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Full Length Research Paper

Folate, MTHFR C677T and A1298C polymorphisms with the relationship with ovarian cancer risk among Chinese females

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Genetic and environmental factors may play roles in the pathogenesis of ovarian cancer; we aimed to evaluate the associations between MTHFR C677T and A1298C polymorphism and folate intake with ovarian cancer. A case-control study with 200 ovarian cancer patients and 200 controls were selected in Chinese population from January, 2003 and January, 2006. Genotyping of the MTHFR 677 C4T and 1298 A4C SNPs was performed on the ABI PRISMs 7500 real-time (RT) polymerase chain reaction (PCR) system. Individuals with the TT genotype of C667T polymorphism had an increased risk of developing ovarian cancer (Adjusted OR = 1.76, 95% CI = 1.03 to 3.38) and an increased risk also found in 1298 CC genotype with OR (95% CI) of 2.14 (1.15 to 5.76). Individuals with daily folate consumption > 335 µg/day had a lower risk of ovarian cancer, with an adjusted OR (95% CI) of 0.50 (0.25 to 0.97). The 1298CC genotype was related to a poor survival of ovarian cancer (HR = 2.11, 95% CI = 1.13-4.61). We observed that high folate intake may have a protective role in the development and prognosis of ovarian cancer, and MTHFR gene polymorphisms may be associated with ovarian cancer risk and its prognosis.

Key words: Methylenetetrahydrofolate reductase gene, ovarian cancer, folate intake.

INTRODUCTION

Ovarian cancer has been ranked as one of the most common cause of death in women from gynecological cancers worldwide (Webb et al., 2011). China has a relatively low incidence of 3 to 5/10^5 females, which is about one-fourth of the incidence in northern European countries (Jin et al., 1993). The wide geographic variation at an international levels of ovarian cancer showed there might be genetic and environmental factors in the carcinogenesis of this cancer.

Folate has been hypothesized to contribute to carcinogenesis because of its dual role in DNA methylation, which has a role in DNA synthesis, integrity and stability, and the folate deficiency usually cause defective DNA repair and chromosomal fragile site expression, leading to chromosomal breaks and micronucleus formation. Thereby folate cancer modulate expression of oncogenes (Aune et al., 2011; Kim, 2004; Larsson et al., 2006). Previous study showed the low folate intake is related to increased risk of several cancers, including breast, esophageal, gastric and colon cancer (Zhang et al., 2011; Zhao et al., 2011; Jessri et al., 2011; Shitara et al., 2010; Kim et al., 2012). .The carcinogenesis of ovarian cancer by folate deficiency is not clear, and may depend on the pathway of folate metabolism.

5, 10-methylene-tetrahydrofolate reductase (*MTHFR)* catalyzes the reduction of 5, 10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, and then methionine synthase catalyzed the reaction of 5-methyltetrahydrofolate and homocysteine to generate

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methionine and tetrahydrofolate. Two MTHFR polymorphisms (C677T and A1298C) have been associated with with changes of enzyme activity (Frosst et al., 1995; Van et al., 1998), leading to an increase in 5, 10-MTHF and a decrease in 5-MTHF. The polymorphisms of this gene have been associated with risk of a variety of cancers, including colon, esophageal, gastric, breast and liver cancer (Kim et al., 2012; Zhao et al., 2011; Neves et al., 2010; Hosseini et al., 2011; Kwak et al., 2008). However, previous studies on the association between MTHFR polymorphisms and ovarian cancer is conflicting (Pawlik et al., 2012; Terry et al., 2010; Zhang et al., 2004). Moreover, there are few studies on the association between survival of cancer and MTHFR polymorphism. Considering the folate pool imbalance and impaired repair mechanisms may result in DNA instability and strand breaks, and the inactive MTHFR polymorphisms under the condition of folate deficiency accelerate the carcinogenesis process of DNA hypomethylation, here we conducted a study to examine the role of folate intake and MTHFR polymorphism on the susceptibility and progression of ovarian cancer.

MATERIALS AND METHODS

Study subjects

This study is a case-control study from Tianjin Medical University Cancer Hospital. All Chinese female cases with newly diagnosed primary ovarian cancer from January, 2003 and January, 2006 in the hospital were invited by face-to-face interviews within one months after diagnosis. A total of 200 cases were selected and they were histopathological confirmed and interviewed. Controls heath people who requested general health examinations in the same hospital during the same period. 200 controls were randomly selected but with frequency matching to cases by age and sex. All the patients were followed up since the January, 2011 of study. The outcome for this study was overall survival, and death from ovarian cancer or other causes were the end point in the present study. Survival time was calculated from the date of diagnosis to the date of last follow-up from any causes.

A self designed questionnaire was conducted to collect information on demographic factors and clinic characteristics, Approval to conduct this study was granted by the Ethics Committee of our hospital. Informed consent was obtained before each interview.

Deoxyribonucleic acid (DNA) extraction

Genotyping of the MTHFR 677 C4T and 1298 A4C SNPs was performed on the ABI PRISMs 7500 real-time (RT) PCR System (Applied Biosystems, Foster City, CA), and primer, probes, and reaction conditions were performed based on previous study (Skibola et al., 1999). The assay was performed under universal conditions, with each reaction containing 50 ng DNA, 0.125 ml 40_ Assay Mix and 2.5 ml TaqMans Universal PCR master mix made to a final volume of 5 ml with sterile water. Thermal cycling conditions were as follows: 501C for 2 min, 951C for 10 min, and 50 cycles of 921C for 15 s and 601C for 1 min. After the PCR reaction, plates were scanned by the ABI PRISMs 7500 PCR system to determine genotypes by allelic discrimination. Genotyping was done by laboratory personnel blinded to case-control status. We also performed the genotyping of internal positive control samples, use of no template controls, and use of replicates for 10% samples for quality control.

Statistical analyses

Differences in the demographicm clinic characteristics and polymorphism carriers between patients and controls were compared by Chi-squared test. Chi-square tests was employed to assess Hardy-Weinberg Equilibrium (HWE) for each SNP among controls. The odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated by unconditional logistic regress, and we adjusted the demographic and clinic characteristics. The assessment of any association between MTHFR genotypes and overall survival was estimated by the survival analysis methods of Kaplan-Meir method and Cox hazard regression model. Kaplan-Meier survival curved were used to plot the survival situation of participants with different MTHFR polymorphisms. Cox proportional hazard regression models were used to test significant findings found by Kaplan–Meier and to generate hazard ratios (HRs) and 95% confidence intervals (CIs) in a multivariate analysis taking into account MTHFR polymorphisms. 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. The continuous variables of folate intake were transferred to three categories as low, moderate and high by using tertile as the cut point. The relative risk [hazard ratio (HR)] and 95% CI were calculated from the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). All statistical analyses were performed by using STATA 10.0 (College Station, TX, USA). All statistical tests were two sided, and differences were taken as significant when the P value was less than 0.05.

RESULTS

The demographical and clinical characteristics of 200 patients and 200 controls are showed in Table 1. The comparision of age, smoking status, drinking status, number of delivery, menopausal status and ovarian cancer in first relatives between cases and controls are summarized in Table 1. There were no significant differences among cases and controls in terms of age distribution, drinking and menopausal status. Ovarian cancer patients had higher proportion of former smoking habit ($p < 0.05$). People who had less number of delivery had higher risk of ovarian cancer, and cancer cases had more relatives with ovarian cancer than in the control group ($p < 0.05$).

Table 2 showed the genotype frequencies of the MTHFR C677T and A1298C genotype polymorphisms in cases and controls and the corresponding ORs with CIs. Polymorphisms in C677T and A1298T showed significant difference between cancer patients and controls. Individuals with the TT genotype of C667T polymorphism had an increased risk of developing ovarian cancer compared with those with CC genotype (Adjusted OR=1.76, 95%CI=1.03 to 3.38). Individuals with 1298 CC gene polymorphism had an increased risk of ovarian cancer compared with AA genotype, with OR (95% CI) of

Table 1. Demographical and clinical characteristics of ovarian cancer patients.

Variable	Cases, N (%)	Controls, N (%)	p value
Age, years [mean, (sd)]	45.6, 9.2	45.2, 8.9	0.33
<30	27(13.5)	26(13.0)	0.99
30-39	50(25.0)	51(25.5)	
40-49	69(34.5)	71(35.5)	
>50	54(27.0)	52(26.0)	
Smoking status			
Smokers	7(3.5)	7(3.5)	<0.05
Former smokers	13(6.5)	3(1.5)	
Nonsmokers	180(90.0)	190(95.0)	
Drinking status			
Drinkers	8(4.0)	9(4.5)	0.42
Former smokers	19(9.5)	12(6.0)	
Nondrinkers	173(86.5)	179(89.5)	
Number of delivery			
0	57(28.5)	23(11.5)	<0.05
1	82(41.0)	70(35.0)	
2	45(22.5)	61(30.5)	
≥3	16(8.0)	46(23.0)	
Menopausal status			
No	95(47.5)	85(42.5)	0.32
Yes	105(52.5)	115(57.5)	
Ovarian cancer in first relatives			
Yes	187(93.5)	199(99.5)	<0.05
No	13(6.5)	1(0.5)	
Tumor type			
Invasive	125(62.5)		
Borderline	73(36.5)		
Missing	2(1.0)		
Chemo-therapy			
Yes	151(75.5)		
No	49(24.5)		
Radio-therapy			
Yes	7(3.5)		
No	193(96.5)		

2.14 (1.15 to 5.76). Individuals with daily folate consumption>335 ug/day had a lower risk of ovarian cancer, with an adjusted OR (95% CI) of 0.50(0.25 to 0.97).

Table 3 showed the association between prognosis of ovarian cancer and MTHFR C677T and folate intake. All the patients were followed up since the diagnosis until the end of January, 2011. Among the 200 patients, 9 were lost to follow-up. Finally, 191 patients were investigation successfully. The median time of follow-up was 34.9 months (minimum and maximum were 2 months and 60 months, respectively). A total of 118 patients (59%) died during the follow-up period. The 1298CC genotype was related to a poor survival of ovarian cancer (HR = 2.11,

Table 2. Frequency distribution and association of MTHFR C677T genotypes with ovarian cancer.

Genotype/Allele	Cases, N (%)	Controls, N (%)	OR[1] (95% CI)	OR[2] (95% CI)
MTHFR C677T				
CC	91 (45.5)	93 (46.5)	1.0 (Ref.)	1.0(Ref.)
CT	83 (41.5)	88 (44.0)	0.96 (0.62-1.49)	1.06 (0.71-1.75)
TT	26 (13)	19 (9.5)	1.39 (0.69-2.87)	1.76 (1.03-3.38)
MTHFR A1298C				
AA	107 (53.5)	112 (56.0)	1.0 (Ref.)	1.0 (Ref.)
AC	77 (38.5)	79 (39.5)	1.02 (0.66-1.57)	1.22 (0.73-1.78)
CC	16 (8.0)	9 (4.5)	1.86 (0.74-4.98)	2.14 (1.15-5.76)
Daily folate consumption (µg/d)				
Mean, (SE)	226.5, 33.7	249.8, 37.4	-	-
<180	44 (22.0)	37 (18.5)	1.0 (Ref.)	1.0 (Ref.)
180-335	91 (45.5)	83 (41.5)	0.92 (0.52-1.62)	0.84 (0.47-1.44)
>335	65 (32.5)	80 (40.0)	0.68 (0.38-1.23)	0.50 (0.25-0.97)

1.None adjusted; 2. Adjusted for age, tobacco smoking, number of delivery and ovarian cancer history.

Table 3. Kaplan-Meier survival estimation of median survival and HRs with MTHFR C677T gene polymorphism.

Genotype	N (%)	Death (%)	Five years survival rate (%)	HR(95% CI), value	P
MTHFR C677T					
CC	91(45.5)	44 (37.3)	51.2	1.0 (reference)	
CT	83(41.5)	52 (44.0)	36.9	1.32 (0.79-2.22)	
TT	26(13)	22 (18.7)	14.4	1.95 (0.96-3.97)	
MTHFR A1298C					
AA	107(53.5)	56 (47.4)	47.3	1.0 (reference)	
AC	77(38.5)	48 (40.2)	37.9	1.39(0.81-2.08)	
CC	16(8.0)	15 (12.4)	7.8	2.11(1.13-4.61)	
Folate intake (µg/d)					
<180	44(22.0)	35 (29.4)	20.5	1.0 (reference)	
180-335	91(45.5)	52 (43.5)	43.1	0.68 (0.35-1.37)	
>335	65(32.5)	32 (27.1)	50.4	0.51 (0.23-1.06)	

[1]Adjusted for age, tobacco smoking, number of delivery and ovarian cancer history.

95% CI = 1.13 to 4.61). We found an non significant inverse relation between intake of folate and the risk of death.

DISCUSSION

The present study indicated that MTHFR C677T and A1298C polymorphisms were associated with susceptibility to ovarian cancer, and high folate consumption is associated with a decreased risk of ovarian cancer. Moreover, MTHFR 1298 CC polymorphism was related to poor prognosis of ovarian cancer compared with AA genotype. Morever, patients with high folate consumption may have better survival than individuals with low diet folate intake. Therefore, we could suggest the folate metabolism has a role in the development and prognosis of ovarian cancer.

MTHFR catalyzes a key step in the folate metabolism, as a result, variations in its activity promote alterations on the levels of circulating folate, and previous studies indicate the folate has a role in carcinogenesis due to its role in critical processes like methylation and DNA synthesis and repair (Choi and Mason, 2000; Giovannucci et al., 2003; Giovannucci et al., 1993). Our study showed the two MTHFR polymorphisms (C677T and A1298C)

could change the activity of folate metabolism, and MTHFR 677TT and 1298CC gene could increase the risk of ovarian cancer. Previous study showed the MTHFR polymorphisms is associated with several cancer risk (Kim et al., 2012; Zhao et al., 2011; Neves et al., 2010; Hosseini et al., 2011; Kwak et al., 2008).

It has been shown that individuals who carry the heterozygote forms C677T and A1298 C have a 50–60% decrease in MTHFR activity compared with those with only wild-type alleles. A reduction in MTHFR activity leads to greater quantities of its substrate 5, 10-MTHF, required for DNA synthesis, and thereby reduces the availability of uracil. Misincorporation of uracil during DNA synthesis may result in double-strand breaks during DNA excision repair. Therefore, the reduced activity could induce the carcinogenesis of cancer. For ovarian cancer, previous study showed the conflicting results (Pawlik et al., 2012; Terry et al., 2010; Zhang et al., 2004), the different results might be variation in ethnicities, patients selection, lifestyle and random by chance.

Folate mediates the synthesis of nucleotides necessary for DNA synthesis, replication, and repair and in DNA methylation reactions (Wang et al., 2008), which could induce the carcinogenesis. Although various epidemiological studies have shown the inverse association between folate intake and the risk of cancers, the role of folate supplement for specific population in the carcinogenesis is still controversial (Kim, 2004). In some animal experiment analysis, dietary folate deficiency cancer inhibit rather than enhance the devlopement of cancer, which is in contrast to the observation in epidemiological studies. Some even demonstrate that an overly abundant intake of folate might instead produce a paradoxical promotion of tumorigenesis among those who harbour pre-existing, undiagnosed precancerous and cancer lesions (Mason, 2009; Aune et al., 2011). Our study also proved the protective role of folate intake, which indicated that the folate intake could decrease the risk of ovarian cancer, and controversial results may be due to ethnicity variation.

Our study showed a significant association between MTHFR A1298C polymorphism and ovarian cancer development and prognosis. However, previous studies showed inconsistent results between this gene polymorphism and ovarian cancer (Goode et al., 2010; Terry et al., 2010). Some biologic data support a potential mechanism of MTHFR A1298C polymorphism for the cancer survival. The balance of the 5,10-MTHF and 5-MTHF controlled by MTHFR may influence the efficacy of chemotherapy with 5-fluorouracil (5-FU). The inactivity 1298CC could increase the amounts of 5, 10-MTHF. Cell line experiments suggest that fluoropyrimidines like 5-FU exert their effect by inhibiting thymidylate synthetase through the formation of a ternary complex, involving 5-FU, thymidylate synthetase, and 5, 10-MTHF (Zhang et al., 1992). Therefore, these MTHFR polymorphisms may enhance the effect of 5-FU by increasing the amount of 5, 10-MTHF. Previous study showed MTHFR 1298A>C and MTHFR diplotypes (for C677T and A1298C) were associated with chemoradiation-related toxicity when 5-FU was used alone (Thomas et al., 2011). In our study, most of the cases got chemotherapy, and our study showed MTHFR1298CC is related to a higher death risk of ovarian cancer, which indicated the interaction between MTHFR A1298C polymorphism and chemotherapy.

In conclusion, we found high folate intake may have a protective role in the development and prognosis of ovarian cancer, and MTHFR gene polymorphisms may be associated with ovarian cancer risk and its prognosis. Further studies in Chinese populations with larger sample sizes is warranted.

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