Genetic Polymorphisms of One-carbon Enzymes Interactively Modify Metabolic Folate Stress and Risks of Hepatocellular Carcinoma Development

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Abstract

Few studies have comprehensively evaluated how genetic modifiers of metabolic one-carbon stress as folate deficiency and hyperhomocysteinemia interact to modify the host-susceptibility of human hepatocellular carcinoma (HCC) development. Genetic polymorphisms of 3 key one-carbon enzymes (methyleneetetrahydrofolate reductase: MTHFR, methionine synthase: MS, and thymidylate synthase: TS) at 5 loci were characterized in a case-control and hospital-based Asian population (n=398). The monophenotypic analysis revealed that the T variant allele at MTHFR 677 loci in relative to the other genotyped variant alleles (MTHFR 1298C, MS2756G, TSEP2R, TSSUPT1494+6bp insertion) was associated with a significant 40% reduction of HCC risks in the dominant model (adjusted ORs: 0.6, 95% CI: 0.4-0.9, P=0.03). Among individuals with low metabolic folate stress (serum folate<6 ng/mL), the MTHFR CC wild-type interacted with the TSEP2R variant alleles to increase HCC risks (OR: 14.6 vs. 0.3, 95% CI: 0.1-0.8), whereas 2-fold reduced HCC were associated with the compound MTHFR T and TSEP2R variant alleles (OR: 0.14 vs. 0.07, 95% CI: 0.02-0.2) (P for interaction: 0.044) after adjustment for serum homocysteine (Hcy) levels. The TSEP2R or TSSUPT+6bp variant alleles interacted with MTHFR T variant allele to reverse its lowering serum folate and elevating Hcy effects (P for trend=0.009 and 0.001, respectively). Taken together, our data demonstrated that MTHFR 677 T and TSEP2R variant allele interacted to alleviate metabolic one-carbon folate stress, which folate-genetic interactions may be the important elements in favor of reduced HCC risks.

Keywords: Metabolic folate stress; Genetic polymorphisms; Hepatocellular carcinoma

Introduction

Serving as one one-carbon donor or acceptor to mediate de novo synthesis of purine and pyrimidine, folate-mediated one-carbon metabolism is critical for normal biochemical and physiological function of the liver [1]. Depleted folate status resulted in one-carbon metabolic and genetic stress including elevated homocysteine (Hcy) levels, aberrant DNA methylation, oxidative DNA, lipid and protein damage in hepatocytes and liver tissues [2,3]. Numerous animal studies have demonstrated that elevated one-carbon metabolic stress by dietary folate deprivation led to hepatocellular carcinoma (HCC) development [3-5]. Despite several hepatic disorders subgroups are reported to frequently suffer from folate deficiency [6-8], there are relatively limited human studies to demonstrate relationships between the increased folate-mediated one-carbon stress and HCC carcinogenesis. A prospective high-risk cohort study showed an association of low blood folate with risks for liver damage and HCC on Caucasian population [9]. Asia subjects in high metabolic one-carbon stress as low serum folate and elevated Hcy levels had significantly increased risks for HCC development [10]. The accumulating evidence suggests an universally important role of elevated one-carbon metabolic stress in human HCC development among different races.

Genetic polymorphisms of one-carbon enzymes have been proposed to modulate metabolic one-carbon stress of human subjects. The most studied key enzyme in folate-mediated one-carbon metabolism is the methylenetetrahydrofolate reductase (MTHFR). It irreversibly converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate for the remethylation of Hcy to methionine by methionine synthetase (MS), providing the precursor of S-adenosylmethionine (SAM) for DNA methylation [11]. A polymorphism at position 677 (C677T) of the MTHFR gene is associated with 30% and 65% reduced enzyme activity in heterozygous (CT) and homozygous (TT) variants, respectively [12]. Another polymorphism at position A1298C of the MTHFR gene is associated with moderately reduced the enzyme activity [13]. Individuals with the MTHFR 677T or 1298C variant alleles commonly have reduced plasma folate levels and increased Hcy concentrations [14,15]. Reduced MS activity by genetic polymorphism at A2756G was associated with elevated Hcy levels [16].

Thymidylate synthase (TS) is the other rate-limiting enzyme which catalyzes the conversion of dUMP to dTMP for DNA synthesis and repair. The potentially functional TS polymorphisms, a 28-bp tandem repeat in the TS 5’-untranslated enhanced region (TSEP) [17], or a 6-bp deletion/insertion at nucleotide 1494 in the 3’-untranslated region of the TS gene (TS 3’-UTR), were correlated with alteration of TS expression [18]. As the TS competes with the MTHFR for their mutual substrate of 5, 10-methylenetetrahydrofolate in one-carbon metabolism, reduced
mRNA expression levels affected by TSER 2R/2R variant allele or TS3'UTR -6/-6 variant alleles [17,19,20] have been associated with lower plasma folate and elevated Hcy levels [18,20–22]. Although the results of the reports are not conclusive, polymorphisms of these one-carbon enzymes not only promoted metabolic stress but also genetic stress such as altered DNA methylation, uracil misincorporation and oxidative DNA damage [23–26], all of which have been proposed as plausible mechanisms in human HCC development.

The full complement of one-carbon enzymes involved in methyl and homocysteine metabolisms is tissue specific. The liver is the major organ with the most active one-carbon metabolism. Hepatic activities of MTHFR, MS and TS could be modulated by availability of their substrate and coenzymes, genetic polymorphisms, rates of one-carbon flux and diseases conditions. Few studies have evaluated how genetic modifiers of metabolic one-carbon stress, mainly emerging from folate deficiency and hyperhomocysteinemia, may interact to modify the host-susceptibility of human HCC development. Several studies reported an increased risk for HCC associated with MTHFR TT variant [27,28], whereas others reported a reduced HCC risk for individuals with TT genotype [29,30]. The genetic MS and TS polymorphisms have been individually linked to several cancer risks including colorectal cancers [31–33], yet with relatively little evidence on HCC development. None of the reported results comprehensively evaluates folate-polymorphic interactions on HCC risks. We test the hypothesis in a case-control and hospital-based study on Asian population in the area where a high HCC incident prevailed. DNAs extracted from peripheral blood monocytes (PBMC) of HCC cases and HCC-free controls (n=398) were genotyped for genetic polymorphisms of 3 key one-carbon enzymes at 5 loci. Serum folate and Hcy levels were assayed for metabolic one-carbon stress. Interactions between one-carbon genetic polymorphisms and metabolic folate stress in modifying risks of HCC development were investigated.

Materials and Methods

Study subjects

The present study was carried out in the period Jan 2005 to Aug 2009. Patients with HCC were recruited from two Medical Centers, Chi-Mei Hospital (CMH) and National Taiwan University Hospital (NTUH) at the Southern and Northern Taiwan, respectively, into the B vitamin and HCC Cancer Prevention Study. Details of recruiting HCC patients with diagnostic criteria for the presence of HCC have been described elsewhere [34]. Briefly, the presence of HCC were diagnosed by two physicians with the data of alpha-fetoprotein elevation (>400 ng/mL), liver imaging (by B-type ultrasonography, computed tomography, magnetic resonance imaging, or/and angiography), and/or histologic exam. In total, 199 HCC patients participated in the entire study. The controls were selected from Community Healthy Exam, who participated in a healthy screening program at Chi-Mei Hospital. The controls were matched by sex with the HCC patients. Exclusion criteria included viral infection, chronic liver diseases, and alcohol abuse as assessed by a medical history, a complete physical examination, and routine laboratory examination. The study protocol was approved by the Joint Ethical Committee of Fu-Jen University, Taiwan National University and Chi-Mei Hospital. All participants have provided written informed consent to participate in the study. The written consent content covered (1) IRB preliminary examine research project; (2) The screened eligible control subjects; (3) The clinical trial participants consent, based on regulations of Taiwan National University and Chi-Mei Hospital IRB or Fu-Jen University Ethics Committee.

Blood biochemical determinations

Within 1 week following the diagnosis of HCC presence and prior to the subsequent treatment of HCC in scheduled consultations, patients donated fasting blood samples. Blood of the controls was collected during the time they received health examination. Peripheral blood samples were taken after a 12 h fasting period, chilled, and transported to the laboratory. Plasma and serum samples were immediately separated upon arrival and were stored at -80°C until further analysis. Lymphocytes were purified from whole blood using standard Ficoll-Hyphaque centrifugation, and were used for molecular genetic analysis in the study. Folate and total homocysteine (tHcy) levels were measured in the serum samples using commercially available radioimmunoassay kits (Becton Dickinson, Orangeburg, NY), and by fluorescence polarization immunoassay (Becton Dickinson) on an Abbott 130 AxSYM system (Becton Dickinson), respectively.

Genetic polymorphisms

The MTHFR C677T, A1298C, and MSA2756G polymorphisms were determined by real-time PCR and melting curve analysis using a Light Cycler instrument (LightCycler, Roche Diagnostics, Mannheim, Germany). The TSER tandem repeat polymorphism and TS 3'UTR polymorphism was analyzed by PCR-RFLP as previously described [17,20]. The forward and reverse primers, the hybridization probes and the amplic products for each genotype were presented in Table 1. Briefly, the reaction buffer consisted of 4 μL of Light Cycler DNA Master Hybridization Probe mix (Taq DNA polymerase, reaction buffer, dNTP mixture, and 10 mM MgCl2), 200 nM probe 1, 400 nM primers, and 50 ng DNA. After a preincubation of the reaction mixture at 95°C for 10 min, thermo cycling was carried out at 95°C for 5 s, 55°C for 5 s, and 72°C for 5 s for 40 cycles. The quality control of each genotype was performed by direct DNA sequencing of separated amplified DNA fragments by electrophoresis in a 2% agarose

| Gene  | Amplified product | Primer and hybridization probe sequence (5’–3’) |
|-------|-------------------|-----------------------------------------------|
| MTHFR C677T | 166 bp | Forward 5'-TGG CAG GTT ACC CCA AAG G-3' |
| Reverse 5'-TTG CTC ATC TAT GGC TAT CTT GCA-3' |
| Probe 1 5'-TGA GGC TGA CCT GAA GCA CCT AGT GAA GGA GAA GGT T-FL |
| Probe 2 5'-LC Red640-CGG GAG CCG ATT TCA TCA T-3' |
| MTHFR A1298C | 183 bp | Forward 5'-CTT TGG GGG AGC TGA AGG ACT AG T-3' |
| Reverse 5'-CAC TTT GTG ACC ATT CCG GTT GT-3' |
| Sensor [A] 5'-CTT CAA AGA CAC TTT CTT CAC TGG TCC T-FL |
| Anchor 5'-640-CTC CCC CCC CCA CAT CTT CAG CAG-3' |
| MSA2756G | 290 bp | Forward 5'-TGT CTC ATC TAT GGC TAT CTT GCA-3' |
| Reverse 5'-GAC ACT GAA GAC CTC TGA TTT GAA CTA-3' |
| Probe 1 5'-GAA GAT ATT AGA CAG GAC CAT TAT GFL-3' |
| Probe 2 5'-640-GTC TCT CAA GGT ATT CGG TTA TCG TGA TAA AAA CAG TTT-3' |
| TSER | 238 bp or 210 bp | Forward 5'-CGT GCC TCC TGT GTT TCC C-3' |
| Reverse 5'-GGG CCG GCC ACA GGC AT-3' |
| TS 3'UTR | 158 bp | Forward 5'-CAA ATC TAA GGG AGC TGA GT-3' |
| Reverse 5'-CAG ATA AGT GGC AGT ACA GA-3' |

Table 1: The primers and hybridization probes sequence of nucleotide for the analysis of one-carbon metabolism genes.
gel at 100 V for 40 min. DNA sequencing was conducted with a Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer, Wellesley, MA, USA) and an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Statistical analyses were performed using the Statistical Analysis System (SAS/STAT version 6.12, SAS Institute, Cary, NC). Chi-square test was used to examine differences in the distribution of selected demographic variables and in genotypic frequencies between cases and controls. Laboratory data of continuous variables were compared using student t tests between cases and controls. We calculated the geometric mean of levels of serum folate and Hcy levels within strata of MTHFR and TS genotypes and case-control status using analysis of covariance. Logistic regression models were used to examine the associations between folate status, MTHFR, MS and TS genotypes, and risk of HCC. The strength of a given parameter associated with HCC was measured by its odds ratio and the corresponding 95% confidence interval and 2-sided P value. Dependent variables such as serum folate and Hcy levels that were not normally distributed were log-transformed. Differences were considered to be statistically significant for P values of <0.05.

Results

Characterization of metabolic one-carbon stress among the study subjects

As metabolic one-carbon status of the study subjects was profiled using blood biochemical markers, mean serum folate levels of the control and HCC cases were 11.7 ± 6.8 and 7.9 ± 5.2 ng/mL, respectively (Table 2). HCC cases had significantly lower serum folate and elevated tHcy concentrations as compared to the healthy control (P<0.001) (Table 2). Metabolic one-carbon stress of the study subjects were characterized by clinical folate deficiency (serum folate <6 ng/mL) and hyperhomocysteinemia (Hcy levels >13 μM). Rates of marginal folate deficiency (43%) and hyperhomocysteinemia (32%) among HCC cases were 3-fold and 5-fold higher than those of the controls (P <0.0001), respectively. The data suggested an elevated metabolic one-carbon stress among HCC cases.

Genetic modifiers of metabolic one-carbon stress in the study subjects

To genotype the subjects, the melting curve-histograms representing for the genetic polymorphisms of MTHFR C677T, MTHFR A1298C, and MS A2756G loci of several HCC patients by use of hybridization probe and real-time PCR analysis were shown in Figure 1. Figure 2 shows the representative gel images for the genetic polymorphism of TSER and TS 3’-UTR using restriction fragment length polymorphism (RFLP) analysis. The quality control of each genotype was confirmed by direct DNA sequencing of amplified DNA products among the designated HCC patients (Figure 3). Genotypic and allelic frequencies of the MTHFR, MS, and TS polymorphisms at 5 loci among the controls and HCC patients are summarized in Table 3. The data revealed that distribution of MTHFR C677T variants was 25% CT and 12% TT in the controls, and 31% CT and 8% TT in the HCC group. The T allele frequencies marginally differed between the controls and HCC cases (29% vs. 23%, P=0.05). Both frequencies of MTHFR A1298C variant allele in the control (20%) and cases (19%), and MS 2756G variant allele in the control (10%) and cases (10%) were not significantly different. Allelic frequencies for the TSER 2R variant allele in the control (17%) and HCC cases (19%) did not significantly differ. Neither did frequencies of the TS 3’UTR polymorphisms for the minor allele +6/+6 bp insertion in the control (23%) and the cases (27%). All of the genotype distributions were in agreement with Hardy-Weinberg Equilibrium in both cases and controls (data not shown).

Single one-carbon polymorphism in relation to HCC risks

Logistic regression analysis for HCC risks in relation to each variant allele of one-carbon polymorphism were examined (Table 4). In relative to the homozygous wild-type C allele at the 677 loci of the MTHFR gene, the compound heterozygous and homozygous variant T allele were associated with a significant 40% reduction of HCC risks (dominant model: Adjusted ORs: 0.6, 95% CI: 0.4-0.9, P=0.03). Conversely, the compound heterozygous or homozygous wild-type C allele had a non-significant 40% increase of HCC risks (recessive model: Adjusted OR: 1.4, 95% CI: 0.7-2.8, P=0.4). Neither in the dominant nor the recessive model, TS polymorphism at single loci of TSER or TS3’UTR were not significantly associated with HCC risks. The MTHFR A1298C variant allele or the MS 2756G variant allele did not correlate with HCC risks (data not shown). For sum of MTHFR and TSER 2R variant alleles, individuals with 3 or 4 mutant alleles exhibited a 50% reduction in risk of HCC as compared to those with no mutant allele, yet without achieving a statistical significance.

Interactions between the single one-carbon polymorphism and metabolic folate stress on HCC risks

We exam how single loci of each one-carbon polymorphism may interact with metabolic one-carbon stress to modify HCC risks by stratifying serum folate status into high metabolic one-carbon stress (deficient folate level <6 ng/mL) or low stress (normal folate level >6 ng/mL). The data are shown in Figure 4. For those with high metabolic stress in folate-deficient status, the compound CT and TT variant genotypes as compared with the CC genotype were significantly associated with 80% reduced risks of HCC (OR: 0.2, 95% CI: 0.1-0.6, P<0.05) (Figure 1A). For those carrying the homozygous MTHFR CC wild-type allele, low metabolic folate stress (normal serum folate >6 ng/mL) was associated with a 90% decrease of HCC risks (OR: 0.1, 95% CI: 0.06-0.3, P<0.05). Low metabolic folate stress in combination with T variant alleles (CT+TT genotype) further reduced HCC risks by 20% as compared with the wild-type CC genotype (OR: 0.08, 95% CI: 0.04-0.2, P<0.05) (Figure 1A). Genetic polymorphisms of TS gene at both 3’ and 5’UTR loci did not modify HCC risks among individuals with high metabolic folate stress (serum folate >6 ng/mL) (Figure 1B and C). Regardless of TSER and TS 3’UTR polymorphisms, individuals with low metabolic folate stress (serum folate >6 ng/mL) had a significant 70-80% reduced HCC risks as compared with those in high metabolic folate stress (Figures 1B and 2C). No significant interactions between

Table 2: Base line and metabolic one-carbon stress of the control and HCC patients

| Age, years | Control subjects | HCC patients | P values |
|------------|------------------|--------------|----------|
| 57.4 ± 9.8 | 62.9 ± 11.4      | <0.0001     |
| Sex, male/female | 133/66 | 138/81 | 0.67    |
| Serum folate, ng/mL | 11.7 ± 6.8 | 7.9 ± 5.2 | <0.0001 |
| Deficiency rate, n (%) | 27 (14) | 86 (43) | <0.0001 |
| Homocysteine, μmol/L | 9.6 ± 3.7 | 12.3 ± 5.8 | <0.0001 |
| Hyperhomocysteinemia, n (%) | 11 (6) | 63 (32) | <0.0001 |

Table 2: Base line and metabolic one-carbon stress of the control and HCC patients

Values are expressed as mean ± SD for continuous variables and proportions (%) for categorical variables. Statistical differences were determined by Wilcoxon test for continuous variables and by χ² test for categorical variables; 2Folate and tHcy values were log transformed for statistical tests; 3Folate deficiency was defined as the serum folate less than 6 ng/mL; 4Hyperhomocysteinemia is defined as the serum Hcy levels greater than >13 μmol/L.
Figure 1: Real-time representative histograms showing genetic polymorphisms at MTHFR C677T, MTHFR A1298C, and MS A2756G loci of the subjects. (A) The MTHFR C677T genotype was monitored at 640 nm and the melting curve showed a single peak at 64°C for CC samples, a single peak at 55°C for TT samples, and two peaks for heterozygous samples. (B) Genotyping the MTHFR C677T polymorphisms among HCC cases (number C24–28) by use of hybridization probe and real-time PCR analysis. (C) The A1298C genotype was monitored at 705 nm and the melting curve showed a single peak at 63°C for AA samples, a single peak at 60°C for CC samples, and two peaks for heterozygous samples. (D) Genotyping the MTHFR A1298C polymorphisms among HCC cases (number C24–28) by use of hybridization probe and real-time PCR analysis. (E) The MS A2756G genotype was monitored at 640 nm and the melting curve showed a single peak at 60°C for A/A samples, a single peak at 55°C for G/G samples, and two peaks for heterozygous samples. (F) Genotyping the MS A2756G MTHFR polymorphisms among HCC cases (number C24–28) by use of hybridization probe and real-time PCR analysis.
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Figure 2: Representative histograms of genetic polymorphism of TSER (A) and TS 3'UTR 1494+6 insertion region (B) using RFLP PCR analysis. (A) Lymphocytic DNAs of the HCC cases (C14, C18 and C29) were assayed for TSER 3R/3R, 3R/2R, and 2R/2R genotypes. (B) Lymphocytic DNAs of the HCC cases (C14, C18 and C29) were assayed for TS 3'UTR 0 bp/0 bp, 0 bp/6 bp insertion, and 6 bp/6 bp.

Interactions between the compound MTHFR and the TS genotype, and the metabolic folate stress on HCC risks.

The interactive effects of the compound MTHFR and TS polymorphisms and metabolic folate stress on HCC risks were assessed (Table 5). As compared with the compound MTHFR 677CC and TS 3R/3R wild-types (OR: 0.14, 95% CI: 0.05-0.4), two-fold increased HCC risk was associated with the compound MTHFR CC allele and TSER 2R variant alleles (OR: 0.3, 95% CI: 0.1-0.8), whereas 2-fold reduced risk was associated with the compound MTHFR T variant allele and TS 2R variant allele (OR: 0.07, 95% CI: 0.02-0.2). The folate-polymeric interactions on HCC risks were significant (P for interaction: 0.04). No significant polymorphic interaction on HCC risks was found for the compound TS3'UTR genotype and MTHFR CT/TT genotypes after adjustment for age, gender, and Hcy levels.

Interactions of the single and the compound TS and MTHFR polymorphisms in modifying metabolic one-carbon stress: serum folate and Hcy levels of the study subjects.

We at last investigate how the MTHFR C677T or/and TSER variant alleles may interact to modify one-carbon metabolic stress of the study subjects, both of which are risk factors for HCC development (Table 6). Among the control subjects, increased numbers of MTHFR 677 T variant alleles in relative to the CC wild-type allele were significantly associated with decreasing serum folate (P for trend: 0.004) and elevated Hcy levels (P for trend: 0.01). No significant effects of the single MTHFR or the TS3'UTR polymorphism on serum folate and tHcy levels of the controls were observed. In relative to the homozgyous major allele genotypes for CC-3R/3R as the reference, the compound CT-3R/3R genotypes were significantly associated with reduced serum folate levels (P=0.02). The compound TT-3R/3R genotypes was associated...
Kuo CS, Cheng CP, Kuo HT, Chen CH, Huang CY, et al. (2016) Genetic Polymorphisms of One-carbon Enzymes Interactively Modify Metabolic Folate Stress and Risks of Hepatocellular Carcinoma Development. J Nutr Food Sci 6: 518. doi:10.4172/2155-9600.1000518

Genetic polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) genes in relation to risk of hepatocellular carcinoma [Logistical regression analysis with the adjustment for age and sex].

Table 3: Genotypic and allelic frequencies between control subjects and patients with hepatocellular carcinoma1-3 [Abbreviation of the genes: MTHFR: Methylenetetrahydrofolate reductase; MS: Methionine synthase; TS: Thymidylate synthase; TSER: Enhancer region of TS; TS3'UTR: Un-translate region at 3'end of TS; 1The 398 subjects were genotyped for the following polymorphisms: 2Differences in genotypic and allelic frequencies between control subjects and HCC patients were tested by χ2 test].

| Genetic polymorphisms                  | Controls                  | HCC patients               | P values |
|----------------------------------------|---------------------------|----------------------------|----------|
| MTHFR C677T, n (%)                     |                           |                            |          |
| CC                                     | 101 (63)                  | 122 (61)                   | 0.11     |
| CT                                     | 40 (25)                   | 62 (31)                    |          |
| TT                                     | 19 (12)                   | 15 (8)                     |          |
| C (%)                                  | 71                       | 77                         | 0.05     |
| T (%)                                  | 29                       | 23                         |          |
| MTHFR A1298C, n (%)                    |                           |                            |          |
| AA                                     | 56 (62)                   | 60 (66)                    | 0.53     |
| AG                                     | 31 (34)                   | 25 (27)                    |          |
| GG                                     | 2 (2)                     |                            |          |
| A (%)                                  | 90                       | 90                         | 1        |
| G (%)                                  | 10                       | 10                         |          |
| TSER, n (%)                            |                           |                            |          |
| 3R/3R                                  | 138 (69)                  | 131 (66)                   | 0.71     |
| 3R/2R                                  | 55 (28)                   | 60 (30)                    |          |
| 2R/2R                                  | 6 (3)                     | 8 (4)                      |          |
| 3R (%)                                 | 83                       | 81                         | 0.46     |
| 2R (%)                                 | 17                       | 19                         |          |
| TS 3'UTR1494, n (%)                    |                           |                            |          |
| 0 bp/0 bp                              | 120 (60)                  | 109 (55)                   | 0.54     |
| 0 bp/+6bp                              | 65 (33)                   | 74 (37)                    |          |
| +6bp/+6bp                              | 14 (7)                    | 16 (8)                     |          |
| 0 bp (%)                               | 77                       | 73                         | 0.29     |
| +6bp (%)                               | 23                       | 27                         |          |

Table 4: Genotypic polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) genes in relation to risk of hepatocellular carcinoma [Logistical regression analysis with the adjustment for age and sex].

| Genotypes | Case/Control | OR (95% CI) | P value |
|-----------|--------------|-------------|---------|
| MTHFR C677T |              |             |         |
| CC         | 223 (122/101)| 1.0         | -       |
| CT         | 141 (62/79)  | 0.7 (0.4-1.0)| 0.5    |
| TT         | 34 (15/19)   | 0.6 (0.3-1.3)| 0.5    |
| P for trend |             |             |         |
| Dominant Model |          |             |         |
| CC         | 223 (122/101)| 1.0         | -       |
| CT/TT      | 175 (77/88)  | 0.6 (0.4-0.9)| 0.03   |
| Recessive Model |          |             |         |
| TT         | 34 (15/19)   | 1.0         | -       |
| CC/CT      | 364 (184/180)| 1.4 (0.7-2.8)| 0.4    |
| TSER       |              |             |         |
| 3R/3R      | 269 (131/138)| 1.0         | -       |
| 3R/2R      | 115 (60/55)  | 1.2 (0.8-1.9)| 0.9    |
| 2R/2R      | 14 (8/6)     | 1.5 (0.5-4.8)| 0.6    |
| P for trend |             |             |         |
| Dominant Model |          |             |         |
| 3R/3R      | 269 (131/138)| 1.0         | -       |
| 3R/2R      | 129 (68/61)  | 1.2 (0.8-1.9)| 0.4    |
| Recessive Model |          |             |         |
| 2R/2R      | 14 (8/6)     | 1.0         | -       |
| 3R/3R + 3R/2R | 384 (191/193)| 0.7 (0.2-2.2)| 0.5    |
| TS3'-UTR   |              |             |         |
| 0/0        | 229 (109/120)| 1.0         | -       |
| 0/+6bp     | 139 (74/65)  | 1.2 (0.8-1.9)| 0.7    |
| +6bp/+6bp  | 30 (16/14)   | 1.3 (0.6-2.8)| 0.7    |
| P for trend |             |             |         |
| Dominant Model |          |             |         |
| 0/0        | 229 (109/120)| 1.0         | -       |
| 0/+6bp + +6bp | 169 (90/79)| 1.2 (0.8-1.9)| 0.3    |
| Recessive Model |          |             |         |
| +6bp/+6bp  | 30 (16/14)   | 1.0         | -       |
| 0/+6bp + 0/0 | 368 (183/185)| 0.9 (0.4-1.9)| 0.7    |
| Sum of TSER and TS 3'-UTR variant alleles | | | |
| 0          | 187 (91/96)  | 1.0         | -       |
| 1          | 168 (85/83)  | 1.0 (0.7-1.6)| 0.8    |
| 0-1        | 43 (23/20)   | 1.2 (0.6-2.5)| 0.6    |
| P for trend |             |             |         |
| Sum of MTHFR and TSER variant alleles | | | |
| 0-1        | 310 (160/150)| 1.0         | -       |
| 2          | 66 (29/37)   | 0.8 (0.5-1.4)| 1.0    |
| 3          | 21 (9/12)    | 0.5 (0.2-1.4)| 1.0    |
| 4          | 1 (1/0)      | 1.0         | -       |
| P for trend |             |             |         |
| Sum of MTHFR and TS 3'-UTR variant alleles | | | |
| 0-1        | 293 (150/143)| 1.0         | -       |
| 2          | 79 (36/43)   | 0.8 (0.5-1.3)| 1.0    |
| 3          | 23 (12/11)   | 1.0 (0.4-2.3)| 0.7    |
| 4          | 3 (1/2)      | 0.5 (0.1-5.5)| 0.6    |
| P for trend |             |             |         |
Discussion

Few studies have comprehensively evaluated how genetic modifiers of metabolic one-carbon stress may modify human HCC risks. We found that the T variant allele at MTHFR 677 loci in relative to the other genotyped variant alleles (MTHFR 1298C, MS 2756G, TSER 2R, TS3'UTR 1494 +6bp insertion) was predominantly associated with a significant 40% reduction of HCC risks (ORs: 0.6, 95% CI: 0.4-0.9, \( P=0.03 \)). Based on the mono-polymorphic assessment, several Caucasian population-based studies reported the protective effects of MTHFR 677TT alleles against HCC development [29,30,35], whereas the other studies on Asia population did not observe such T allele-polymermic protective effect [27,28]. The possibility for the inconclusive results may in part, if not all, be due to lack of evaluation on the compound polymorphic interactions among the key one-carbon enzymes, corroboration of which activities determined the hepatic one-carbon flow, modulated one-carbon metabolic stress and may interactively modify HCC risks [27-33]. One study by Yuan et al. [30] has demonstrated that the maximum number of mutant alleles in the 3 polymorphic loci of MTHFR1298, 677, and TS3'UTR was associated with a significant 62% reduced HCC risks on Caucasians and a non-significant 38% reduced HCC risks on Asians. Similarly, we observed a non-significant 50% reduced HCC risks associated with maximum numbers of variant alleles in the 3 polymorphic loci of MTHFR677T, TS3'UTR, and TSER (Table 4). A wide ethnic variation of variant allele frequency reported for TS polymorphisms may in part account for the discrepant observations among different races in modifying HCC risks. The TS 2R variant allele is less common in Asians (<20%, Table 2) than Caucasians (40%) [36]. The TS 3'UTR +6/+6 allele was the major allele in Caucasians, whereas this +6/+6 allele constitutes the minor, and possibly the variant allele genotype in Asians [30,32].

In addition to one-carbon genetic modifiers of HCC risks, metabolic one-carbon stress plays the key role in modulating HCC development in rodents [3-5] and humans [9,10]. The major first-time finding in the present study was to demonstrate that the folate-genetic polymorphisms interacted to modify the host-susceptibility of HCC development. Only among those with low metabolic folate stress, the compound CC genotype and TSER 2R variant allele was associated with 2-fold increased HCC risks, whereas the compound T and 2R variant allele was correlated with 2-fold reduced HCC risk (\( P \) for interaction: 0.044). How folate-genetic interaction may differentially modify HCC risk is unclear. Several mechanisms are plausible. It has been proposed that reduced MTHFR activity by CT and TT genotypes in the absence of other mutations in TS may deviate one carbon flow from remethylation process to de novo thymidylate synthesis, part of metabolic one-carbon stress plays the key role in modulating HCC development in rodents [3-5] and humans [9,10]. The major first-time finding in the present study was to demonstrate that the folate-genetic polymorphisms interacted to modify the host-susceptibility of HCC development. Only among those with low metabolic folate stress, the compound CC genotype and TSER 2R variant allele was associated with 2-fold increased HCC risks, whereas the compound T and 2R variant allele was correlated with 2-fold reduced HCC risk (\( P \) for interaction: 0.044). How folate-genetic interaction may differentially modify HCC risk is unclear. Several mechanisms are plausible. It has been proposed that reduced MTHFR activity by CT and TT genotypes might deviate one carbon flow from remethylation process to de novo thymidylate synthesis, part of which one carbon flow can be distributed toward purine synthesis with the compound T and 2R variant allele. In particular under normal folate status which provides sufficient one-carbon sources, such one-carbon redistribution by the compound T and 2R variant alleles was reinforced to enrich dNTP pool in favor of DNA repair [25]. Given the fact that the compound MTHFR T and TSER 2R variant alleles reversed blood folate-lowering and Hcy-elevating effects of TT allele alone among those with normal blood folate level (Table 6), our observation supported the compound T and 2R variant alleles effect on one-carbon redistribution hypothesis. Enhanced provisions of a better supply of thymidylate and purines by the compound T and 2R variant allele are critical for damaged DNA repair, a defensive mechanism of clearing mutagenic lesions for HCC carcinogenesis [24,31]. It has been reported that the MTHFR 677TT genotype was associated with reduced mis-incorporation dU contents of healthy human subjects [23], with reduced DNA mutations of colon tumour [33], and the compound CT/CG genotype was associated with a significantly reduced risk of developing colorectal cancer [26].

![Figure 4: Modifying effects of serum folate levels and genetic polymorphisms on HCC risks.](Image)

Serum folate levels of the study subjects were stratified into deficient (<6 ng/mL) or normal status (>6 ng/mL), and polymorphic effects on HCC risks on (A) MTHFR677T, (B) TS 3'UTR or (C) TSER polymorphisms were assessed by logistic regression analysis. HCC risks were adjusted by age, gender and serum Hcy levels [Data were shown by odds ratio (OR) and the corresponding 95% confidence interval (CI) in parenthesis. Statistical significance was set 2-sided \( P \) value <0.05 when 95% CI <1.0].
**Table 5:** Interactions between MTHFR C677T genotypes, TS genotypes and folate metabolic stress on HCC risk. 

| Genotype | Folic acid (ng/mL) | Homocysteine (umol/L) |
|----------|-------------------|-----------------------|
|          | mean | SD | P | mean | SD | P |
| **MTHFR** |       |    |   |       |    |   |
| CC       | 101  | 14.3 | 9.7 | (referent) | 101  | 9.13 | 2.3 | (referent) |
| CT       | 79   | 12.2 | 7.9 | 0.10 | 79   | 9.06 | 2.3 | 0.56 |
| TT       | 19   | 10.0 | 7.5 | 0.23 | 19   | 11.6 | 4.5 | <0.01 |

P for trend: - - - 0.004 - - - 0.01

**TSER** |       |    |   |       |    |   |
| 3R/3R    | 138  | 12.8 | 8.2 | (referent) | 138  | 9.41 | 2.8 | (referent) |
| 3R/2R    | 55   | 14.0 | 10.4 | 0.06 | 55   | 9.11 | 2.3 | 0.16 |
| 2R/2R    | 6    | 11.8 | 10.5 | 0.54 | 6    | 10.0 | 2.3 | 0.80 |

P for trend: - - - 0.30 - - - 0.40

**TS3'-UTR** |       |    |   |       |    |   |
| 0/0      | 120  | 13.1 | 9.5 | (referent) | 120  | 9.27 | 2.6 | (referent) |
| 0/+6     | 65   | 12.9 | 7.5 | 0.15 | 65   | 9.49 | 3.1 | 0.62 |
| +6/+6    | 14   | 13.9 | 10.3 | 0.77 | 14   | 9.30 | 1.5 | 0.05 |

P for trend: - - - 0.70 - - - 0.70

**TSER-MTHFR** |       |    |   |       |    |   |
| 3R/3R-CC | 76   | 14.3 | 9.2 | (referent) | 76   | 9.21 | 2.3 | (referent) |
| 3R/2R-CC | 23   | 14.7 | 11.6 | 0.40 | 23   | 8.87 | 2.1 | 0.78 |
| 2R/2R-CC | 2    | 10.8 | 4.3 | 0.36 | 2    | 9.10 | 0.8 | 0.22 |
| 3R/3R-CT | 51   | 11.4 | 6.6 | 0.02 | 51   | 9.09 | 2.5 | 0.28 |
| 3R/2R-CT | 24   | 13.8 | 9.6 | 0.91 | 24   | 8.75 | 1.8 | 0.15 |
| 2R/2R-CT | 4    | 12.3 | 13.3 | 0.35 | 4    | 10.5 | 2.8 | 0.45 |
| 3R/3R-TT | 11   | 8.25 | 4.5 | 0.06 | 11   | 12.2 | 5.2 | <0.01 |
| 3R/2R-TT | 8    | 12.4 | 10.1 | 0.69 | 8    | 10.8 | 3.4 | 0.73 |
| 2R/2R-TT | 0    | -    | -    | -    | 0    | -    | -    |

P for trend: - - - 0.70 - - - 0.70

**TS3'UTR-MTHFR** |       |    |   |       |    |   |
| 0/0-CC   | 60   | 14.5 | 10.6 | (referent) | 60   | 9.11 | 2.0 | (referent) |
| 0/+6-CC  | 33   | 15.0 | 8.6 | 0.44 | 33   | 9.04 | 2.9 | 0.15 |
| +6/+6-CC | 8    | 10.1 | 5.5 | 0.16 | 8    | 9.62 | 1.2 | 0.06 |
| 0/0-CT   | 50   | 12.4 | 8.4 | 0.23 | 50   | 8.95 | 2.5 | 0.10 |
| 0/+6-CT  | 25   | 10.5 | 4.5 | <0.01 | 25   | 9.44 | 2.0 | 0.84 |
| +6/+6-CT | 4    | 20.9 | 13.9 | 0.52 | 4    | 8.05 | 1.6 | 0.50 |
| 0/0-TT   | 10   | 8.00 | 4.8 | 0.08 | 10   | 11.8 | 4.1 | 0.001 |
| 0/+6-TT  | 7    | 11.3 | 8.4 | 0.42 | 7    | 11.7 | 5.8 | <0.01 |
| +6/+6-TT | 2    | 15.4 | 16.4 | 0.46 | 2    | 10.5 | 1.1 | 0.23 |

Table 6: Interactions of the TS or/and MTHFR polymorphisms in modifying metabolic one-carbon stress of the control subjects. Values are expressed as mean ± SD for continuous variables. Within each genotype or the compound genotypes, the statistical differences between the referent (wild-type genotype) and the variant genotypes were determined by t test at P<0.05. P for trend was analyzed by contrast for linear for continuous variables. Differences were considered to be statistically significant at P<0.05. Folate and tHcy values were log transformed for statistical tests.
TT genotypes with reduced oxidative DNA damage of lymphocytes of HCC patients [26]. The reported protection against genetic instability due to the reduced MTHFR activity by T allele may serve as the mechanistic basis to confer host-susceptibility of HCC development [37].

Without being in compound with TT genotype, the 2R/2R double repeat in TSER than the triple repeat alone is associated with lower TS expression in HeLaS3 cells [17] or in tumour tissue [19], may restrict TS activity to induce deregulation of DNA synthesis, repair and cell cycle progression [18,38,39]. The compound MTHFR CC wild-type allele with TSER 2R variant allele did not enhance substrate provision of 5, 10-methylene-THF to compensate reduced TS activity. By the observation that the compound MTHFR CC wild-type and TSER 2R variant alleles displayed an adverse effect in folate status (Table 6), the study extended to support the differential folate-genetic interaction in one-carbon folate flow in modifying HCC risk.

It should be noted that the significant T and R allelic interaction of modulating blood folate status and modifying HCC risks was only observed among those with normal folate status, but not for those with folate-deficiency. Similarly, the compound MTHFR T and TS3UTR +6/+6bp variant alleles reversed blood folate-lowering and Hcy-elevating effects of TT-allele alone among those with normal blood folate level (Table 6). As the TS 3'UTR +6/+6bp alleles in relative to the 0/0bp alleles were associated with higher expression of TS transcripts [20] and higher levels of serum folate levels [18], the enhanced channeling one-carbon flow toward DNA synthesis and repair by such combined variant genotypes of MTHFR and TS may favor in recycling one-carbon unit to tetrahydrofolate for reentering the one-carbon metabolism and balancing overall one-carbon flux for each metabolic cycle. As modulation of metabolic one-carbon stress by the compound MTHFR and TS variant genotypes is only effective under sufficient folate levels of the control subjects, the data suggest a threshold effect of metabolic one-carbon stress for such folate-polymorphic interactions in the protection against HCC progression. In fact, individuals with low metabolic one-carbon stress (serum folate ≥ 6 ng/l) had significantly 80-90% reduced HCC risks regardless any examined MTHFR and TS genotype at any tested loci (Figure 4). The beneficial effects of sufficient folate status in supplying one-carbon units for each metabolic cycle of one-carbon metabolism may maximize the favorable one-carbon flux by altered one-carbon enzymatic activity due to different genetic polymorphisms. Our data also provide the plausible explanation why results of the current literature investigating the MTHFR or/and TS polymorphic effects in cancer risks are inconsistent [27-29,32,36]. Without consideration of the preexisting one-carbon folate status with the interactive polymorphic impact as the whole, their real functional associations with cancer risks cannot be truly explicated.

Our findings should be interpreted with a few limitations. Due to the unavailability of tumor specimens, analysis of polymorphic-genetic-folate interactions by germline genotypes of MTHFR and TS, and serum folate levels may not reflect the true relationships in the target HCC tissues. Small sample sizes may be underpowered for detecting a small but significant association. Larger sample sizes of the studies are needed to clarify whether the TSER or 3'UTR 1494ins6 polymorphism could truly affect one-carbon folate status in the cases and controls. Lastly, the inherent limitations associated with cross-sectional study designs do not depict the causal effect of MTHFR and TS polymorphisms, folate status and HCC development. Further prospectively designed studies are warranted.

In summary, our data suggest a strong polymorphic effect by reduced MTHFR activity genotype in serum folate and Hcy levels, both in magnitude levels and functional profiles, was differentially influenced by TS variant allelic interaction in normal folate-dependent threshold levels. The compound polymorphic impact of T and 2R variant allele in reduced HCC risk of folate-sufficient individuals supports the hypothesis that deviation of one carbon flux in favor of thymidylate and purine synthesis, possibly for DNA repair, may be a key anticancer mechanism of HCC carcinogenesis. As TS is a target for chemotherapeutic drugs such as 5-fluorouracil, and its mRNA and protein expression levels as the prognostic indicators for certain cancers [40,41], further studies on effects of polymorphic-folate interaction in HCC survival and prognosis are warranted. Since MTHFR and TS genetic polymorphisms interacted to modulate metabolic stressor of folate status, one-carbon polymorphic identification should be useful to serve as pre-diagnostic markers of metabolic stress in providing alternative strategies in HCC prevention and prognosis.

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Author Disclosures

Chang-Sheng Kuo and Chin-Pao Cheng contribute equally to the study. CS Kuo, CP Cheng, HT Kuo, CH Chen, CY Huang, CC Chen and RFS Huang: No conflicts of interest.

References

1. Shane B (1995) Folate chemistry and metabolism. In: Folate in Health and Disease, LB Bayliss, editor. New York: Marcel Dekker.

2. Kim YI, Pogribny IP, Basnakian AG, Miller JW, Selhub J, et al. (1997) Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr 65: 46-52.

3. James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, et al. (2003) Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J Nutr 133: 3740S-3747S.

4. Mikol YB, Hoover KL, Creasia D, Poirier LA (1983) Hepatocarcinogenesis in rats fed methyl-deficient, amino acid-defined diets. Carcinogenesis 4: 1619-1629.

5. Pogribny IP, James SJ, Jernigan S, Pogribna M (2004) Genomic hypomethylation is specific for preneoplastic liver in folate/methyl deficient rats and does not occur in non-target tissues. Mutat Res 548: 53-59.

6. Tkaczkewski W, Niedzielska H, Malafiej E, Dwomiaik D, Draminski M (1971) Studies of serum folic acid level in patients with viral hepatitis. Pol Med J 10: 1081-1084.

7. Wu A, Chanarin I, Slavin G, Levi AJ (1975) Folate deficiency in the alcoholic-its relationship to clinical and haematological abnormalities, liver disease and folate stores. Br J Haematol 29: 469-478.

8. Bosy-Westphal A, Petersen S, Hinrichsen H, Czech N, Muller JM (2001) Increased plasma homocysteine in liver cirrhosis. Hepatol Rev 20: 28-38.

9. Welzl TM, Katki HA, Sakoda LC, Evans AA, London WT, et al. (2007) Blood folate levels and risk of liver damage and hepatocellular carcinoma in a prospective high-risk cohort. Cancer Epidemiol Biomarkers Prev 16: 1279-1282.

10. Wu MY, Kuo CS, Lin CY, Lu CL, Syu Huang RF (2009) Lymphocytic mitochodrial DNA deletions, biochemical folate status and hepatocellular carcinoma susceptibility in a case-control study. Br J Nutr 102: 715-721.

11. Selhub J (1999) Homocysteine metabolism. Annu Rev Nutr 19: 217-246.

12. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, et al. (1994) Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping, and mutation identification. Nat Genet 7: 551-554.

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13. Lievers KJ, Boers GH, Verhoef P, den Heijer M, Kluftmans LA, et al. (2001) A second common variant in the methylene-tetrahydrofolate reductase (MTHFR) gene and its relationship to MTHFR enzyme activity, homocysteine, and cardiovascular disease risk. J Mol Med (Berl) 79: 522-528.

14. Bailey LB, Gregory JF 3rd (1999) Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J Nutr 129: 919-922.

15. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R (1998) A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 64: 169-172.

16. Guerhani S, Oliveira E, Marrackchi R, Ben Slama MR, Staxi M, et al. (2007) Methylene-tetrahydrofolate reductase and methionine synthase polymorphisms and risk of bladder cancer in a Tunisian population. Cancer Genet Cyto-genet 176: 48-53.

17. Horie N, Alba N, Oguro K, Hojo H, Takeishi K (1995) Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5'-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct Funct 20: 191-197.

18. Trinh BN, Ong CN, Coetzee GA, Yu MC, Laird PW (2002) Thymidylate synthase: a novel genetic determinant of plasma homocysteine and folate levels. Hum Genet 111: 299-302.

19. Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, et al. (2001) Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. Pharmacogenomics J 1: 65-70.

20. Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, et al. (2000) Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiol Biomark Prev 9: 1381-1385.

21. Kealey C, Brown KS, Woodside JV, Young I, Murray L, et al. (2005) A common insertion/deletion polymorphism of the thymidylate synthase (TYSM) gene is a determinant of red blood cell folate and homocysteine concentrations. Hum Genet 111: 347-353.

22. Chen J, Hunter DJ, Stampfer MJ, Kyle C, Chan W, et al. (2003) Polymorphism in the thymidylate synthase promoter enhancer region modifies the risk and survival of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2: 958-962.

23. DeVos L, Chanson A, Liu Z, Ciappio ED, Parrell LD, et al. (2008) Associations between single nucleotide polymorphisms in folate uptake and metabolizing genes with blood folate, homocysteine, and DNA uracil concentrations. Am J Clin Nutr 88: 1149-1158.

24. Chiang EP, Wang YC, Tang FY (2007) Folate restriction and methylenetetrahydrofolate reductase 677T polymorphism decreases adedmeth synthesis via folate-dependent remethylation in human-transformed lymphoblasts. Leukemia 21: 651-658.

25. Quinlivan EP, Davis SR, Shelnutt KP, Henderson GN, Ghandour H, et al. (2005) Methylene-tetrahydrofolate reductase 677C ≥ T polymorphism and folate status affect one-carbon incorporation into human DNA deoxynucleosides. J Nutr 135: 389-396.

26. Kuo CS, Huang CY, Kuo HT, Cheng CP, Chen CH, et al. (2014) Interrelationships among genetic C677T polymorphism of 5,10-methylenetetrahydrofolate reductase, biochemical folate status, and lymphocytic p53 oxidative damage in association with tumor malignancy and survival of patients with hepatocellular carcinoma. Mol Nutr Food Res 58: 329-342.

27. Zhu Z, Cong WM, Liu SF, Xian ZH, Wu WQ (2008) A study on the association of MTHFR C677T polymorphism with genetic susceptibility to hepatocellular carcinoma. Zhonghua Gan Zang Bing Za Zhi 14: 196-198.

28. Mu LN, Cao W, Zhang ZF, Cai L, Jiang QW, et al. (2007) Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms and the risk of primary hepatocellular carcinoma (HCC) in a Chinese population. Cancer Causes Control 18: 665-75.

29. Saffroy R, Pham P, Chiappini F, Gross-Goupil M, Castera L, et al. (2004) The MTHFR 677C > T polymorphism is associated with an increased risk of hepatocellular carcinoma in patients with alcoholic cirrhosis. Carcinogenesis 25: 1443-1448.

30. Yuan JM, Lu SC, Van Den Berg D, Govindarajan S, Zang QZ, et al. (2007) Genetic polymorphisms in the methylenetetrahydrofolate reductase and thymidylate synthase genes and risk of hepatocellular carcinoma. Hepatology 46: 749-756.

31. Ulrich CM, Bigler J, Bostick R, Fosdick L, Potter JD (2002) Thymidylate synthase promoter polymorphism, interaction with folate intake, and risk of colorectal adenoma. Cancer Res 62: 3361-3364.

32. Zhou JY, Shi R, Yu HL, Zeng Y, Zheng WL, et al. (2012) The association between two polymorphisms in the TS gene and risk of cancer: a systematic review and pooled analysis. Int J Cancer 131: 2103-2116.

33. Ulrich CM, Curtin K, Potter JD, Bigler J, Caan B, et al. (2005) Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 14: 2509-2516.

34. Kuo CS, Lin CY, Wu MY, Lu CL, Huang RF (2008) Relationship between folate status and tumor progression in patients with hepatocellular carcinoma. Br J Nutr 100: 596-602.

35. Jin F, Qu LS, Shen XZ (2009) Association between the methylenetetrahydrofolate reductase C677T polymorphism and hepatocellular carcinoma risk: a meta-analysis. Diagn Pathol 4: 39.

36. Marsh S, Collie-Duguid ES, Li T, Liu X, McLeod HL (1999) Ethnic variation in the thymidylate synthase enhancer region polymorphisms among Causasian and Asian population. Genomics 58: 310-312.

37. Lau JWY, Leow CK (1999) Surgical management. In: Leong ASY, Liew CT, Lau JWY, Johnson PJ, editors. Hepatocellular carcinoma: diagnosis, investigation and management.

38. Chu E, Allegra CJ (1996) The role of thymidylate synthase as a RNA binding protein. Bioessays 18: 191-198.

39. Kastanos E, Zajac-Kaye M, Dennis PA, Allegra CJ (2001) Downregulation of p21WAF1 expression by thymidylate synthase. Biochem Biophys Res Commun 285: 195-200.

40. Moertel CG (1994) Chemotherapy for colorectal cancer. N Engl J Med 330: 1136-1142.

41. Shintani Y, Ohta M, Hirabayashi H, Tanaka H, luchi K, et al. (2003) New prognostic indicator for non-small-cell lung cancer, quantitation of thymidylate synthase by real-time reverse transcription polymerase chain reaction. Int J Cancer 104: 790-795.