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DNA adduct level in lung tissue may act as a risk biomarker of lung cancer

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Abstract

Lung cancer is a leading cause of mortality in Taiwan. We hypothesised that high susceptibility to DNA damage in the target organ acts as a risk biomarker for the development of lung cancer. To verify this hypothesis, the aromatic/hydrophobic DNA adduct levels of non-tumorous adjacent lung tissues from 73 primary lung cancer patients and 33 non-cancer controls were evaluated by ^{32}P-postlabelling assay. Wilcoxon rank sum test showed that DNA adduct levels in lung cancer patients (49.58±33.39 adducts/10^8 nucleotides) were significantly higher than those in non-cancer controls (18.00±15.33 adducts/10^8 nucleotides, $P < 0.001$). The DNA adduct levels among lung cancer and non-cancer samples were not influenced by smoking behaviour and cigarette consumption. Our data also showed that the polymorphisms of cytochrome P4501A1 *(CYP1A1)* MspI, glutathione S-transferase M1 *(GSTM1)* and the combination of both genetic polymorphisms were not related to the DNA adduct levels. Interestingly, positive association between CYP1A1 protein expression and DNA adduct levels was found when CYP1A1 protein expression in lung specimens from lung cancer patients was examined by immunohistochemistry. Multivariate linear regression analysis indicated that the DNA adduct level was not associated with gender, smoking behaviour, or genetic polymorphisms of *CYP1A1* and *GSTM1*. Moreover, multivariate logistic regression analysis showed that persons with high DNA adduct levels ($>$48.66 adducts/10^8 nucleotides) had an approximately 25-fold risk of lung cancer compared with persons with low DNA adduct levels ($\leqslant$48.66 adducts/10^8 nucleotides). In conclusion, DNA adduct levels in lung tissue may be a more reliable lung cancer susceptibility biomarker than DNA adduct levels in leucocytes. In addition, higher susceptibility to DNA damage in lung cancer patients may partly play a role in the development of lung cancer.

Keywords: Aromatic/hydrophobic DNA adducts; Lung cancer

1. Introduction

Malignant neoplasms have been the leading cause of death in Taiwan since 1982, with lung cancer the leading cause of cancer mortality according to the Department of Health, ROC, Life Statistics, 1996. Cigarette smoking is widely accepted as the major cause of lung cancer. The risk of lung cancer increases with the number of cigarettes smoked and duration of smoking. However, smoking behaviour cannot fully explain the epidemiological characteristics of lung cancer in Taiwanese women, of whom less than 4% are smokers [1,2]. The Taiwanese male smoking population increased from 55% in 1986 to 59% in 1990. In contrast, in the United States, the smoking prevalences in both males and females have decreased from 52% and 34% in 1965 to 27% and 24% in 1991, respectively. However, the lung cancer incidences in that country have increased from 71 and 17 persons/100 000/year to 75 and 43 persons/100 000/year in males and females, respectively [3]. In Taiwan, the lung cancer incidences for males and females have also increased annually to 26 and 12 persons/100 000/year in 1995 according to the Department of Health, ROC, Cancer Registry Annual Report,

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1998. Thus, a large proportion of lung cancer incidence might be associated with factors other than smoking, especially in non-smokers [4]. Formation of DNA adducts by covalent binding to chemicals with electrophilic properties is suggested to be the initiating event leading to mutation and/or neoplastic transformation [5]. Based on this rationale, DNA adduct formation has been used as a dosimeter to assess human exposure to smoking and environmental pollutants, as well as genotoxic effects resulting from exposure [5]. A linear relationship has been found between DNA adduct levels in human lung and daily or lifetime cigarette consumption [6]. On the contrary, some data indicate that DNA adduct levels in lung tissues from smoking lung cancer patients are not associated with the number of cigarettes smoked [7,8]. DNA adduct levels have even been reversely correlated with cigarette consumption [9]. DNA adduct level in white blood cells has also been used as an exposure biomarker and indicator of lung cancer risk [10–13]. However, the adduct level in non-target leucocytes does not really reflect the degree of DNA damage in the target organ of the lung and thus cannot properly be used to estimate the risk. Thus, we examined the DNA adduct levels in lung tissue from lung cancer patients and non-cancer controls using ^{32}P-postlabelling. The polymorphisms of cytochrome P4501A1 (*CYP1A1*) and glutathione-S-transferase (*GSTM1*) may be associated with DNA adduct levels in lung tissues from lung cancer patients [14–16]. Therefore, in this study, *CYP1A1* and *GSTM1* polymorphisms and the protein expression of both genes in lung specimens were also analysed by polymerase chain reaction (PCR) and immunohistochemistry to verify the effects of the genotype and phenotype of both genes on the formation of DNA adducts in lung tissue.

2. Patients and methods

2.1. Study subjects

73 primary lung cancer patients including 38 (53%) non-smokers, 32 (44%) smokers and 3 (4%) patients of unknown smoking status, who had undergone thoracic surgery at Veterans General Hospital-Taichung were enrolled. 33 non-cancer patients with lung disease, including pneumothorax, tuberculosis, chest wall deformity and cryptococcal infection, who had undergone thoracic surgery at Chen-Kung University Hospital, Tainan or Changhua Christian Hospital, Changhua, served as control subjects. None of the subjects had received radiation therapy or chemotherapy prior to surgery. The non-tumorous areas surrounding the tumorous lung tissues were resected for the analysis of DNA adduct levels by ^{32}P-postlabelling assay. Information on smoking history was obtained from the patients by interview with informed consent. Smokers and non-smokers were current smokers who smoked up to the day of pulmonary surgery and lifetime non-smokers, respectively.

2.2. DNA extraction

Frozen tissues were homogenised in 10 mM Tris, 0.1 M NaCl, 25 mM EDTA (pH 8.0) and 0.5% sodium dodecyl sulphate (SDS) on ice. The aqueous supernatant was incubated with RNase A and RNase T1 (250 μg/ml, Sigma Chemical Co.) at 37°C for 60 min followed by digestion with proteinase K (10 μg/ml, Merck) at 55°C for 12 h. The digest was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v), then sodium acetate (0.3 M final concentration) was added to the aqueous supernatant. DNA was precipitated with 100% ethanol at −20°C and dissolved in water. For ^{32}P-postlabelling analysis, the solutions of DNA were adjusted to 1 mg/ml in distilled water.

2.3. Quantitation of DNA adducts

Aromatic/hydrophobic DNA adduct levels were evaluated by ^{32}P-postlabelling assay [17], after enrichment of adducted nucleotides using nuclease P1. The assay was modified for first clean-up and transfer chromatography, as described by Gupta [18]. Hydrolysis of DNA, treatment with nuclease P1 and ^{32}P-postlabelling of nucleotides were carried out as previously described [19]. The separation of the adducts was performed by three-dimensional thin layer chromatography (TLC). The plates were developed in the first direction with 0.65 M sodium phosphate (pH 6.0), in the second direction with 3.6 M formic acid and 8.5 M urea adjusted to pH 3.5 with lithium hydroxide, and in the third direction with 0.8 M LiCl, 0.5 M Tris, and 8 M urea (pH 8.0). Radioactive zones of the TLC plates were located by screen-intensified autoradiography at −80°C for 4 days. These radioactive zones were cut out, scraped off and counted by liquid scintillation counting. After adjusting for the similar surface area of radioactive zones, this value obtained from scintillation counting served as a background count, and was subtracted from the counts of radioactive zones. Each sample was analysed in triplicate. The limit of sensitivity was 1 adduct/10^9 unmodified nucleotides. The relative adduct labelling (RAL) was calculated according to a previous report (19): RAL = counts per minute (c.p.m.) in adduct nucleotides)/(c.p.m. in total nucleotides).

2.4. Polymorphisms of CYP1A1 and GSTM1

Genotyping of the MspI polymorphism of the *CYP1A1* gene was performed by PCR amplification

using the primer set of 5′-TAGGAGTCTTGTCTCAG CCT-3′ and 5′-CAGTGAAGAGGTGTAGCCGCT-3′ [20]. The amplified products were digested with MspI and analysed by electrophoresis on a 1.5% agarose gel. Detailed information of the PCR assays can be found elsewhere [20]. Genotypes of *GSTM1* were determined by the presence or absence of PCR product, according to the method of Groppi and colleagues [21]. Two primers, 5′-GAAGGTGGCCTCCTCCTTGG-3′ and 5′-AATTCTGGAT TGTAGCAGAT-3′, were used for PCR. If samples had no PCR product, the PCR experiment was repeated by adding a set of *β-actin* primers together with the *GSTM1* primers, to confirm that the absence of *GSTM1* PCR product represented a null genotype.

2.5. Immunohistochemistry

The CYP1A1 and GSTM1 proteins were immunohistochemically assessed on air-dried 5 μm formalin-fixed, paraffin-embedded sections using commercially available anti-human CYP1A1 (Chemicon International Inc., USA) and GSTM1-1 (Xford Biomedical Research Inc., USA) rabbit polyclonal antibodies respectively. Briefly, the sections were placed in a microwaveable container, submerged in 10 mM citrate buffer (pH 6.01), wrapped in vented cling film and incubated for two 5-min periods at maximum power in a domestic microwave. Following microwaving, the sections were allowed to equilibrate to room temperature in the buffer, then were rinsed in distilled water. The CYP1A1 and GSTM1 antibodies were respectively applied to the sections at a 1:1000 dilution for 1 h at room temperature. Immunoreactivities of both CYP1A1 and GSTM1 were demonstrated using the universal labelled streptavidin–biotin (LSAB), horseradish peroxidase (HRP) kit (Dako) according to the manufacturer's instructions. The sections were counterstained in haematoxylin and scored semiquantitatively following scanning of the entire tumour field. Cytosolic immunoreactivities of CYP1A1 and GSTM1 in the non-tumour field were scored as negative (0%), '+' (< 15% cytosolic positive), '++' (15–50% cytosolic positive) or '+++' (> 50% cytosolic positive).

2.6. Statistical analysis

The differences in DNA adduct levels between lung cancer patients and lung cancer controls of different gender, smoking status and genetic polymorphisms of *CYP1A1* and *GSTM1* were calculated by Wilcoxon rank sums test, χ^2 test and Kruskal–Wallis H test. The association between DNA adduct levels and cigarette consumption of smokers was statistically analysed by the Spearman rank correlation analysis. Multivariate linear regression analysis was used to assess which variable was important for the DNA adduct levels. Multivariate logistic regression analysis was performed to calculate the risk of lung cancer in association with age, gender, smoking status, genetic polymorphisms of *CYP1A1* and *GSTM1* and DNA adduct level.

3. Results

3.1. Higher susceptibility to DNA damage in lung cancer patients than in non-cancer controls

In this study, we collected 73 primary lung cancer patients and 33 non-cancer controls. The distributions of gender, smoking status, *CYP1A1* polymorphism, *GSTM1* polymorphism, and the combination of *CYP1A1* and *GSTM1* polymorphisms between lung cancer and non-cancer control groups did not differ

Table 1
Characteristics of study subjects in this experiment[a]

Characteristics	Lung cancer patients ($n=73$)	Non-cancer controls ($n=33$)	P value
Mean age (years)±SEM	57.89±7.12	49.27±17.18	0.160
Sex	*n* (%)	*n* (%)	
Male	51 (70)	24 (73)	
Female	22 (30)	9 (73)	0.764
Tumour type			
Adenocarcinoma	48 (66)	–	
Squamous	25 (34)	–	
Tumour stage			
I	30 (41)	–	
II	11 (15)	–	
III	29 (40)	–	
IV	3 (4)	–	
Smoking status			
Non-smoking	38 (53)	22 (67)	
Smoking	32 (44)	11 (33)	0.234
Unknown	3 (4)	0	
CYP1A1 polymorphism			
m1/m1 (A)	24 (33)	8 (24)	
m1/m2 (B)	35 (48)	15 (45)	
m2/m2 (C)	14 (19)	10 (30)	0.400
GSTM1 polymorphism			
Negative (−)	34 (47)	17 (52)	
Positive (+)	39 (53)	16 (48)	0.637
CYP1A1/*GSTM1*			
A/−	24 (33)	11 (33)	
A/+	35 (48)	12 (36)	
BC/−	10 (14)	6 (18)	
BC/+	4 (5)	4 (5)	0.525

[a] The difference of age between lung cancer and non-lung cancer patients was calculated by Wilcoxon rank sums test. The other status differences between lung cancer and non-lung cancer patients were calculated by χ^2 test. A value of less than 0.05 was taken to be significant.

(Table 1). The susceptibility was evaluated by the comparison of DNA adduct levels in non-tumorous lung tissues between lung cancer patients and non-cancer controls. DNA adduct levels in lung cancer patients were significantly higher than in non-cancer controls ($P < 0.001$, Table 2). Amongst different parameters including gender, smoking status, polymorphisms of *CYP1A1*, *GSTM1* and the combination of both genetic polymorphisms, the DNA adduct levels were significantly different between cases and controls except the combination of both genetic polymorphisms A/− and A/+ (Table 2). Among 73 lung cancer patients and 33 non-cancer controls, DNA adduct levels ranged from 2.4 to 147.1 and from 3.4 to 88.8 adducts/10^8 nucleotides, respectively. The individual variations in DNA adduct levels in lung cancer patients were approximately 2-fold those of non-cancer controls. These results suggest that lung cancer patients have a higher susceptibility to DNA damage than non-cancer controls. Moreover, when the control group were split into either infectious disease control or non-infection control groups. We found that the DNA adduct levels of 11 infection controls (19.51±11.74 adduct/10^8 nucleotides) did not differ from those of 22 non-infection controls (17.25±17.05 nucleotides/10^8 nucleotides; $P = 0.302$).

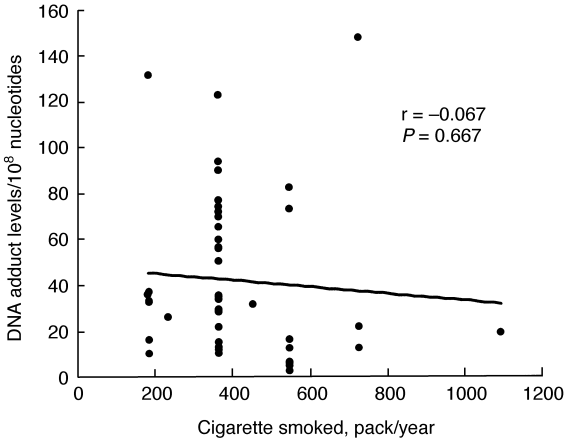

Fig. 1. The correlation between DNA adduct levels in lung tissues and cigarette consumption of smokers analysed by Spearman rank correlation.

Table 2
Comparison of DNA adduct levels between lung cancer patients and non-cancer controls with different gender, smoking status and polymorphisms of *CYP1A1* and *GSTM1*[a]

Parameter	DNA adduct levels/10^8 nucleotides mean±SEM				*P* value
	n	Lung cancer	*n*	Non-cancer	
Type	73	49.58±33.39	33	18.00±15.33	< 0.001
Gender					
Female	22	58.03±32.65	9	16.44±11.20	< 0.001
Male	51	45.94±33.36	24	18.59±16.79	< 0.001
P value		0.118		0.827	
Smoking status					
Non-smoking	38	49.28±30.73	22	19.01±17.90	< 0.001
Smoking	32	49.03±37.21	11	15.99±8.52	0.002
P value		0.719		0.836	
CYP1A1 polymorphism					
m1/m1 (A)	24	37.99±24.22	8	17.61±10.78	0.014
m1/m2 (B)	35	50.24±30.80	15	21.38±20.60	0.001
m2/m2 (C)	14	67.78±45.27	10	13.25±6.36	0.0001
P value		0.081		0.455	
GSTM1 polymorphism					
Negative (−)	34	50.06±40.72	17	16.94±9.86	< 0.001
Positive (+)	39	43.93±24.55	16	19.14±19.88	< 0.001
P value		0.350		0.845	
CYP1A1/*GSTM1*					
A/−	8	50.96±29.79	4	18.05±12.32	0.073
A/+	16	31.51±18.73	4	17.17±10.90	0.249
BC/−	26	57.62±43.92	13	16.59±9.54	< 0.001
BC/+	23	52.58±24.72	12	19.79±22.46	0.001
P value		0.083		0.956	

[a] The difference of *CYP1A1* polymorphism and *CYP1A1*/*GSTM1* polymorphisms were calculated by Kruskal-Wallis H test, and the others were calculated by Wilcoxon rank sum test.

Table 3
The association between protein expressions of CYP1A1 and GSTM1 and DNA adduct levels[a]

Protein expression	DNA adduct/10^8 nucleotides			*P* value
	Low ($n=22$)	Medium ($n=22$)	High ($n=20$)	
CYP1A1				
−	8	10	9	
+	7	5	3	
++	7	6	2	
+++	0	2	6	0.036
GSTM1				
−	3	5	10	
+	2	1	0	
++	8	9	3	
+++	9	8	7	0.131

[a] The protein expressions of CYP1A1 and GSTM1 were evaluated by immunohistochemistry, and the criteria of protein expression levels were described in the text. 64 of 73 lung specimens from cases were available in this study. The adduct levels of lung cancer patients were divided into three categories as follows: Low: 15.85±7.21 (2.4–29.27 adducts/10^8 nucleotides); Medium: 45.07±10.73 (31.18–62.60 adducts/10^8 nucleotides); High: 89.06±26.62 (64.46–147.09 adducts/10^8 nucleotides). The association between the protein expressions of CYP1A1 and GSTM1 and DNA adduct levels was statistically analysed by χ^2 test.

3.2. The DNA adduct level was not associated with smoking behaviour or cigarette consumption

Previous reports have indicated that the DNA adduct levels in lung tissues from lung cancer patients are associated with smoking behaviour [6,22]. However, our data showed that the DNA adduct levels in case and control groups did not differ between smokers and non-smokers ($P=0.719$ for case group, $P=0.836$ for control group, Table 2). Further linear correlation regression analyses showed no association between DNA adduct levels and cigarette consumption (pack/year) in the entire smoking population including cases and controls ($r=-0.067$, $P=0.667$, Fig. 1). These results suggest that smoking behaviour and cigarette consumption did not influence the DNA adduct level in the lung tissues of our study subjects.

3.3. The DNA adduct levels were associated with *CYP1A1* protein expression, but not associated with CYP1A1 *and* GSTM1 *polymorphisms and GSTM1 protein expression*

Involvement of CYP1A1 and GSTM1 in the activation and detoxification of polycyclic aromatic hydrocarbons (PAH) is well recognised [23,24]. The genotypes of both genes have been shown to be associated with the DNA adduct levels in some previous reports. In this study, our results showed that no correlations between the DNA adduct levels in lung tissues and the genetic polymorphisms of *CYP1A1* and *GSTM1*, or between the DNA adduct levels in lung tissues and the combination of *CYP1A1* and *GSTM1* polymorphisms (Table 2). No paraffin block of lung specimens from non-cancer controls can be offered. Thus, the association between DNA adduct levels and protein expressions of CYP1A1 and GSTM1 was restricted in lung cancer patients (Table 3). Our data showed that CYP1A1 protein expression was positively correlated with DNA adduct levels ($P=0.036$), but not with GSTM1 protein expression ($P=0.131$).

3.4. High DNA adduct level is a significant risk marker for lung cancer

To elucidate which variables, among gender, smoking status and polymorphisms of *CYP1A1* and *GSTM1*, affected the formation of DNA adducts in lung tissue, multivariate linear regression analyses were performed. The protein expressions of CYP1A1 and GSTM1 were not included as a variable as the immunostaining data was not available for all of the study population. The DNA adduct levels were approximately normally distributed after log transformation. Our results indicated that the DNA adduct levels in lung tissues from cases and controls were not influenced by any of the above variables (Table 4). Multivariate logistic regression analysis was used to verify whether the DNA adduct level acted as an independent risk biomarker for lung cancer. Among the variables studied were DNA adduct levels, age, gender, smoking status and polymorphisms of *CYP1A1* and *GSTM1*. The cut-off of two SDs above

Table 4
Multivariate logistic regression analysis of the risk of lung cancer in association with DNA adduct level, age, gender, smoking status and genetic polymorphisms of *CYP1A1* and *GSTM1*

Variables	Groups (*n*) unfavourable (*n*)/favourable (*n*)	OR (95% CI)	*P* value
DNA adduct level	>48.66 (34)/⩽48.66 (72)	25.19 (2.99–211.99)	0.003
Age (years)		1.06 (1.01–1.12)	0.02
Sex	Female (31)/male (75)	0.95 (0.24–3.69)	0.94
Smoking status	Smoking (43)/non-smoking (60)	1.36 (0.38–4.94)	0.64
CYP1A1 polymorphism	B,C (74)/A (32)	0.72 (0.38–1.37)	0.32
GSTM1 polymorphism	Positive (55)/negative (51)	1.64 (0.55–4.90)	0.38

the mean adduct level in the non-cancer control group was used to divide all study subjects into 'high' and 'low' DNA adduct groups. Our data showed that persons with high DNA adduct levels (>48.66 adducts/10^8 nucleotides) had an approximately 25-fold risk of lung cancer compared with the persons with low DNA adduct levels ($\leqslant 48.66$ adducts/10^8 nucleotides) (95% confidence interval (CI) = 2.99–211.99, $P = 0.003$). The odds ratios of the other variables except age (OR = 1.06, 95% CI = 1.01–1.12, $P = 0.02$) did not reach statistical significance (Table 4). Thus, DNA adduct level in the lung tissue acted as a significant risk biomarker for the development of lung cancer.

4. Discussion

Direct comparisons of DNA adduct levels in lung tissue of lung cancer patients and non-cancer controls have rarely been performed. Only two reports evaluated the DNA adduct levels in lung tissues from lung cancer and non-cancer patients. However, both studies have shown no difference in the level of aromatic DNA adducts of non-tumorous bronchial tissues between the lung cancer and non-cancer groups [22,25]. This finding was not consistent with our data. In our study, the DNA adduct levels in lung cancer patients were significantly higher than in non-cancer controls regardless of the smoking status. This conflicting result may be caused by different lung specimens from different populations. In previous reports, comparisons of the susceptibility to DNA damage between cases and controls mostly focused on smoking-induced DNA adducts in non-target human white blood cells [10–12,26]. Although one study showed that the DNA adduct level in white blood cells of cases was significantly higher than in controls [26]. DNA adduct levels in non-target white blood cells can not really reflect the DNA damage in the target lung tissue.

The adjacent lung tissue surrounding the tumour is not normal and has sustained some genetic alterations including *TP53* mutations that may cause a decrease in the capacity for DNA repair [27]. This may be a possible reason to explain why the tissue from the lung cancer patients had higher DNA adduct levels than the normal lung tissue from non-cancer control subjects who suffered from respiratory diseases other than malignancy. In this study, 33 such patients were used as controls to assess whether higher DNA damage in the lung cancer patients may be involved in lung carcinogenesis. We consider that bacterial infection may alter the DNA adduct levels in the lung tissue through interfering with metabolic activation, detoxification and DNA repair capabilities. In order to understand the possible effects of various respiratory diseases on DNA adduct levels, the control subjects were divided into infection diseases, such as tuberculosis, cryptococcus infection and non-infection diseases, such as pneumothorax and chest wall deformity. However, the DNA adduct levels were not significantly different in these two groups. This result suggests that the DNA adduct level in lung tissue might be not influenced by bacterial infection. Thus, at least in this study, the influence of various respiratory diseases on DNA adduct levels can be excluded.

Our data showed that smoking habit and/or cigarette consumption did not influence the DNA adduct levels in our cases or controls. Previous reports have indicated that the DNA adduct levels in lung tissues and leucocytes from lung cancer patients are significantly correlated with cigarette consumption [6,22]. However, some data do not support this finding. The lack of an association between the DNA adduct level and cigarette smoking found in this study may be due to two possible reasons. First, a relatively low number of smokers (43 of 106, 40.7%) were enrolled compared with similar studies reported previously. Although smoking is, in general, the major cause of lung cancer, less than 40% of the lung cancer incidence in Taiwan is caused by smoking [2]. Secondly, approximately half of the non-smokers in our study population were female who had been found to have a high susceptibility to DNA damage derived from active smoking and environmental carcinogen exposure (data not shown). The adduct level of the non-smoking group may, therefore, be increased by the high adduct levels in these non-smoking females leading to non significant differences in the adduct levels between the smoking and non-smoking groups. In addition, a non-linear exposure–DNA adduct relationship was observed in human white blood cells and rat lung tissues under high exposure conditions [29,30]. A recent report indicated that DNA adduct levels in white blood cells were correlated with the average concentrations of PAH in the ambient air of workers who smoked cigarettes, whereas in non-smokers no such relationship was found. They concluded that saturation of DNA adduct formation may occur, leading to non-linear response relationships [29]. It is believed that exposure to increased doses of carcinogen from environmental factors other than smoking may saturate the metabolic activation and induction of DNA repair processes or detoxification enzymes or other mechanisms may be involved in the alteration of DNA damage level. Our previous reports indicated that PAHs might be responsible for the mutagenicity of airborne particulates in Taiwan [31,32]. The levels of PAHs in airborne particulates in Taiwan are higher than those in other countries, especially the levels of benzo[a]pyrene, benzo[b]-fluoranthrene and benzo[g,h,i]perylene [31,32]. These genotoxic/carcinogenic compounds from environmental pollution may influence the formation of DNA adducts in lung tissues. Thus, we suggest that no difference in

DNA adduct levels between the lung tissues of smokers and non-smokers in our study, may be caused by a non-linear DNA adduct formation following exposure to high doses of carcinogens from the combination of smoking and environmental exposure.

The association between *CYP1A1* and *GSTM1* polymorphisms and the DNA adduct levels in lung tissues and white blood cells has been extensively investigated to predict individual lung cancer susceptibility [11,14,15]. Conflicting conclusions among several previous studies may have resulted from differences in race, sample size, smoking status or other confounding factors. A case–control study reported that individuals with the susceptible *CYP1A1* MspI genotype combined with a deficient *GSTM1* genotype are at remarkably high risk for lung cancer [33]. However, smoking-associated bulky DNA adduct levels in bronchial tissue from lung cancer patients were very similar for four combinations of *GSTM1* and *CYP1A1* genetic polymorphisms. This result suggests that *GSTM1* and *CYP1A1* polymorphisms do not influence the individual susceptibility to DNA damage. Moreover, Garcia-Closas and colleagues [34] reported that there is not enough evidence to support a substantial modification of the effect of pack-years on lung cancer risk by the *CYP1A1* and *GSTM1* genotypes. Our case–control study showed that *CYP1A1* MspI was not associated with risks for overall lung cancer subjects. Interestingly, when the cases were stratified according to histological type, there was significant association between homozygote variant of *CYP1A1* (m2/m2, C) and squamous cell carcinoma [35]. An early study demonstrated that the presence of PAH–dGMP adducts in lung tissues from non-cancer autopsy donors are associated with the *GSTM1* null genotype, but not with the *CYP1A1* exon 7 variant [36]. Ryberg and associates [37] recently indicated that the polymorphism of *GSTM1* in 135 male patients and 342 controls showing the null genotype is associated with a slightly increased lung cancer risk. In this study, we did not observe any association between DNA adduct levels and *CYP1A1*, *GSTM1* or between DNA adduct levels and the four combinations of polymorphisms in our study population (Table 2). A similar conclusion was reported by To-Figueras and colleagues [38] who showed that Northwestern Mediterraneans with the *GSTM1* null genotype did not have an increased lung cancer risk. In the phenotype study, the activity of aryl hydrocarbon hydroxylase linked to *CYP1A1* in lung tissues from smoking lung cancer patients was found to have a significant correlation with DNA adduct levels [39]. Our immunohistochemistry data in lung tissue specimens from lung cancer patients showed that CYP1A1 protein expression was significantly associated with the DNA adduct levels, but not GSTM1 protein expression (Table 3). These results suggest that CYP1A1 protein expression in DNA adduct formation may be more important than its polymorphism under heavy environmental pollution. In this study population, the DNA adduct levels were not associated with the genotype and phenotype of *GSTM1*.

DNA adduct levels in the target organ reflect the overall endpoint of DNA damage through metabolic activation, detoxification, DNA repair and cell proliferation pathways after carcinogen exposure including smoking and environmental factors. High DNA adduct levels are associated with high mutation frequencies of *TP53* and *K-ras* and tumour incidence in animal models [40,41]. Therefore, DNA adduct levels in non-target white blood cells have been used as a lung cancer risk biomarker [10–12]. A molecular epidemiological case–control study of lung cancer revealed that persons with high DNA adduct levels in peripheral leucocytes had a 7.7-fold relative risk when compared with persons with low DNA adduct levels, when the DNA adduct level was adjusted for age, gender, ethnicity and season [26]. Our study demonstrated that the DNA adduct level in the target lung tissue of the lung cancer patients had a higher susceptibility to DNA damage than tissue from non-cancer controls. Multivariate logistic regression analysis showed that persons with high DNA adduct levels had an approximately 25-fold relative risk when compared with persons with low DNA adduct levels. The relative risk estimated from DNA adduct levels the target organ of the lung was 3-fold the relative risk from peripheral leucocytes. This result showed that the evaluation of DNA adduct levels in the target organ may be more reliable for the estimation of cancer risk than the evaluation of DNA adduct levels in non-target organs.

In conclusion, the results of the DNA adduct evaluations in lung tissues are consistent with a constitutional susceptibility to lung cancer. Thus, DNA adduct levels in target lung tissue may be a more reliable lung cancer susceptibility biomarker than DNA adduct level in non-target leucocytes. In addition, higher susceptibility to DNA damage in lung cancer patients may partly play a role in the development of lung cancer.

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