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Is folic acid associated with lung cancer development? A cross-sectional study in NHANES

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Abstract

Objective The folic acid is used as an adjuvant dietary supplement in cancer treatment, but its potential benefits or adverse effects in lung cancer (LC) management remain unclear. This study aimed to examine the relationship between different forms of folic acid and LC based on a national population-based survey and conduct a thorough analysis of the potential use of folic acid in cancer treatment.

Methods Cross-sectional analysis from the 2007–2018 National Health and Nutrition Examination Survey (NHANES) was assessed. A cohort of 27,631 participants was identified and weighted. Information on folic acid levels and malignancy was determined through laboratory tests and interviews. To address potential confounding variables, a 1:2 propensity score matching (PSM) was employed, and 201 participants were included. This study utilized restricted cubic splines (RCS) to explore the none-linear relationship between various forms of folic acid and the incidence of LC.

Results Significant associations were observed between clinical characteristics of the participants and LC in both unweighted and weighted analyses. Following PSM, total folate, dietary folate, 5-formyltetrahydrofolate (5-formylTHF) and 5,10-methylenetetrahydrofolate (5,10-methenylTHF) were significantly associated with LC risk ($p < 0.05$). The RCS analysis suggested that there was a significant non-linear association between 5,10-methenylTHF and the odds of developing LC. Additionally, total folate, folic acid, 5,10-methenylTHF, and RBC folate influenced the likelihood of developing LC in a certain dose range.

Conclusions The development of LC was associated with total folate, dietary folate, 5-formylTHF and 5,10-methenylTHF levels. Folic acid, total folate, 5,10-methenylTHF, and RBC folate were positively correlated with LC within a certain range.

Keywords Folic acid, Lung cancer, Restricted cubic splines, NHANES, Cross-sectional study

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Introduction

Lung cancer (LC), the leading cause of cancer-related mortality worldwide, claims approximately 350 lives daily—exceeding the combined death toll of breast, prostate, and colorectal cancers [1, 2]. The lethality of this malignancy stems from its insidious onset, frequently delaying diagnosis until advanced disease [3], thereby imposing a catastrophic burden on global public health [4, 5]. While tobacco smoking remains the predominant risk factor, emerging evidence highlights the critical roles of genetic susceptibility and modifiable lifestyle factors, particularly dietary habits, in LC pathogenesis [6]. Therefore, further research is necessary to identify and address the influencing factors of LC and develop effective interventions.

Folic acid, a water-soluble B vitamin, is a cofactor in the essential single-carbon pathway responsible for providing methyl groups to choline phospholipids, creatine, adrenaline, and DNA [7, 8]. Extensive research has been conducted on the relationship between folic acid and various tumors, such as colorectal cancer [9, 10], endometrial cancer [11], breast cancer [12], bladder cancer [13], LC [14], and pancreatic cancer [15]. Numerous studies demonstrated that folic acid supplementation exerted dual modulatory effects in certain cancers. On the one hand, folic acid supplementation may enhance immune function, improve the ability of the body to combat cancer [14], and mitigate the genotoxic effects [16]. And folic acid supplementation reduces the probability of cancer occurrence by repairing DNA and preserving DNA integrity and stability [17], since aberrations in DNA potentially increase the risk of cancer development. Primary dietary sources of folate (e.g., leafy greens, legumes, citrus fruits) with alkaline nature, may inherently reduce dietary acid load (DAL). Studies suggested that elevated DAL was associated with increased cancer risk [18], potentially through suppressing folate activity or disrupting metabolic pathways, thereby destabilizing DNA integrity and triggering abnormal cell proliferation, which may elevate lung cancer risk. Thus, folate supplementation may counteract the harmful effects caused by high DAL. On the other hand, increased folate levels can enhance tumor cell proliferation and elevate the risk of cancer by perturbing the homeostasis of single-carbon metabolism or modifying the specific methylation patterns of DNA and histone promoters [7, 19]. These conflicting findings underscore the urgency of clarifying dose-response relationships between folic acid and LC.

Given the uncertain dose-response relationship between folic acid and LC, alongside conflicting evidence on its therapeutic efficacy in tumors, this study analyzed associations of 11 distinct folate forms with LC occurrence and explored novel clinical perspectives, through utilizing data from the National Health and Nutrition Examination Survey (NHANES) database. Our findings aimed to reconcile prior discrepancies, inform targeted nutritional guidelines, and identify high-risk populations warranting tailored interventions.

Methods

Research population

The NHANES database (https://wwwn.cdc.gov/nchs/nhanes/) is a nationally representative cross-sectional survey conducted in the United States that aims to assess nutritional status, programs, and policies to meet public health needs [20]. This database utilizes personal interviews, standardized physical examinations, and laboratory tests to comprehensively evaluate nutritional status [21]. By employing a complex multistage probability sampling design, the NHANES database provides nationally representative samples for analysis. Therefore, we used the NHANES database to assess correlations between various forms of folic acid and LC development.

The NHANES database has been collecting detailed and targeted data on dietary supplements since 2007. Therefore, this study used data from NHANES for a total of 10 cycles between 2007 and 2018 (2007–2008, 2008–2009, 2009–2010, 2010–2011, 2011–2012, 2013–2014, 2015–2016, 2016–2017, and 2017–2018) with 59,842 individuals. After excluding individuals younger than 20 years of age and those with missing information on dietary folate or missing information at baseline, 27,631 individuals were enrolled in this study. A flowchart of the screening process was shown in Fig. 1. Noteworthy, data of THF, 5-formylTHF, 5,10-methenylTHF, UMFA, and MeFox were selected since 2011 to 2018, because the NHANES database was launched with these data collection starting form 2011.

Screening methods for covariates

This study collected baseline information from the NHANES database, including age, sex, race, marital status, education, BMI, hypertension, diabetes, smoking status, alcohol consumption, and the ratio of family income to poverty (PIR). Home interviews collected information on age, sex, race, marital status, income, education, and smoking status. At the Mobile Examination Center (MEC), professionals collected information on height, weight, and alcohol consumption. BMI was calculated by dividing weight by height squared (kg/m^2). Alcohol consumption was defined as consumption of at least 15 drinks every 12 months. Participants were classified as smokers if they had smoked more than 100 cigarettes in their lifetime. Diseases, such as hypertension and diabetes, were classified based on a clear diagnosis provided by a specialized physician or health professional. In addition, participants provided personal interview data on diabetes, pre-diabetes, use of insulin or oral hypoglycemic

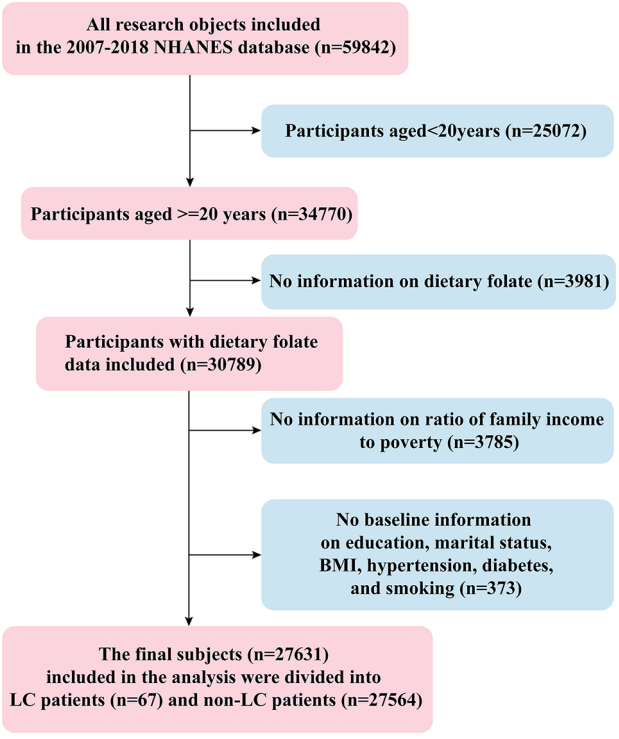

Fig. 1 Flow chart of participant selection in the analytic cohort within NHANES

medications, and diabetic retinopathy. Trained interviewers asked these questions using a computer-assisted personal interview (CAPI) system.

Folic acid concentration determination

Different forms of folic acid in serum, namely, 5-methyltetrahydrofolate (5-methylTHF), 5-formyltetrahydrofolate (5-formylTHF), pteroylglutamic acid (folic acid), tetrahydrofolate (THF), 5,10-methylenetetrahydrofolate (5,10-methenylTHF), unmetabolized folic acid (UMFA), and pyrazino-s-triazine derivative of 4-alpha-hydroxy-5-methyltetrahydrofolate (MeFox), were determined using isotope-dilution high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) [22]. Serum folate levels were calculated by adding the folic acid, 5-formylTHF, THF, and 5,10-methylTHF levels. MeFox was not included in the total folate calculation because it was already present in the body. Information on dietary folate was obtained through two 24-hour dietary recall interviews with the participants. RBC folate was determined using a microbiological assay. The first dietary recall interview was conducted in person at the MEC, and the second interview was conducted via telephone 3–10 days later. These data include over-the-counter personal and prescribed dietary supplements. The comprehensive Dietary Investigator's Procedures Manual provided by NHANES presents a complete explanation of the methods and examination procedures used to gather dietary data.

Malignancy status

The malignancy status was determined by a trained professional interviewer using the CAPI system to evaluate the diagnosis of malignant disease. The interviews were conducted using the following questions: "Has your doctor or other health professional told you that you have a malignant neoplasm or any type of malignant neoplasm?". If the participant answered "yes.", they were classified as having a malignant tumor. They would then be asked, "What is the type of malignant tumor?". Their responses would help identify the type of malignant tumor. Patients included in this study were currently or previously diagnosed with LC.

Statistical methods

Categorical variables were expressed as number and percentage; chi-square test was used to test the comparative variability between groups. For quantitative data, normality was assessed using the Shapiro-Wilk test. If the data conformed to a normal distribution, they were expressed as mean ± standard deviation, and the differences were compared using independent samples or paired samples t-test. If the data were not normally distributed, they were expressed as median and interquartile range. Differences between two independent groups were compared using a nonparametric test (Mann-Whitney U test), and paired samples were compared using the Wilcoxon signed-rank test.

The NHANES database is weighted using the MEC weighting process, whose variable is named WTMEC2YR. To reduce the selection bias in patients with LC and healthy individuals, this study used propensity score matching (PSM) to correct for confounders. PSM is a logistic or probit regression with intervening factors as the dependent variables, and all observed non-study factors as independent variables to give the probability of an individual being diagnosed with LC under the given covariates, yielding the probability that an individual will be diagnosed with LC. The caliper was set to 0.1. The restricted cubic spline (RCS), a continuously smoothed segmented cubic polynomial, was used to explore the dose-response relationship between different folic acid concentrations and LC by choosing the position and number of nodes and fitting a spline function, RCS (X), such that the continuous variable X exhibits a smooth curve over the entire range of values. $p < 0.05$ was considered to be statistically significant, and statistical analyses were performed using SPSS software (version 24.0; IBM, Armonk, NY, USA) and R software v. 4.2.2.

Results

Baseline characteristics of participants

This study utilized data from the NHANES database spanning 10 cycles (2007–2018) to analyze the association between folic acid and LC. A total of 59,842 participants were initially included as of December 31, 2018; 27,631 eligible participants remained after screening for missing information and age discrepancies. Among these, 67 LC patients and 27,564 non-oncology participants were included in this study. The participant characteristics are found in Table 1. Of the LC patients, 77.6% were aged 60 years or older, 56.7% were female, and 62.7% were non-Hispanic white. Furthermore, 44.8% of the LC patients were married. Most of the patients with LC had attained a high school education or higher (74.6%), and the majority had a BMI of 25 kg/m^2 or above (59.7%). In addition, many patients had hypertension (58.2%), but fewer had diabetes mellitus (26.9%). Most patients with LC were smokers (89.6%), with no reported consumption of more than 15 alcoholic drinks in a 12-month period. Most patients had moderate economic status (65.7%). Notably, significant differences in age, race, marital status, BMI, hypertension, diabetes, smoking, and alcohol

Table 1 Baseline characteristics of NHANES participants during 2007–2018 (before weighted)

Variables	Total, *N* (%)	Non-LC, *N* (%)	LC, *N* (%)	*P* value
Total	27,631	27,564 (99.8)	67 (0.2)	
Age (years)				< 0.001
<60	18,492 (66.9)	18,477 (67.0)	15 (22.4)	
≥60	9139 (33.1)	9087 (33.0)	52 (77.6)	
Sex				0.186
Male	13,441 (48.6)	13,403 (48.6)	38 (56.7)	
Female	14,190 (51.4)	14,161 (51.4)	29 (43.3)	
Race				0.002
Non-Hispanic white	11,908 (43.1)	11,866 (43.0)	42 (62.7)	
Non-Hispanic black	5853 (21.2)	5840 (21.2)	13 (19.4)	
Other	9870 (35.7)	9958 (35.8)	12 (17.9)	
Marital status				< 0.001
Married	14,163 (51.3)	11,433 (51.3)	30 (44.8)	
Unmarried	7325 (26.5)	7318 (26.5)	7 (10.4)	
Other	6143 (22.2)	6113 (22.2)	30 (44.8)	
Education				0.677
Less than high school	6418 (23.2)	6401 (23.2)	17 (25.4)	
High school or above	21,213 (76.8)	21,163 (76.8)	50 (74.6)	
BMI (kg/m^2)				0.031
<25	7850 (28.4)	7823 (28.4)	27 (40.3)	
≥25	19,781 (71.6)	19,741 (71.6)	40 (59.7)	
Hypertension				< 0.001
Yes	10,023 (36.3)	9984 (36.2)	39 (58.2)	
No	17,607 (63.7)	17,580 (63.8)	28 (41.8)	
Diabetes				< 0.001
Yes	3602 (13.0)	3584 (13.0)	18 (26.9)	
No	23,384 (84.7)	23,339 (84.7)	45 (67.2)	
Borderline	645 (2.3)	641 (2.3)	4 (6.0)	
Smoking status				< 0.001
≥100 Sticks/lifetime	12,358 (44.7)	12,298 (44.6)	60 (89.6)	
< 100Sticks/lifetime	15,273 (55.3)	15,266 (55.4)	7 (10.4)	
Alcohol drinks				0.003
< 14 drinks/past 12 Mos	17,676 (64.0)	17,646 (64.0)	30 (44.8)	
≥ 15 drinks/past 12 Mos	145 (0.5)	145 (0.5)	0 (0.0)	
Other/unknown	9810 (35.5)	9773 (35.5)	37 (55.2)	
PIR				0.057
<1	5943 (21.5)	5929 (21.5)	14 (20.9)	
1-<4	14,699 (53.2)	14,655 (53.2)	44 (65.7)	
≥4	6989 (25.3)	6980 (25.3)	9 (13.4)	

Bold indicates P less than 0.05. PIR, Ratio of family income to poverty

consumption were observed between the LC and control groups ($p < 0.05$).

Analysis of weighted baseline information

The NHANES database employs a sophisticated multistage sampling methodology, resulting in the non-independent sampling of individuals at varying probabilities. This study conducted comparisons and analyses of weighted between-group disparities by adjusting baseline characteristic data using the WTMEC2YR. By applying weights, the sample was made representative of the civilian, non-institutionalized population of the United States. The sample weight served as an indicator of the proportion of the population represented by the sampled individuals. Table 2 shows the final weighted sample of participants. The results revealed significant differences between the LC and control groups in terms of age, race, marital status, hypertension, diabetes, smoking, and PIR ($p < 0.05$).

Correlation between different forms of folic acid and LC

To mitigate the influence of confounding variables, the LC group was subjected to 1:2 PSM with control group incorporating age, race, BMI, marital status,

Table 2 Baseline characteristics of NHANES participants during 2007–2018 (weighted)

Variables	Total, *N* (%)	Non-LC, *N* (%)	LC, *N* (%)	*P* value
Total	1167493597.4	1165180591.8	2313005.6	
Age (years)				< 0.001
<60	864652239.0 (74.1)	863995927.9 (74.2)	656311.1 (28.4)	
≥60	302841358.4 (25.9)	301184663.9 (25.8)	1656694.5 (71.6)	
Sex				0.705
Male	563237574.7 (48.2)	562060205.2 (48.2)	1177369.5 (50.9)	
Female	604256022.7 (51.8)	603120386.6 (51.8)	1135636.1 (49.1)	
Race				0.004
Non-Hispanic white	795881181.6 (68.2)	793974653.4 (68.1)	1906528.2 (82.4)	
Non-Hispanic black	126354381.0 (10.8)	126141797.7 (10.8)	212583.3 (9.2)	
Other	245258034.8 (21)	245064140.7 (21)	193894.1 (8.4)	
Marital status				< 0.001
Married	646727213.6 (55.4)	645553879.0 (55.4)	1173334.6 (50.7)	
Unmarried	307483951.9 (26.3)	307374345.1 (26.4)	109606.8 (4.7)	
Other	213282431.9 (18.3)	212252367.7 (18.2)	1030064.2 (44.5)	
Education				0.263
Less than high school	174696953.7 (15)	174170747.5 (14.9)	526206.2 (22.7)	
High school or above	992796643.7 (85)	991009844.3 (85.1)	1786799.4 (77.3)	
BMI (kg/m^2)				0.100
<25	342028683.6 (29.3)	341133886.7 (29.3)	894796.9 (38.7)	
≥25	825464913.8 (70.7)	824046705.1 (70.7)	1418208.7 (61.3)	
Hypertension				0.006
Yes	372250731.0 (31.9)	371085353.6 (31.8)	1165377.4 (50.4)	
No	795242866.4 (68.1)	794095238.2 (68.2)	1147628.2 (49.6)	
Diabetes				0.001
Yes	112734525.9 (9.7)	112181295.3 (9.6)	553230.6 (23.9)	
No	1029752802.8 (88.2)	1,028,185,321 (88.2)	1567481.8 (67.8)	
Borderline	25006268.7 (2.1)	24813975.5 (2.1)	192293.2 (8.3)	
Smoking status				< 0.001
≥100Sticks/lifetime	516976168.5 (44.3)	514917643.3 (44.2)	2058525.2 (89)	
< 100 Sticks/lifetime	650517428.9 (55.7)	650262948.5 (55.8)	254480.4 (11)	
Alcohol drinks				0.072
< 14 drinks/past 12 Mos	825744396.0 (70.7)	824505486.1 (70.8)	1238909.9 (53.6)	
≥ 15 drinks/past 12 Mos	5321825.5 (0.5)	5321825.5 (0.5)	0 (0)	
Other/unknown	336427375.9 (28.8)	335353280.2 (28.8)	1074095.7 (46.4)	
PIR				0.003
<1	168176319.8 (14.4)	167896544.8 (14.4)	279775.0 (12.1)	
1-<4	571139272.5 (48.9)	569445200.4 (48.9)	1694072.1 (73.2)	
≥4	428178004.9 (36.7)	427838846.5 (36.7)	339158.4 (14.7)	

Bold indicates P less than 0.05. PIR, Ratio of family income to poverty

hypertension, diabetes, smoking, and alcohol consumption as covariates (Fig. 2). The post-treatment baseline characteristics between groups are presented in Table 3. Following PSM, no statistically significant differences were observed in the baseline characteristics between the LC group ($n = 67$) and the control group ($n = 134$) across all variables ($p > 0.05$). Folate levels, including total folate, folic acid, dietary folate, serum folate, 5-formylTHF, 5,10-methenylTHF, RBC folate, 5-methylTHF, UMFA, THF, and MeFox levels, were analyzed after adjusting for baseline information to facilitate comparison (Table 4). The findings indicated a significant correlation between total folate, dietary folate, 5-formylTHF and 5,10-methenylTHF levels and the development of LC ($p < 0.05$).

Visual depiction of correlation between folic acid and LC

To further investigate the relationship between various forms of folate and LC, an RCS visual representation was used. The results depicted in Fig. 3 revealed that total folate and 5,10-methenylTHF were statistically significant factors for LC ($p < 0.05$). Additionally, 5,10-methenylTHF had significant non-linear correlation with LC for non-linear test respectively ($p < 0.001$), but no significant non-linear relationship was observed between the other forms of folate and the likelihood of developing LC. For total folate, the development of LC was positively associated with increasing total folate levels below 284.8 μg. Importantly, at total folate levels below 189 μg, the non-protective effect was significant ($p < 0.05$), and total folate emerged as a significant factor in this context. The threshold point at which the odds ratio (OR) equaled 1 was precisely at 284.8 μg, indicating a critical cut-off point. For 5,10-methenylTHF, the cut-off point (OR) equaled 1 at concentrations equal to 0.14 nmol/L; at concentrations greater than 0.14 nmol/L, LC prevalence increased with increasing concentration. In the case of other forms of folate, they were statistically significant under certain concentrations. Folic acid levels below 107.6 μg were positively correlated with the incidence of LC, and a statistically significant difference was observed when folic acid levels were below 34.8 μg ($p < 0.05$). Similarly, after the concentrations of RBC folate between 1175.0 and 1623.9 nmol/L, the incidence of LC increased noticeably as concentrations increased.

Discussion

LC, as the leading cause of cancer-related mortality with a persistently low 5-year survival rates, underscores the urgent need for novel strategies in early detection and targeted therapies [23]. Emerging evidence highlights folate metabolism as a critical player in carcinogenesis, given its dual role in nucleotide biosynthesis and DNA methylation regulation [24]. Our study further elucidated the complex relationship between folate status and LC through a comprehensive analysis of 67 LC patients and 27,564 healthy controls from NHANES (2007–2018), incorporating RCS to explore the none-linear relationship.

The dual nature of folate in cancer biology is rooted in its metabolic functions. Since folate serves as a vital nucleic acid synthesis cofactor in the one-carbon group transfer metabolic pathway, rapidly proliferating cells such as tumor cells that lack folic acid experience reduced proliferation [25]. Beyond its role in nucleotide synthesis, folate metabolism profoundly influences DNA methylation. Folic acid, via its derivative 5-methyltetrahydrofolate (5-MTHF), serves as the primary methyl

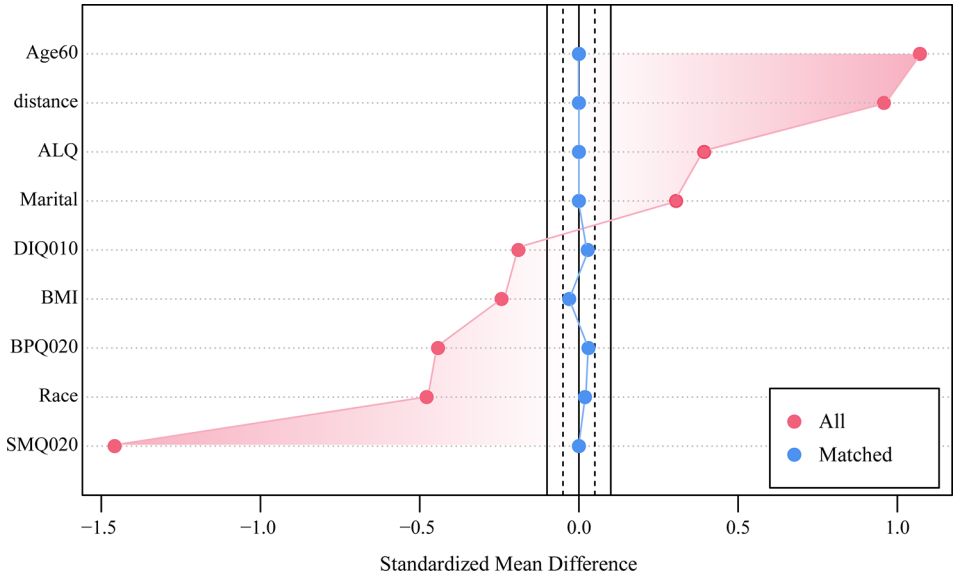

Fig. 2 Propensity score-matching analysis

Table 3 Baseline characteristics after propensity score matching analysis

Variables	Total, *N* (%)	Non-LC, *N* (%)	LC, *N* (%)	*P* value
Total	201	134 (66.7)	67 (33.3)	
Age (years)				1.000
<60	45 (22.4)	30 (22.4)	15 (22.4)	
≥60	156 (77.6)	104 (77.6)	52 (77.6)	
Sex				0.542
Male	120 (59.7)	82 (61.2)	38 (56.7)	
Female	81 (40.3)	52 (38.8)	29 (43.3)	
Race				0.904
Non-Hispanic white	130 (64.7)	88 (65.7)	42 (62.7)	
Non-Hispanic black	36 (17.9)	23 (17.2)	13 (19.4)	
Other	35 (17.4)	23 (17.2)	12 (17.9)	
Marital status				0.777
Married	96 (47.8)	66 (49.3)	30 (44.8)	
Unmarried	22 (10.9)	15 (11.2)	7 (10.4)	
Other	83 (41.3)	53 (39.6)	30 (44.8)	
Education				0.507
Less than high school	57 (28.4)	40 (29.9)	17 (25.4)	
High school or above	144 (71.6)	94 (70.1)	50 (74.6)	
BMI (kg/m^2)				0.469
<25	74 (36.8)	47 (35.1)	27 (40.3)	
≥25	127 (63.2)	87 (64.9)	40 (59.7)	
Hypertension				0.919
Yes	118 (58.7)	79 (59.0)	39 (58.2)	
No	83 (41.3)	55 (41.0)	28 (41.8)	
Diabetes				0.975
Yes	56 (27.9)	38 (28.4)	18 (26.9)	
No	133 (66.2)	88 (65.7)	45 (67.2)	
Borderline	12 (6.0)	8 (6.0)	4 (6.0)	
Smoking status				0.600
≥100Sticks/lifetime	183 (91.0)	123 (91.8)	60 (89.6)	
< 100 Sticks/lifetime	18 (9.0)	11 (8.2)	7 (10.4)	
Alcohol drinks				0.231
< 14 drinks/past 12 Mos	102 (50.7)	72 (53.7)	30 (44.8)	
Other/unknown	99 (49.3)	62 (46.3)	37 (55.2)	
PIR				0.108
<1	46 (22.9)	32 (23.9)	14 (20.9)	
1-<4	113 (56.2)	69 (51.5)	44 (65.7)	
≥4	42 (20.9)	33 (24.6)	9 (13.4)	

PIR, Ratio of family income to poverty

Table 4 Correlation of different forms of folic acid with LC

Variables	Non-LC (*n* = 134)	LC (*n* = 67)	*P* value
Total folate, μg	340.5 (251.0, 497.8)	279.0 (196.0, 438.0)	**0.013**
Folic acid, μg	131.0 (71.3, 234.3)	104.0 (51.0, 215.0)	0.165
Dietary folate, μg	185.5 (134.0, 272.3)	167.0 (119.0, 211.0)	**0.036**
Serum folate, nmol/L	43.5 (24.8, 61.4)	44.5 (27.1, 72.2)	0.396
RBC folate, nmol/L	1111.0 (889.5, 1630.0)	1350.0 (845.5, 1695.0)	0.661
5-methylTHF, nmol/L	36.5 (25.9, 54.8)	30.1 (24.9, 64.0)	0.897
UMFA, nmol/L	0.65 (0.48, 1.09)	0.72 (0.57, 1.27)	0.414
5-formylTHF, nmol/L	0.14 (0.14, 0.14)	0.14 (0.14, 0.21)	**0.001**
THF, nmol/L	0.77 (0.55, 1.15)	0.83 (0.53, 1.20)	0.710
5,10-methenylTHF, nmol/L	0.14 (0.14, 0.14)	0.22 (0.14, 0.22)	**< 0.001**
MeFox, nmol/L	1.78 (1.00, 2.77)	1.74 (1.13, 3.48)	0.517

Bold indicates P less than 0.05. 5-methylTHF, 5-Methyl-tetrahydrofolate; UMFA, Unmetabolized Folic Acid; 5-formylTHF, 5-Formyl-tetrahydrofolate; THF, Tetrahydrofolate; 5,10-methenylTHF, 5,10-Methenyl-tetrahydrofolate; MeFox, Mefox oxidation product

donor for the synthesis of S-adenosylmethionine (SAM), the universal methyl group source for DNA and histone methylation. Aberrant DNA methylation patterns, including global hypomethylation and promoter-specific hypermethylation, are hallmarks of LC progression. Hypomethylation of repetitive genomic elements, such as LINE-1 retrotransposons, has been linked to chromosomal instability and activation of proto-oncogenes in lung tumors. Furthermore, hypermethylation of tumor suppressor genes (e.g., CDKN2A/p16, RASSF1A) driven by folate-mediated SAM availability may silence critical anti-cancer pathways and immune evasion through hypermethylation of immune-related genes. Another potential explanation is that potentially mediated by mechanisms such as methionine cycling acceleration via VCIP135-mediated stabilization of methionine adenosyltransferase IIα [26] and impaired immune surveillance through reduced NK cell toxicity [27, 28]. Conversely, folate metabolism intersects with DNA repair mechanisms implicated in LC. Folate deficiency reduces thymidylate synthesis, leading to uracil misincorporation into DNA and subsequent base excision repair (BER) activation [29, 30]. Chronic BER activity in folate-depleted states generates DNA strand breaks and oxidative stress, exacerbating mutagenesis in bronchial epithelial cells exposed to tobacco carcinogens. Notably, polymorphisms in folate-dependent DNA repair enzymes (e.g., MTHFR C677T) modulate LC risk, with the TT genotype associated with reduced DNA methylation capacity and increased susceptibility to smoking-induced damage. These mechanisms may explain the population-specific discrepancies observed in our study, where high folate levels exhibited both protective and carcinogenic effects depending on a discrepancy potentially attributable to population-specific factors including baseline folate status, smoking exposure patterns, and ethnic differences in folate metabolism [31]. This also suggested that folic acid was found to protect against the development of squamous cell carcinoma [25], reverse chemotaxis in the bronchial epithelial cells [32], and decrease susceptibility of respiratory epithelial cells to tobacco carcinogens following repeated exposure to cigarette smoke [33]. Furthermore, it has been suggested that diets rich in

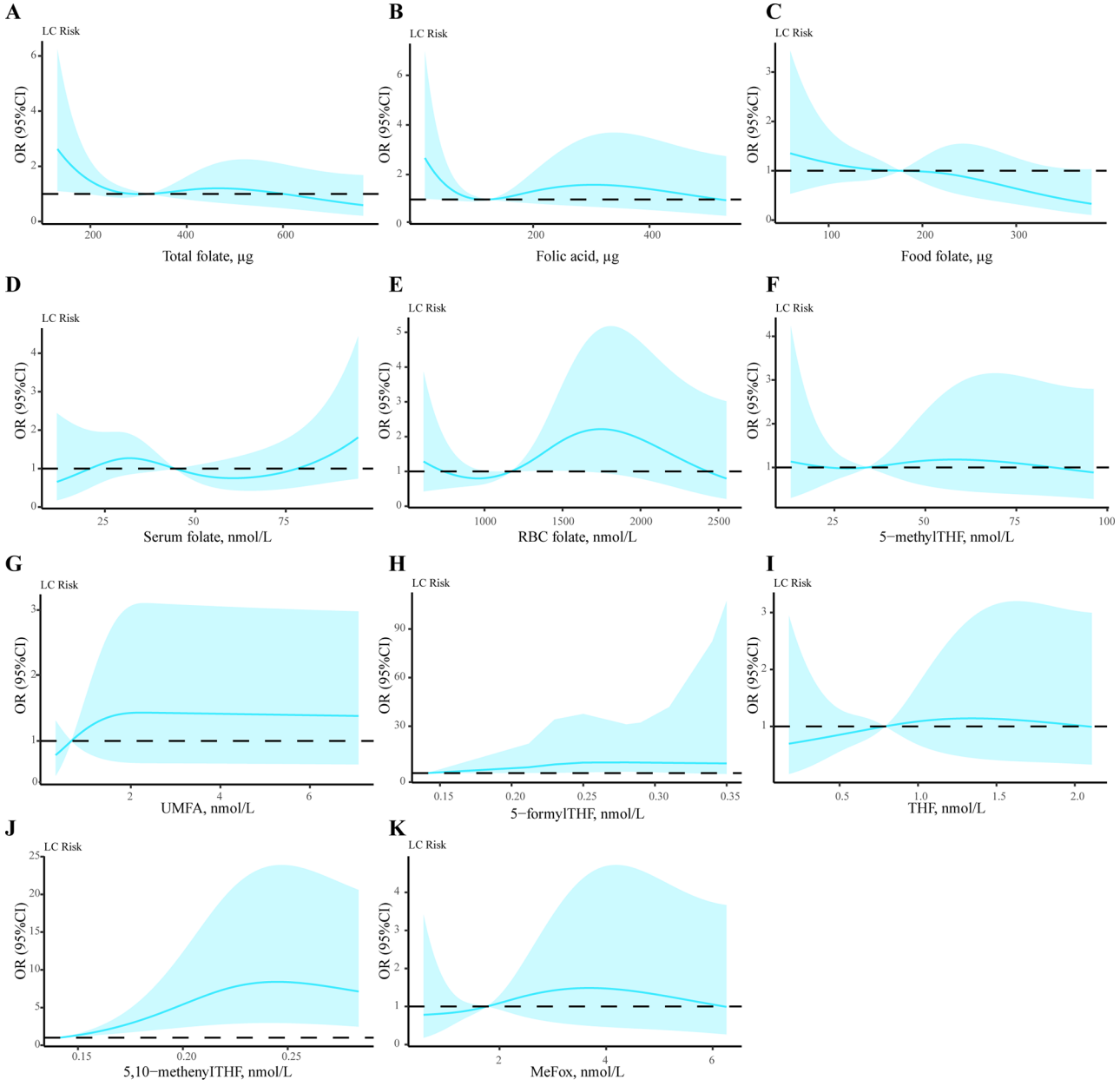

Fig. 3 Dose-response relationship between folate intake and LC. **A**: Total folate **B**: Folic acid **C**: Dietary folate **D**: Serum folate **E**: RBC folate **F**: 5-methylTHF **G**: UFMA **H**: 5-formylTHF **I**: THF **J**: 5,10-methenylTHF **K**: MeFox. The solid line indicates the estimated risk of LC, and the dashed line indicates the fitted 95% CI. LC, lung cancer; OR, odds ratio

folate-containing foods (e.g., dark leafy greens, legumes) are often associated with reduced cancer risk [34]. This paradox may stem from the synergistic effects of phytochemicals—such as flavonoids, carotenoids, and glucosinolates—abundant in folate-rich plant foods which exert antioxidant, anti-inflammatory, and epigenetic regulatory effects independent of folate's metabolic pathways. Obviously, unlike synthetic folic acid supplements, whole-food folate sources deliver a complex nutrient-phytochemical milieu that modulates bioavailability and metabolic outcomes. Our findings align with this dichotomy: total folate levels exhibited a positive correlation with LC within specific ranges.

Distinct folate derivatives demonstrated varying associations with LC, reflecting their metabolic roles [35] (Fig. 4). Serum folate reflects short-term status, whereas RBC folate serves as a long-term biomarker [36]. While unmetabolized folic acid (UMFA) showed no association with LC, elevated RBC folate—a marker of long-term 5-MTHF storage—correlated with increased LC incidence in specific ranges (1175.0–1623.9 nmol/L), which supports the hypothesis that sustained folate exposure

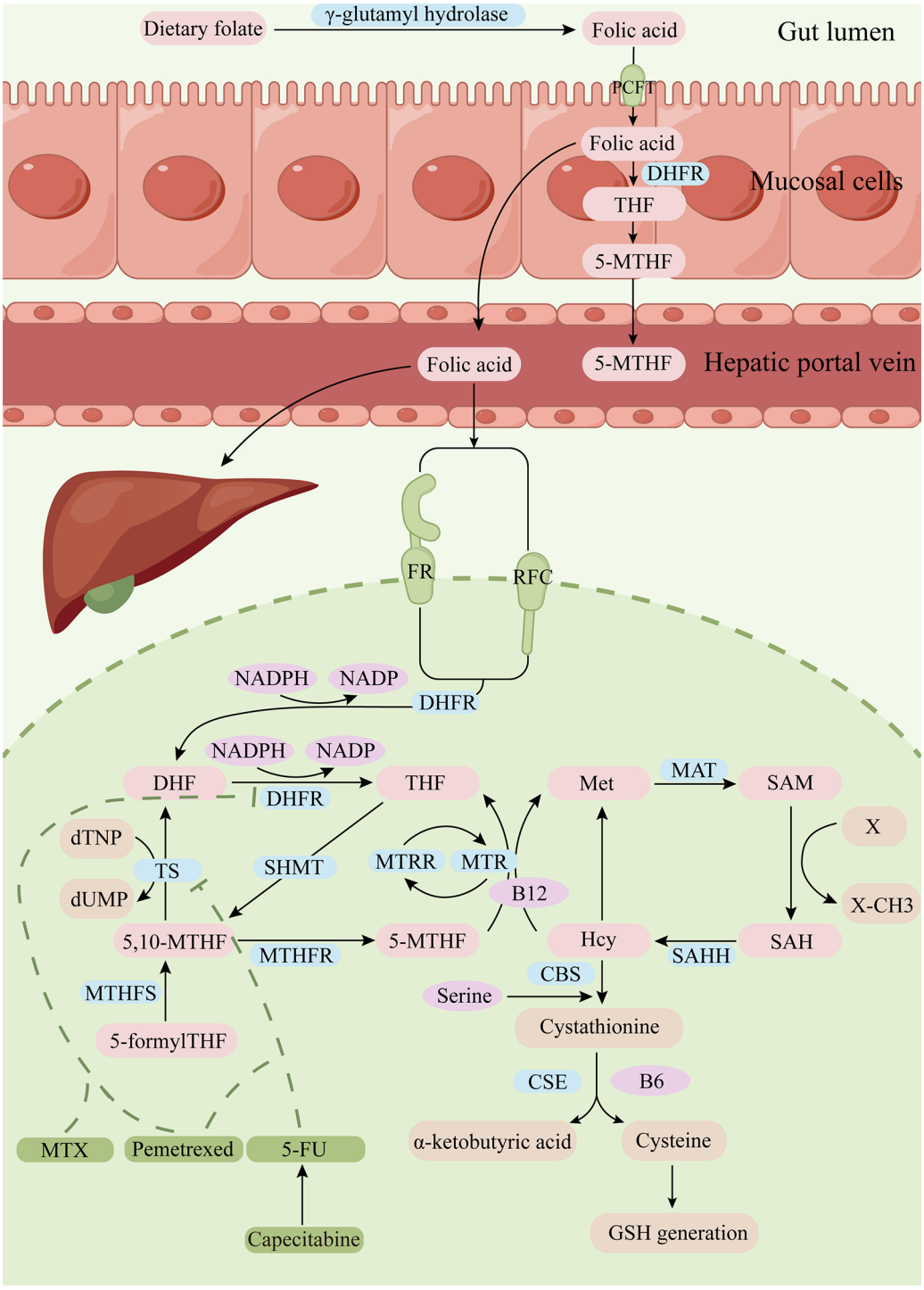

Fig. 4 Schematic of folate-mediated one-carbon metabolism with targets of relevant pharmacologic agents indicated

modulates carcinogenesis. This suggests that chronic folate surplus may disrupt methylation homeostasis by saturating the methionine cycle. Excess 5-MTHF drives methionine synthase (MTR)-dependent homocysteine remethylation, depleting tetrahydrofolate (THF) pools required for purine synthesis and diverting one-carbon units toward SAM production. Overactive methionine cycling may promote LC progression by hypermethylating tumor suppressor promoters, generating oncometabolites like S-adenosylhomocysteine (SAH), which inhibit methyltransferases, and fueling glutathione synthesis to counteract oxidative stress in tumor cells [37]. Similarly, dietary folate showed significant association ($p = 0.036$) without clear dose-response relationship, echoing

experimental evidence that chronic folate deprivation enhances metastatic susceptibility in murine models [38]. These findings emphasize the need for longitudinal assessments of folate status in LC stratification. A study indicating that starvation of A549 and T.T cells by removing folic acid, leading to depletion of endogenous THF, demonstrated an effect against tumor cell growth. And this inhibitory effect could be reversed by adding folic acid or 5-methyl THF to the cell culture medium [39]. However, no direct link between THF and LC was found in this study.

In addition to the dosages covered in this article, other factors of folic acid supplementation are equally critical in influencing its effects. Research have speculated that the timing of folic acid supplementation was crucial. Preclinical studies suggest folate supplementation may suppress early carcinogenesis by correcting DNA hypomethylation and repair deficits in premalignant lesions [40]. However, once oncogenic drivers (e.g., KRAS mutations) are established, folate surplus may accelerate tumor growth by providing nucleotide precursors and enhancing methylation-dependent gene silencing [41, 42]. Furthermore, various cancer risk factors such as age, sex, vitamin B12 levels, alcohol consumption, smoking habits, and genetic polymorphisms in enzymes related to folic acid metabolism can interact with folic acid. Discrepancies in these factors may result in the contrasting effects of folic acid supplementation. For example, individuals with a 3-repeat polymorphism in the promoter region of thymidylate synthase (TS) gene—a crucial enzyme in folate metabolism—had a 2-fold lower risk of developing colorectal cancer if they had a daily folate intake exceeding 440 μg per day. However, in the context of a 2-repeat polymorphism, a heightened risk of 1.5-fold was linked to elevated folate intake, with a comparable trend observed for vitamin B12 [43]. Consequently, folic acid supplementation should not be approached unilaterally, but should take into account the effects of multiple factors [44] (see Fig. 4)

Translational implications of folate biology are rapidly evolving. As a biomarker [45] and targeted drug carrier [46], it enables therapies like anti-folate receptor (FR) antibodies, antibody-drug conjugates, folate-drug couplings, and FA-modified nanoparticles [47]. Disrupted folate metabolism, a key mechanism for antifolates (e.g., methotrexate) and fluoropyrimidines, inhibits cancer proliferation by blocking enzymes like DHF reductase and TS [48, 49]. MTR dysfunction disrupts methylation and folate cycling; MTR-targeted drugs exploit the "methyl-folate trap" to treat cancers with high nucleotide demand. Folic acid also aids cancer diagnosis via FR-targeted imaging (e.g., radionuclides) and detection of FR + circulating tumor cells. Additionally, SLC19A1, a folate transporter, determines tumor carbon source preferences and marks SHMT1-dependent cancers [50].

In summary, whether in vitro, vivo or clinical experiments, there are different opinions on folic acid and several mechanisms could explain their phenomena. What is certain, however, is that the effects of folic acid on cancer and the future prospects for its use in cancer treatment cannot be ignored. Consequently, further comprehensive research is required on elucidating the effects of folic acid and the influence of varying folic acid doses. The results and references discussed in this study can contribute to subsequent clinical studies.

Strengths and limitations

Using data from a nationally representative sample of U.S. adults, this investigation revealed an association between 11 forms of folate and the incidence of LC. Among these, 5,10-menthylTHF, folic acid, total folate and RBC folate intake influenced the incidence of LC over a range of concentrations. Therefore, the contents of this study provided a reference for the dosage of folic acid that can be given to LC patients. These findings offered valuable insights for future research on the role of folate in LC.

There are certain limitations to this study that warrant attention and improvement. Specifically, the sampling strategy employed in NHANES database may have restricted generalizability of the findings to a broader population, as the study only included samples from adults in the US. Future studies with larger cohorts are warranted to validate these results. Regarding the study on the association between folic acid intake and lung cancer incidence, while our PSM-adjusted analysis enhanced internal validity by reducing confounding, the cross-sectional design inherently limits causal interpretation. Future longitudinal or cohort studies are required to validate these associations and explore potential causal mechanisms. Furthermore, NHANES lacks data on cancer treatment specifics (e.g., chemotherapy agents or targeted therapies), and certain drugs (e.g., methotrexate or pemetrexed) may influence folate metabolism, potentially confounding our results. While this study could not account for such interactions, this issue warrants further investigation in future prospective studies with detailed treatment records. In addition, disparities have been observed between dietary folate intake and serum folate concentrations [51]. Our study delineates a nuanced relationship between folate status and LC, influenced by metabolic derivatives, exposure duration, and population characteristics. While affirming folate's potential as both a therapeutic target and a risk modulator, these findings caution against simplistic interpretations of folate supplementation. Future research should integrate omics approaches to unravel gene-nutrient interactions and

validate biomarkers. Longitudinal studies are warranted to clarify temporal effects of folate exposure and optimize its clinical application in LC prevention and treatment. Finally, the fluctuating folate intake among patients with cancer, in contrast to the stable intake among the general population, may have impacted accuracy of the experimental findings. In the future, we will plan to conduct further research on the response of LC to different doses of folic acid in order to overcome these limitations.

Conclusions

This study used NHANES data from 2007 to 2018 to demonstrate correlations between the occurrence of LC and 5-formylTHF, 5,10-methenylTHF, total and dietary folate. 5,10-mecthenylTHF, folic acid, total folate and RBC folate exhibited a positive correlation with the incidence of LC in a certain range of concentrations. Additional reference values for the usage of folic acid supplements were offered by this finding. It was helpful to ascertain the appropriate usage and dosage of folic acid for clinical purposes, as well as to formulate tailored dietary and medication recommendations for diverse populations.

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Author contributions
Xuxiang Lu, Yang Zhang, and Fei Jing designed research; Binbin Li and Fei Xu conducted research; Fei Xu analyzed data; Binbin Li wrote the paper. Wei Zhang had primary responsibility for final content. All authors read and approved the final manuscript.

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Data availability
The survey data are publicly available on the internet for data users and researchers throughout the world http://www.cdc.gov/nchs/nhanes/.

Declarations

Ethical approval and consent to participate
The studies involving human participants, human materials, or human data were reviewed and approved by the National Center for Health Statistics. The patients/ participants provided their written informed consent to participate in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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