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# Association of Low Activity of UGT1A7 with Lung Cancer in Taiwan: A Preliminary Case Control Study

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## ABSTRACT

This study was aimed to evaluate the correlation between the polymorphisms of the *UDP-glucuronosyltransferases1A7* (*UGT1A7*) gene and the risk of lung carcinogenesis in the Taiwanese population. A total of 230 lung cancer patients and 230 age- and gender-matched healthy individuals were enrolled in this case control study. *UGT1A7\*1* was defined as a high activity allele, while *UGT1A7\*2*, *UGT1A7\*3* and *UGT1A7\*4* were categorized as low activity alleles. The relationship between *EGFR* mutations and *UGT1A7* polymorphisms was investigated in this study. The frequency of *UGT1A7\*2* and *UGT1A7\*3* was significantly higher in the lung cancer group than in the control group ( $p = 0.03$ , odds ratio (OR) = 1.44, 95% confidence interval (CI) = 1.04 - 1.99 for *UGT1A7\*2*; and  $p = 0.01$ , OR = 1.56, 95% CI = 1.11 - 2.18 for *UGT1A7\*3*). The frequency of lower activity alleles was significantly higher in the lung cancer group than in the control group ( $p = 0.03$ , OR = 1.58, 95% CI = 1.04 - 2.40 for the high-activity allele/low-activity allele (H/L) group; and  $p < 0.01$ , OR = 2.23, 95% CI = 1.29 - 3.84 for the low-activity allele/low-activity allele (L/L) group). This difference was only significant in the male subgroup, with odds ratio of 1.87 (95% CI = 1.05 - 3.36,  $p = 0.03$ ) for the H/L group and 2.638 (95% CI = 1.28 - 5.42,  $p < 0.01$ ) for the L/L allele group. Yet, pathologic type and epidermal growth factor receptor (EGFR) mutations did not affect the distribution of *UGT1A7* in the patient group. The results suggested that the polymorphisms of the metabolic gene, *UGT1A7*, may contribute to reduced enzyme activity and subsequently affect the detoxification of carcinogens. It is therefore concluded that *UGT1A7* polymorphism is associated with lung carcinogenesis for the Taiwanese population.

Key words: *UGT1A7* polymorphisms, lung cancer, risk factor, EGFR mutation, gene, enzyme

## INTRODUCTION

Lung cancer is responsible for 1.3 million deaths worldwide annually<sup>(1)</sup>. It is ranked the number-one cause of all cancer deaths in both men and women in Taiwan<sup>(2)</sup>. Tobacco smoking is an important risk factor for lung cancer. In particular, the activity of xenobiotic enzymes has been linked to the modulation of risk<sup>(3,4)</sup>. Tobacco smoke contains more than 60 known carcinogens such as nicotine, nitrosamines and polycyclic aromatic hydrocarbons. These carcinogens are metabolized by both hepatic and extrahepatic enzymes, such as cytochrome P450 enzymes or uridine 5'-diphospho-glucuronosyltransferases (UGTs)<sup>(4-6)</sup>. The *UGT1A* gene complex

is located on human chromosome 2 at 2q37, which encoded nine functional proteins (UGT1, UGT1A3-UGT1A10)<sup>(7,8)</sup>. UGT1A7, a member of the UGT1 family, plays important roles in the conjugation and detoxification of several tobacco carcinogens, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)<sup>(9)</sup> and benzo-[a]-pyrene (BaP)<sup>(10,11)</sup>. The catalytic activity of UGT1A7 is determined by its polymorphisms at codon 129, 131 and 208. *UGT1A7\*1* (wild type) has the highest activity, followed by *UGT1A7\*2* (K<sup>129</sup>K<sup>131</sup>W<sup>208</sup>), *UGT1A7\*4* (N<sup>129</sup>R<sup>131</sup>R<sup>208</sup>) and *UGT1A7\*3* (K<sup>129</sup>K<sup>131</sup>R<sup>208</sup>)<sup>(11)</sup>. In recent reports in Japan, the presence of *UGT1A7* polymorphisms has been suggested to be a risk factor in orolaryngeal cancer<sup>(12)</sup>, proximal GI tract cancer<sup>(13)</sup>, hepatocellular carcinoma<sup>(14)</sup>, colorectal cancer<sup>(15,16)</sup>, pancreatic cancer<sup>(17)</sup> and lung cancer<sup>(6)</sup>.

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Non-smoking women have a high incidence rate of lung adenocarcinoma in Taiwan and other Asian countries, indicating that there may be risk factors other than smoking, which leads to lung cancer. Alteration of genes such as over-expression of oncogenes<sup>(18)</sup>, silencing of tumor suppressor genes<sup>(19,20)</sup> and defects in DNA repair mechanism<sup>(21)</sup> may also contribute to lung carcinogenesis. Most Asian female patients with lung cancer are non-smokers and they often represent a distinct type. Adenocarcinoma and epidermal growth factor receptor (EGFR) overexpression are the most predominant<sup>(22)</sup>. EGFR has been identified as an important prognostic factor in the advanced stage of lung cancer. The clinical outcomes after the therapy of EGFR tyrosine kinase inhibitor can be predicted by EGFR mutations<sup>(23)</sup>.

The purpose of this study was to examine the relationship between *UGT1A7* polymorphism and *EGFR* mutations in the Taiwanese population and to determine whether *UGT1A7* polymorphisms are potentially associated with a higher risk of lung cancer. The distribution of *UGT1A7* alleles, genotypes and their predicted phenotypes in lung cancer patients were compared with the controls. *EGFR* mutations in the lung cancer patients were also analyzed.

## MATERIALS AND METHODS

### I. Patients and Clinical Specimens

A total of 230 lung cancer patients from Wan Fang Hospital, Taipei Medical University were studied between 2004 and 2009 according to a protocol approved by the Institutional Review Board (IRB). All patients who participated in this study signed the letter of informed consent and had the willingness and ability to give informed consent. A confirmed diagnosis of primary lung cancer and ages between 18 and 90 were the specific inclusion criteria in this study. The peripheral blood and clinical information of the patients were collected after obtaining informed consent. Of the 230 patients, 197 cancer-containing tissues were collected from bronchoscopic biopsies (70.6%), proven malignant pleural effusion (26.9%) or lobectomy (2.5%). A total of 230 healthy volunteers who were matched with the

cancer patients by age and sex, and had no history of cancer or disease associated with *UGT1A7* polymorphism, such as Gilbert's syndrome, pancreatic diseases, hyperbilirubinemia and hepatitis C, were randomly recruited into the study over the same period of time.

### II. Determination of Genotypes

In order to determine the *UGT1A7* genotyping, genomic DNA from peripheral blood mononuclear cells was extracted by proteinase K digestion, followed by the conventional phenol-chloroform method as previously described<sup>(24)</sup>. Genomic DNA from paraffin-embedded tissues was extracted by commercial kits (DEXPAT<sup>TM</sup>, Takara, Shiga, Japan) for the detection of *EGFR* mutation.

The identification of each subject's *UGT1A7* alleles was performed by PCR-restriction fragment length polymorphism (RFLP) analysis for the nucleotide 622 polymorphic sites and nested PCR was performed for nucleotide 387 polymorphic positions. The use of these combined analyses allowed the differentiation of all genotypes formed by *UGT1A7\*1*, *UGT1A7\*2*, *UGT1A7\*3* and *UGT1A7\*4* alleles, except *UGT1A7\*1/UGT1A7\*3* and *UGT1A7\*2/UGT1A7\*4*. Since the frequency of the *UGT1A7\*4* allele in the Chinese population was less than 3%<sup>(11,17,25)</sup>, the subjects who exhibited either the *UGT1A7\*1/UGT1A7\*3* or *UGT1A7\*2/UGT1A7\*4* genotypes were considered to have the *UGT1A7\*1/UGT1A7\*3* genotypes, according to previous reports<sup>(12,26)</sup>.

The PCR reaction for generating *UGT1A7* 622T→C and *UGT1A7* 387T→G DNA fragments was performed in a thermal cycler (PC806, ASTEC, Japan) in the presence of 0.2 mM of dNTPs (Protech, Taiwan), 1 x Taq buffer (Protech, Taiwan), 0.04 U/μL of Taq polymerase (Protech, Taiwan), 1 μL of forward and reverse primers (Table 1) and 0.5 μL of DNA prepared in a total volume of 25 μL<sup>(26)</sup>. The reaction was performed under the following conditions: 1 cycle at 94°C for 3 min, followed by 34 cycles at 94°C for 30 s; 55°C for 30 s; 72°C for 1 min, and 1 cycle at 72°C for 10 min, and then cooling down at 4°C. Primary and nested PCR amplifications were utilized to determine *UGT1A7* 387T→G DNA fragments and to further confirm by restriction enzymes

**Table 1.** Primer sequences used in *UGT1A7* genotyping

Position (cDNA)	Primers	Sequence	Restriction Enzyme	Result (bp)
622 T→C	U7F3	5'-TGTC CCCAGACTTCTCTTAG-3'	RsaI	WT: 447
	U7R3	5'-GCTACCC AACAATTAAGTGA-3'		MT: 54+393
387 T→G	1 <sup>st</sup> PCR			
	U7F1	5'-TGAATGAATAAGTACACGCC-3'		
	U7R2	5'-TAGGGGGCAAATAAATGTTTC-3'		
	Nested PCR			
	387F	5'-AAATTGCAGGAGTTTGC*TTA-3'	AflIII	WT: 159
387R	5'-TGGCAAATATTCCCCTGGC-3'		MT: 142+17	

digestion. The UGT1A7 622T→C DNA fragments were digested by RsaI (Promega, USA). DNA fragments were separated by 3% agarose gel electrophoresis at 100 V for 40 min. While UGT1A7 387T→G DNA fragments were digested by AflIII (New England BioLabs, UK), sizes were fractioned by 8% polyacrylamide gel electrophoresis at 100 V for 95 min. Both were then visualized by ethidium bromide staining. According to previous literatures on UGT1A7 enzyme activity, UGT1A7\*1 was defined as a higher activity allele and UGT1A7\*2, UGT1A7\*3 and UGT1A7\*4 were categorized as lower activity alleles<sup>(10,11)</sup>. The predicted phenotypes were categorized into high-activity allele/high-activity allele (H/H), high-activity allele/low-activity allele (H/L), and low-activity allele/low-activity allele (L/L)<sup>(27)</sup>.

The detection of *EGFR* mutations in exon 18-21 was performed by PCR. Table 1 represents the primers used in the study and the representative results have been previously described<sup>(28)</sup>. Direct sequencing of the mutant bands was also used to validate the accuracy of detection.

### III. Statistical Analysis

SPSS 13.0 (SPSS Inc, Chicago, IL, USA) for Windows was used to analyze continuous and categorical data. The continuous data were analyzed by Student's *t*-test. Multivariable logistic regression was applied to calculate the odds ratios for comparing the differences in the predicted phenotypes, genotypes and variant allele frequencies between patients and normal controls. Two-tailed  $p < 0.05$  was considered as statistically significant. The Hardy-Weinberg equilibrium of alleles at individual loci was also evaluated.

## RESULTS

### I. Clinical and Histological Data

Table 2 depicts the demographic characteristics of cancer patients and normal controls. Both groups of lung cancer patients and healthy individuals consisted of 128 males (55.7%) and 102 females (44.3%). The majority of patients ( $n = 223$ , 96.9%) were diagnosed with non-small cell lung cancer (NSCLC) at an advanced stage. Among the patients, 77 (33.5%) were diagnosed with stage III B and 113 (49.1%) with stage IV.

### II. Association of UGT1A7 Polymorphism with Lung Cancer

The alleles at the individual loci fulfilled the Hardy-Weinberg distribution in both patient and control groups ( $p > 0.05$ ). The frequencies of UGT1A7\*2 and UGT1A7\*3 were significantly higher in the lung cancer group. ( $p = 0.03$ , odds ratio (OR) = 1.44, 95% confidence interval (CI) = 1.04 - 1.99 for UGT1A7\*2; and  $p = 0.01$ , OR = 1.56, 95% CI = 1.11 - 2.18 for UGT1A7\*3, respectively, Table 3). Subgroup analysis that compared male lung cancer patients with male controls revealed a similar result, with an odds

ratio of 1.59 (95% CI = 1.06 - 2.43,  $p = 0.03$ ) for UGT1A7\*2, and 1.63 (95% CI = 1.05 - 2.54,  $p = 0.03$ ) for UGT1A7\*3. However, the frequencies of UGT1A7\*2 and UGT1A7\*3 were not significantly different between the female lung cancer patients and their corresponding controls.

There was a significant difference in the distribution of genotypes between the patients and controls. Cancer patients had a higher percentage of UGT1A7\*1/\*3, UGT1A7\*2/\*2 and UGT1A7\*3/\*3 genotypes than the controls, suggesting that genotypes of lower activity might be a potential risk factor for the development of lung cancer ( $p = 0.02$ , OR = 1.83, 95% CI = 1.10 - 3.04 for UGT1A7\*1/\*3;  $p < 0.01$ , OR = 3.89, 95% CI = 1.44 - 10.51 for UGT1A7\*2/\*2; and  $p = 0.04$ , OR = 2.92, 95% CI = 1.04 - 8.20 for UGT1A7\*3/\*3). Results of further analysis on the predicted phenotypes showed that the frequency of cancer patients having at least one low-activity allele was significantly higher than the controls ( $p = 0.03$ , OR = 1.58, 95% CI = 1.04 - 2.40 for the H/L group; and  $p < 0.01$ , OR = 2.23, 95% CI = 1.29 - 3.84 for the L/L group, Table 4).

Comparing the subgroup analysis of male patients with that of male controls, similar results were revealed for the H/L and L/L allele groups, with an OR of 1.87 (95% CI = 1.05 - 3.36,  $p = 0.03$ ) and 2.64 (95% CI = 1.28 - 5.42,  $p < 0.01$ ), respectively. However, this trend was not observed in female patients. These results suggested a positive

**Table 2.** Demographic characteristics of enrolled patients with lung cancer and the control participants

	Lung cancer (n = 230)	Control (n = 230)
Sex (%)		
Male	128 (55.7)	128 (55.7)
Female	102 (44.3)	102 (44.3)
Age (year)	67.74 ± 12.23	67.70 ± 13.15
Pathology Classification (%)		
Non-small cell lung cancer	223 (96.9)	
Adenocarcinoma	181 (78.8)	
Squamous cell carcinoma	32 (13.9)	
Others	10 (4.3)	
Small cell lung cancer	7 (3.0)	
Lung cancer stage (%)		
IA	14 (6.1)	
IB	9 (3.9)	
IIA	2 (0.9)	
IIB	5 (2.2)	
IIIA	3 (1.3)	
IIIB	77 (33.5)	
IV	113 (49.1)	
Limited (small cell type)	1 (0.4)	
Extensive (small cell type)	6 (2.6)	

**Table 3.** Comparison of the allelic frequency of *UGT1A7* between lung cancer patients and the control group

Allele Frequency	Lung cancer (n = 460)	Control (n = 460)	OR	95% CI	p - value
*1	0.516 (237)	0.613 (282)	ref	-----	-----
*2	0.250 (115)	0.207 (95)	1.44	1.04 - 1.99	0.03*
*3	0.230 (106)	0.176 (81)	1.56	1.11 - 2.18	0.01*
*4	0.004 (2)	0.004 (2)	1.19	0.17 - 8.51	0.86
Female (n = 204 in each group)					
*1	0.554 (113)	0.627 (128)	ref	-----	-----
*2	0.225 (46)	0.202 (41)	1.27	0.78 - 2.08	0.34
*3	0.211 (43)	0.162 (33)	1.48	0.88 - 2.48	0.14
*4	0.010 (2)	0.009 (2)	1.13	0.16 - 8.17	0.90
Male (n = 256 in each group)					
*1	0.484 (124)	0.602 (154)	ref	-----	-----
*2	0.270 (69)	0.211 (54)	1.59	1.04 - 2.43	0.03*
*3	0.246 (63)	0.187 (48)	1.63	1.05 - 2.54	0.03*
*4	0.000 (0)	0.000 (0)	-----	-----	-----

\*  $p < 0.05$  compared with control.

**Table 4.** Comparison of predicted phenotypes between lung cancer patients and the control group

UGT1A7 Predicted phenotype	Lung cancer (n = 230) (%)	Control (n = 230) (%)	OR	95% CI	p - value
H/H	59 (25.7)	86 (37.4)	ref	-----	-----
H/L	119 (51.7)	110 (47.8)	1.58	1.04 - 2.40	0.03*
L/L	52 (22.6)	34 (14.8)	2.23	1.29 - 3.84	< 0.01*
Female (n = 102 in each group)					
H/H	31 (30.4)	38 (38.3)	ref	-----	-----
H/L	51 (50.0)	50 (49.0)	1.28	0.70 - 2.37	0.43
L/L	20 (19.6)	13 (12.7)	1.94	0.83 - 4.50	0.13
Male (n = 128 in each group)					
H/H	28 (21.9)	47 (36.7)	ref	-----	-----
H/L	67 (52.3)	60 (46.9)	1.87	1.05 - 3.36	0.03*
L/L	33 (25.8)	21 (16.4)	2.64	1.28 - 5.42	< 0.01*

\*  $p < 0.05$  compared with control.

correlation between these alleles and the presence of lung cancer. These alleles encoded protein with low catalytic activity of UGT1A may contribute to increased risk of lung cancer in male but not female patients.

A stratified analysis of smoking rate in the male lung cancer patients was performed to evaluate the effect of smoking on these patients. Of the 128 male patients, 108 (85.3%) were smokers. The smoking rates of the male lung cancer patients were 82.1%, 86.6% and 93.3% in the H/H, H/L and L/L groups, respectively, showing no statistically significant difference ( $p = 0.43$ ). This suggests that smoking was not the confounding factor for the observed differences in *UGT1A* genotypes in males.

Although the frequencies of genotypes and predicted

alleles were different between the lung cancer patients and the controls, further analysis failed to demonstrate a significant association between *UGT1A7* polymorphisms and pathologic types or stages. The patient numbers identified with H/H, H/L and L/L *UGT1A7* genotypes were 26 (23.0%), 58 (51.3%) and 29 (25.7%) for those with stage IV NSCLC, and 34 (30.9%), 57 (51.8%) and 19 (17.3%) for earlier stages of NSCLC, respectively ( $p = 0.21$ ). Of the 181 patients with adenocarcinoma, 52 (28.7%), 92 (50.8%) and 37 (20.4%) patients were identified with H/H, H/L and L/L *UGT1A7* genotypes, while in other types of lung cancer, the patient numbers were 8 (16.3%), 28 (57.1%) and 13(26.5%), respectively ( $p = 0.19$ ).

### III. Association between *UGT1A7* Polymorphism and *EGFR* Mutations

Of the 197 tissue samples collected from the cancer patients, 72 (36.5%) cancer patients showed *EGFR* mutations. The mutations of exon 19, exon 20 and exon 21 accounted for 36.1% (n = 26), 4.1% (n = 3) and 59.7% (n = 43), respectively. The results were consistent with prior studies, which indicated that the female gender and non-smoking status were risk factors for *EGFR*<sup>mut</sup> lung cancer. The mutation occurred in 46 (51%) out of 91 females and 26 (25%) out of 106 males, showing a positive association with lung cancer (OR = 3.15, 95% CI = 1.72 - 5.75,  $p < 0.01$ ). In addition, the mutation rate was 57.3% (n = 55) in the non-smokers, which was significantly higher than the smokers (16.8%, n = 7,  $p < 0.01$ ; OR = 6.63; 95% CI = 3.43 - 12.82). However, there was no significant difference in the distribution of *UGT1A7* polymorphisms for the patients with or without *EGFR* mutations.

## DISCUSSION

The relationship between *UGT1A7* genotypes and the risk of lung cancer in the Taiwanese population was evaluated in this study. Our results were consistent with a previous Japanese study that showed a strong correlation between the *UGT1A7* genotype and lung cancer<sup>(6)</sup>. In addition to reports revealed by the previous study, our results indicated that patients with predicted low-activity phenotypes, i.e. one or two of the *UGT1A7*\*2, *UGT1A7*\*3 and *UGT1A7*\*4 alleles, were associated with an increased risk of lung cancer in the Taiwanese population. This association was found in the male subgroup for the first time by subgroup analysis in this study. The stratified analysis showed that the smoking rates among the three groups with *UGT1A7* H/H, H/L and L/L genotypes were similar, indicating that smoking was not a confounding factor of genotype variables. Since the smoking rate of male lung cancer patients (85.3%) was much higher than the rate of male adults (35.4%) in Taiwan, the accumulation of tobacco carcinogens was expected to be increased in those with low activity of *UGT1A7* expression, leading to elevated lung carcinogenesis in these patients<sup>(29)</sup>.

Our finding was in line with the known consequence of low *UGT1A7* activity, leading to the accumulation of carcinogens as a result of reduced capacity of conjugation and detoxification against them. In addition to lung cancer, low-activity alleles of *UGT1A7* have also been implicated in colorectal cancer<sup>(15,16)</sup>, with dietary carcinogen intake<sup>(30)</sup>, smoking and alcohol consumption<sup>(25)</sup> as modifying factors. Likewise, similar trends have been found in the susceptibility of proximal gastrointestinal tract cancers and orolaryngeal cancers for the smokers in particular<sup>(12,23)</sup>. The frequency of the wild type *UGT1A7*\*1 allele in the present study was more than 50% of the population, which was similar to previous studies on the *UGT1A7* genotypes

in the Chinese, Taiwanese and Japanese populations<sup>(6,25,26)</sup>, but higher than that in Caucasians<sup>(12)</sup>. This suggested that ethnic differences in the *UGT1A7* genotypes may exist between Caucasians and Asians.

The association between lung cancer and *UGT1A7*, a gene that metabolizes carcinogens from smoking, was not significant in female patients and this finding is disclosed for the first time in this report. The finding echoes a previous molecular epidemiologic discovery on a pathogenesis of lung cancer unrelated to the detoxication of carcinogens, especially derived from female patients who smoke<sup>(31)</sup>. The smoking rate of the female patients was 11.8% and not significantly different from that of Taiwanese female adults<sup>(29)</sup>, indicating that the *UGT1A7* gene had a low impact on interrupting the cancer development in the female patients. Previous research also found that the female gender was a sole risk factor independent of smoking for *EGFR*<sup>mut</sup> NSCLC<sup>(32)</sup>. With significantly higher incidence of *EGFR* mutations found in Asian female patients as shown in our study and previous reports, other risk factors and carcinogenesis correlated to *EGFR* mutations should be considered to prevent lung cancer in this subpopulation<sup>(33)</sup>. On the other hand, the suspicion that low activity alleles of *UGT1A7* may be more common among the patients with *EGFR* wild type than those with *EGFR* mutations was ruled out by the observation that the mutations of *EGFR* and the polymorphisms of *UGT1A7* were unrelated.

No single factor or class of genes shall determine the development of lung cancer as it is a complex disease involving several genetic, biological, psychological, social and environmental factors. For example, inherited germline mutation in tumor suppressor gene *P53* significantly increases the risk of developing lung cancer<sup>(19)</sup>. A higher risk in non-small cell lung cancer was associated with functional polymorphism in the promoter region of the *MDM2* gene, one that controls *MDM2* transcription and *P53* activity<sup>(20)</sup>. Alteration in DNA repair proteins, such as *ERCC2*, results in impaired DNA repair capability and also increases the risk of lung cancer<sup>(21)</sup>. Apart from *UGT1A7*, other carcinogen metabolic genes, such as cytochrome P450, particularly the *CYP1A1* subfamily polymorphisms, are also involved in increasing the risk of lung cancer<sup>(4,5)</sup>. Individuals with combined *NAT1* rapid and *NAT2* slow genotype seemed to have a significantly higher risk for lung adenocarcinoma<sup>(34)</sup>, or the *NAT2* slow genotype when combined with the *GSTM1* null genotype may increase the susceptibility to adduct formation, gene mutation and lung cancer risk<sup>(35)</sup>. With the consideration of the significant genes involving the development of lung cancer, better prediction and prevention of the disease may be achieved. Correlations of toxin-metabolizing genes and risk factors should be investigated to predict the risk of lung cancer in future studies.

The results of the current study suggest that *UGT1A7* polymorphism is associated with lung cancer in a Taiwanese subpopulation. Lower activity alleles and predicted phenotypes are significantly associated with an elevated risk of lung cancer, with the exclusion of female patients. Based on this observation, the determination of *UGT1A7* polymorphisms

may provide additional information for high risk groups and support in developing preventive strategies against lung cancer, especially in male smokers. Further studies on a larger population should be performed to confirm these findings.

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