RESEARCH COMMUNICATION

Folate Intake, Methylenetetrahydrofolate Reductase Polymorphisms in Association with the Prognosis of Esophageal Squamous Cell Carcinoma

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Abstract

Aim: An epidemiological study was conducted based on an esophageal cancer patient's cohort to investigate the association of folate intake and MTHFR C677T polymorphism with the prognosis of esophageal cancer in a Chinese population. Methods: 167 patients aged 37-75 years who had histological confirmed diagnosis of esophageal squamous cell cancer were collected from Jan. 2006 to Jan. 2008. MTHFR genotypes at the C677T site were analyzed by PCR-based RFLP methods, and the folate intake was computed by multiplying the food intake (in grams) and the folate content (per gram) of food in our questionnaire. Results: We found associations between the prognosis of esophageal cancer and smoking status, T and N stages. Individuals carrying the MTHFR 677CT and TT genotypes showed a shorter survival time than with the CC genotype, with adjusted HRs (95% CI) of 1.20 (0.56-2.15) and 2.29 (1.30-4.28), respectively. Similarly, those carrying MTHFR 677T allele had a 1.86-fold risk of death. A higher folate concentration showed a significant decreased risk of death, with an HR (95% CI) of 0.45 (0.18-0.87). Individuals with high folate intake and the MTHFR 677CC genotype showed a significant decreased risk of esophageal cancer (0.43, 0.25-0.89). Conclusion: Our findings supports the hypothesis that high folate intake and active MTHFR C677T polymorphism may exert protective roles in the prognosis of esophageal cancer in the Chinese population.

Keywords: Folate intake - methylenetetrahydrofolate reductase - esophageal cancer - polymorphisms - prognosis

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Introduction

Esophageal cancer is the sixth most common cancer worldwide in 2002 (Blount et al., 2007). Its rates showed a wide international geographic variation in the incidence and mortality of esophageal cancer (Choi and Mason, 2000; Kim, 2004; Blount et al., 2007), suggesting that the role of genetic and environmental factors in the pathogenesis of this cancer (Choi and Mason, 2000).

Possible risk factors for esophageal cancer include cigarette smoking, alchol drinking, hot-temperature food items, chronic mucosal irritation, and a family history of cancers (Wang et al., 2007; Falk, 2009; Morita et al., 2010). Deficiency of nutrients, such as folate, vitamin and trace elements, was also found to be associated with an increased risk for several cancers, including esophageal cancer, whereas a high intake of fruits and vegetables has been considered to be effective in prevention (Morita et al., 2010). Folate is a water-solution B vitamin, and folate deficiency can influence the carcinogenesis of esophageal cancer through two ways: one is inducing misincorportion of uracil into DNA to result in chromosomal breaks and mutations. Another is leading the alteration in DNA methylation and thus to alter the expression of tumor suppressor genes (Choi and Mason, 2000; Kim, 2004; Blount et al., 2007). Previous epidemiologic studies have shown that folate deficiency could increase the risk of human cancers (Yang, 2000; Mayne et al., 2001; Chen et al., 2002). However, the role of dietary folate in esophageal cancers is still controversial and the studies in Chinese population were scarce till now.

Except for an inadequate folate intake, Methylenetetrahydrofolate reductase (MTHFR), functional polymorphisms in folate metabolism, may also play a role in the susceptibility of esophageal cancer risk. It facilitates the conversion of 5, 10-methylene THF to 5-methyl THF, and leads point mutations and/or chromosomal breaks. Also, it may cause decline of 5-methyl THF to induce a decrease of the conversion of homocysteine to methionine, which could result in a carcinogenesis process of DNA hypomethylation (Stern et al., 2000; Fang and Xiao, 2003; Blount et al., 2007). Approximately 60 polymorphisms have been described in the MTHFR gene (Leclerc and Rozen, 2007). The most common functional variant and most studied to date is the thermolabile MTHFR C677T polymorphism (rs1801133). The C677T variant is a C

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to T transition in exon 4 at nucleotide 677, resulting in the conversion of alanine to valine at position 222 of the MTHFR amino acid sequence (Frosst et al., 1995; Choi and Mason, 2000). Heterozygotes (CT) and homozygotes (TT) for the C677T variant have, respectively, 65% and 30% of the MTHFR enzyme activity observed in homozygous wild type subjects (CC) (Bailey et al., 1999). As a result, TT homozygotes have been associated with lower serum folate levels and higher homocysteine levels than their wild type homozygous counterparts. An impact of this polymorphism on several types of cancer including colorectal cancer, acute lymphocytic leukemia, lung, prostrate, gallbladder and esophageal cancers has been conducted (Boccia et al., 2007; Suzuki et al., 2007; Cao et al., 2008; Yu et al., 2008; Bai et al., 2009). However, evidence for the potential association between the MTHFR C677T polymorphism and esophageal cancer is conflicting (Stolzenberg-Solomon et al., 2003; Yang et al., 2005; He et al., 2007; Umar et al., 2010).

The role of folate intake and MTHFR C677T polymorphism on survival of patients has also been reported in different types of malignancies, including endometrial cancer, breast cancer, rectal cancer and esopheageal cancer (Shrubsole et al., 2001; Jiang et al., 2005; Xu et al., 2007). However, the results is inconsistent (Miller et al., 1988; Shen et al., 2001; Jain et al., 2008; Upadhyay et al., 2008). Low folate intake and inactive of MTHFR C677T polymorphism has been found to be significantly related with poor survival of patients with advanced gastric cancer or esophageal cancer who were treated with first-line fluorouracil-based chemotherapy (Shitara et al., 2010; Lu et al., 2011). Study conducted in India population showed no association between MTHFR C677T polymorphism and esophageal cancer risk (Umar et al., 2010). Moreover, the association between folate intake and MTHFR C677T polymorphism in relation to esophageal cancer prognosis has not been explore in Chinese population (Sun et al., 2011).

Hence, we conducted an epidemiological study based on an esophageal cancer patient's cohort to investigate the association of folate intake and MTHFR C677T polymorphism with the prognosis of esophageal cancer in a Chinese population.

Materials and Methods

Study population

The procedures for case recruitment were described previously (Sun et al., 2011). Briefly, 167 patients aged 37-75 years who had histological confirmed diagnosis of esophageal squamous cell cancer in the General Hospital of Chengdu Military Area from Jan. 2006 to Jan. 2008, which accounted for about more than 90% of cases under esophageal cancer surgery in this hospital during the same period. All the recruited patients were residents in Chengdu for more than five years, with pathologically confirmed esophageal cancer and informed consent. All cases recruited in this study were examined by endoscopy and histologically confirmed.

Informed consent was obtained from all participants before investigation.

Data collection

A self-administered structured questionnaire was used in our study, consisting 65 items. Information were collected about demographic (age, sex and family history of cancer) and clinical characteristics (histopathology, tumor location, and lymph modes status), tobacco usage, smoking, alcohol-drinking habits and dietary habits (including 45 foods/food groups). For each food item, we collected the frequency and quantity of consumption, and calculated the daily intake by multiplying the frequency reported for the consumption of each food item by the specified portion size. The folate intake was computed by multiplying the food intake (in grams) and the folate content (per gram) of food in our questionnaire, and then the sum of all folate intake from various foods/food groups was calculated as the total folate intake. Face to face interview was performed for all subjects by trained interviewers. Completed questionnaires were obtained from 167 cases. Cancer patients were asked to refer about habits a year before the disease diagnosed.

Sample collection

Each patients were required to provide 5ml venous blood for DNA extraction. MTHFR genotypes at the C677T site were analyzed by PCR-based RFLP methods, as described previously (Sun et al., 2011).. Briefly, a total volume of 10 ul of PCR product was obstained through 200ng of genomic DNA and 20 pmol of each primer. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 65 s, at 60°C for 65 s, at 72°C for 90 s, and a final extension at 72°C for 5 min. After transient centrifugation, agarose electrophoresis was conducted. The PCR products included 173-bp fragments of 677C/C wild-type homozygotes; 173-, 125-, and 48-bp fragments of 677C/T heterozygotes, and 125- and 48-bp fragments of 677T/T homozygotes.

Statistical analysis

Statistical analyses were performed by using Stata version 8 (Stata, College Station, TX). Cox proportional hazards model was applied to estimate the risk between folate intake and MTHFR C677T polymorphism and esophageal cancer by calculating hazard ratio (HRs) and 95% confidence intervals (95% CIs). The primary death of esophageal cancer was defined as the failure event and the time of survival was the time between diagnosis and death. The cause of death was defined by specialists based on the clinical documents and reports by cancer registration. If the patient died of other causes rather than esophageal cancer, he/she was censored at the date of death. All survived patients were censored at the date of the last follow-up. Alcohol exposure was categorized into four levels, former drinkers, non-drinkers (never drinker), moderate drinkers and heavy drinkers. Individuals who quit drinking more than one year were considered as former drinkers, individuals who drank alcoholic beverages 5 days or more per week with an amount of 50 g or more ethanol on each occasion while moderate drinkers were defined as drinkers consuming less frequently and/or lower amounts. Smoking status was also divided into four categories considering

Table 1. Characteristics of Patients with Esophageal Cancer and Matched Controls

Variables	Cases N(%)	Death, N(%)	HR (95% CI)	P value
Mean age (years)	53.2±4.7	92(55.1)	-	0.35
Sex				
Male	116(69.5)	54(58.7)	1.0(Reference)	-
Female	51(30.5)	38(41.3)	1.23(0.74-1.65)	0.11
Alcohol drinking status				
Never	49(29.2)	25(27.3)	1.0(Reference)	-
Former	19(11.6)	9(10.3)	0.98(0.42-1.43)	0.42
Moderate	69(41.5)	36(38.9)	1.21(0.67-1.67)	0.52
Heavy	30(17.7)	22(23.5)	1.42(0.83-1.84)	0.67
Smoking status				
Never	71(42.4)	35(37.6)	1.0(Reference)	-
Former	16(9.3)	8(9.2)	1.15(0.67-1.34)	0.06
Moderate	55(32.9)	31(33.9)	1.40(0.74-1.83)	0.32
Heavy	26(15.4)	18(19.3)	1.79(1.13-2.30)	<0.05
Site				
Upper	34(20.3)	21(23.3)	1.0(Reference)	-
Middle	93(55.6)	48(52.5)	0.94(0.51-1.43)	0.2
Low	40(24.1)	40(24.2)	0.85(0.48-1.38)	0.24
TNM stage				
T				
T1	21(12.6)	8(8.2)	1.0(Reference)	-
T2	64(38.2)	25(27.6)	1.43(0.74-2.45)	0.15
T3	62(37.4)	40(43.5)	2.47(1.20-3.96)	<0.05
T4	20(11.8)	19(20.7)	3.47(1.78-4.87)	<0.05
N				
N0	98(58.6)	33(36.4)	1.0(Reference)	-
N1	69(41.4)	59(63.6)	2.33(1.45-4.20)	<0.05
M				
M0	164(98.5)	89(96.7)	1.0(Reference)	-
M1	3(1.5)	3(3.3)	1.92(0.17-7.37)	0.48
Chemo-therapy				
No	154(92.2)	90(97.7)	1.0(Reference)	-
Yes	13(7.8)	2(2.3)	0.34(0.04-2.10)	0.27
Radio-therapy				
No	136(81.4)	81(88.2)	1.0(Reference)	-
Yes	31(18.6)	11(11.8)	0.76(0.32-2.85)	0.16

cumulative exposure to tobacco: former smokers, non-smokers (never smokers), moderate smokers and heavy smokers. Individuals who quit smoking more than one year were considered as former smokers, individuals with pack-years (PYs) ≤40 were regarded as moderate smokers, and smokers with PYs>40 were regarded as heavy smokers. The differences between folate intake categories and MTHFR C677T polymorphism were compared by using the Kaplan-Meier curves and Log-rank test. All tests were two-sided with significance at P<0.05.

Results

Of 167 patients, all the patients were followed up.

Table 2. Relationship Between Folate Consumption and MTHFR C677T Polymorphisms and Esophageal Cancer Risk

MTHFR C677T	Cases N(%)	Death, N(%)	HR (95% CI)[1]	P value
Folate intake				
Mean (SE)	268.3(20.9)	-	-	-
<230	65(38.9)	42(45.2)	1.0(Reference)	-
230-300	60(36.2)	37(40.1)	0.87(0.56-1.62)	0.29
>300	42(24.9)	14(14.7)	0.45(0.18-0.87)	<0.05
MTHFR C677T				
CC	75(45.2)	37(40.2)	1.0(Reference)	-
CT	74(47.74)	41(44.6)	1.20(0.56-2.15)	0.17
TT	19(11.1)	14(15.2)	2.29(1.30-4.28)	<0.05
CT/TT	92(54.8)	55(59.8)	1.86(1.13-3.17)	<0.05

[1]Adjusted for age, sex, smoking, drinking, tumor sites, TNM stage, chemo-therapy and radio-therapy

Demographical characteristics of patients are shown in Table 1. The mean age of cases was 53.2±4.7 years, respectively. Majority of the patients were males (69.5%). Patients were followed up since the diagnosis until the end of Nov. 2011. The median time of follow-up was 39 months (minimum and maximum were 3 months and 60 months, respectively). 92 patients died of esophageal cancer during the follow-up period. As showed in Table1, we found association between the prognosis of esophageal cancer and smoking status, with the HR (95% CI) of 1.79 (1.13-2.30). The association between clinical characteristics and the prognosis of esophageal cancer were also showed in Table 1. Both T and N stages were related to the survival time, and a significant trend was found between them. Patients at higher TNM stage showed a greater risks of death, and the P for trend were 0.012 and 0.008, respectively (Data not shown in tables).

We further analyzed role of MTHFR C677T polymorphisms in the prognosis of esophageal cancer. Individuals carrying the MTHFR 677CT and TT genotypes showed a shorter survival time than the CC genotype. A significantly increased risk of death was found, with the adjusted HRs (95% CI) of 1.20 (0.56-2.15) and 2.29 (1.30-4.28), respectively. Similarly, those carrying MTHFR 677T allele had a 1.86-fold risk of death. The mean adjusted daily folate consumption was 268.3±20.9 ug/day in cancer cases, and a higher folate concentration showed a significant decreased risk of death, with HRs (95% CI) of 0.45 (0.18-0.87) (Table 1).

The impact of combination of MTHFR C677T polymorphisms and folate intake on esophageal cancer risk showed in Table 3. Among the high folate intake group, the MTHFR 677CC genotype showed a significant decreased risk of esophageal cancer, with the the adjusted

Table 3. The MTHFR C677T Polymorphisms and Esophageal Cancer Risk According to Drinking, Smoking and Folate Intake

Daily folate consumption	Genotype								
	CC Cases	Death, N(%)	HR(95% CI)[1]	CT Cases	Death, N(%)	HR(95% CI)[1]	TT Cases	Death, N(%)	HR (95% CI)[1]
<230 ug/day	27(36.0)	19(45.2)	1.0(Reference)	31(41.9)	11(37.8)	1.0(Reference)	7(50.0)	7(28.6)	1.0(Reference)
230-300 ug/day	28(37.3)	18(42.9)	0.66(0.32-1.55)	29(39.2)	19(43.2)	1.87(0.82-2.95)	3(21.4)	4(50.0)	1.74(0.92-2.23)
>300 ug/day	20(26.7)	5(11.9)	0.43(0.25-0.89)	14(18.9)	7(18.9)	1.62(0.55-2.43)	4(28.6)	3(21.4)	1.38(0.80-1.90)

[1]Adjusted for age, sex, smoking, drinking., tumor sites, TNM stage, chemo-therapy and radio-therapy

HRs (95% CI) of 0.43 (0.25-0.89). Significant interaction was found between folate consumption and MTHFR genotype (p=0.029, data was not showed in tables).

Discussion

The present study demonstrates the importance of folate consumption and genetic variation in In our study, we observed the MTHFR C677T polymorphisms were associated with susceptibility in the prognosis of esophageal cancer, and high folate consumption is associated with a significant decreased risk of esophageal cancer. Our study showed esophageal cancer patients with high folate consumption may have better survival after surgery than individual with low consumption of folate. We found a high risk of death in patients carrying MTHFR 677TT genotypes. There were possible two mechanisms of folate intake and increased risk of death. The first involved alteration of the normal methylation process, and the second may be an imbalance in the steady-state lelves of DNA precursors, leading to aberrant DNA synthesis, stability and repair, and chromosomal changes (Lamprecht and Lipkin, 2003; Ulrich, 2010). Regarding DNA methylation, Global and regional DNA hypomethylation have been identified in several cancer, including esophageal cancer, and they are known to induce proto-oncogene activation and chromosomal instability, while regional DNA hypermethylation is associated with transcriptional silencing of tumor suppressor genes. Therefore, DNA methylation modifications, which are possibly mediated by dietary folate intake levels, may affect the prognosis of esophageal cancer. The second possible mechanism by which folate intake influences esophageal cancer prognosis involves thymidine synthesis, which may be important for maintaining DNA stability (Turek and Jagodzinski, 2005; Esteller, 2008). In additional, the activity of MTHFR, folate metabolic enzyme, is also involved in the folate metabolic and DNA methylation process. As a key enzyme in folate metabolism, the product of MTHFR serves as the carbon donor for the methylation of homocysteine tomethionine, which is catalyzed by the enzyme MTR (Sarbia et al., 2006). Therefore, the MTHFR C677T may play a role in the prognosis of esophageal cancer.

In the present study, we observed a significant independent effect of folate intake and the MTHFR C677T polymorphism on patient's survival, which is inconsistent to previous studies on gastrointestinal malignancies (Umar et al., 2010; Lu et al., 2011). Umar reported MTHFR C677T did not seem to have significant role in survival of esophageal in Indian population. However, another study conducted in China showed the folate intake and MTHFR C677T polymorphism may be related to the survival of esophageal cancer. The variation in the role of MTHFR C677T polymorphism may be due to the difference in ethnicity. The prevalence of variant genotypes of MTHFR C677T polymorphisms varies to a great extent among different human population (Miller et al., 1988). The frequency of MTHFR 677TT in German is 13% (Wilcken et al., 2003), whereas 1% in Africans, 2.2% in India and 15% in Japan (Yang et al., 2005; Umar et al., 2010). In our study, the frequency of MTHFR 677TT genotype in our study is ???%, which is in line with the previous studies from Chinese population (Zhang et al., 2004; Wang et al., 2007). The variation of frequency of MTHFR C677T decides the difference in the association between MTHFR C677T polymorphism and esophageal cancer risk. Another reason might be other factors influence the activity of the key enzymes involved in folate metabolism.

Our study also showed there was significant interaction between folate status and MTHFR C677T polymorphism on the prognosis of esophageal cancer. Previous study showed folate status and MTHFR C677T polymorphism on genomic DNA methylation in periopheral blood mononuclear cell and found that the MTHFR C677T polymorphism could influence DNA methylation status through an interaction with folate status (Friso et al., 2002), which was in line with our study. This finding indicates that esophageal cancer prognosis might be influenced by the folate intake and related gene polymorphism.

In conclusion, our study indicates that the high consumption of folate intake and active MTHFR C677T polymorphism may have a protective role in the prognosis of esophageal cancer. Gene-environment interaction is found between folate intake and MTHFR C677T genotypes. This study provides more information on the gene biomarker for of the prognosis of esophageal cancer in Chinese population.

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