smoke may help to understand the individual variations in susceptibility to carcinogen exposure.

In this study, we have also observed a significantly higher level of lipid peroxidation-related DNA adducts, i.e., MDA adducts in pancreatic cancer patients than controls. Formation of these endogenous adducts may be a consequence of carcinogen exposure and other oxidative stress. It is known that reactions between oxygen and the conjugated double bonds in polyunsaturated fatty acids lead to reactive oxygen species and lipid peroxidation (21). Many of the lipid peroxidation products, including MDA, readily form DNA adducts and have been shown to be mutagenic. In addition, dietary fats also affect the level of membrane (lipid)-bound enzymes, such as drug-metabolizing enzymes that regulate carcinogen metabolism (22). The synergistic interaction between lipid peroxidation and carcinogen metabolism has been demonstrated in our previous studies in breast cancer (23, 24). Normal adjacent breast tissues from breast cancer patients who displayed a bulky aromatic adduct also had significantly higher levels of MDA-DNA adducts than those who did not have the bulky aromatic adduct (24). The coincident higher levels of both aromatic and MDA adducts observed in patients with pancreatic cancer as compared to controls in the present study further support such a possible interaction.

Although the role of high-fat diets in human pancreatic cancer is not conclusive, and although detailed dietary histories are not available from these patients, it is tempting to speculate that the levels of lipid peroxidation-related DNA adducts are associated with high dietary fat intake. The positive relationship between BMI and total DNA adducts is consistent with this hypothesis. A previous study in women at high risk for breast cancer has demonstrated a significant reduction of oxidative DNA damage following intervention with a low-fat diet (25).

Fig. 2. Levels of total DNA adducts in A tissues (n = 13) and tumors (n = 20) of patients with pancreatic cancer and in C tissues of healthy organ donors and patients with nonpancreatic cancers (n = 24). The average relative adduct labeling (RAL) × 10⁻⁸ value in cancer patients was 8-fold of that in controls. Box boundaries, the 25th and 75th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, median; dotted line, mean.

Fig. 3. Correlation between BMI and DNA adduct levels in tumor tissues. A, total aromatic adducts on the lower maps; B, total DNA adducts on maps derived from all three cut-outs. The correlation coefficients for A and B are 0.58 (P = 0.03) and 0.51 (P = 0.06), respectively. RAL, relative adduct labeling.
The association between BMI and levels of DNA adducts in the pancreas is a novel observation. To our knowledge, no such correlation has ever been reported in other human cancers, although BMI has been associated with risk of developing several human malignancies (26–28). It is possible that the association between BMI and DNA adducts is a reflection of effect of dietary fat intake, although epidemiological studies have not shown a consistent correlation between dietary fat intake and pancreatic cancer. This issue needs to be addressed in future prospective studies.

As observed in our previous breast cancer studies (23, 24), a significantly lower level of DNA adducts was detected in tumors as compared to normal adjacent tissues from pancreatic cancer patients. This phenomenon may be explained by two possible mechanisms: (a) the accelerated cell turn over rate of tumors relative to normal pancreatic tissue or the extensive surrounding fibroblastic stroma contributed to a “dilution effect” on DNA adducts in tumors; and (b) metabolic differences between pancreatic ductal cells in tumors and the pancreatic acinar cells in normal adjacent tissues lead to the lower levels of DNA adducts in tumors.

In summary, a significantly higher level of DNA adducts was detected in patients with pancreatic cancers as compared to controls without pancreatic cancer. The DNA damage detected provides further evidence supporting a role of smoking and lipid peroxidation in human pancreatic cancer.

References
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