Association Between Gene Expression of Metabolizing Enzymes and Esophageal Squamous Cell Carcinomas in China

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Epidemiological studies have indicated that the incidence of esophageal squamous cell carcinoma (ESCC) is associated with environmental exposure to mutagens and carcinogens. To determine whether the basal expression level of genes involved in metabolism of carcinogens is associated with the risk of ESCC, a case–control study of 100 patients with newly diagnosed, untreated ESCC and 117 healthy controls was performed, and the relative expression levels of four metabolism genes (*CYP2E1*, *GSTP1*, *MTHFR*, and *NQO1*) were determined with quantitative real-time reverse transcription–polymerase chain reaction in peripheral blood mononuclear cells (PBMCs). Analyzed with the mean of relative expression level in the controls as the cut-off point, the result exhibited that the increased risk for ESCC was significantly associated with reduced expression of *GSTP1* (odds ratio [OR]=3.644, 95% confidence interval [CI: 1.947–6.823) and *NQO1* (OR=1.870, 95% CI: 1.046–3.345). When adjusted for age, sex, smoking status, and alcohol use, the increased risk for ESCC was significantly associated with reduced expression of *GSTP1*, *MTHFR*, and *NQO1*, and *GSTP1* mRNA showed a steady association with the risk for ESCC (OR=2.640) in the model of stepwise regression analysis. Reduced expression of *GSTP1* in PBMCs was significantly associated with the risk for ESCC, suggesting an important etiology clue to the early progression of ESCC in the Huaian population of China.

Introduction

MANY STUDIES HAVE indicated that the incidence of esophageal squamous cell carcinoma (ESCC) is associated with environmental and genetic factors (Kuwano *et al.*, 2005; Malik *et al.*, 2010). Increasing our understanding of the role of genetic factors in determining human susceptibility to the carcinogenic effects of environmental agents has become a major research goal in molecular epidemiology. Some xenobiotics present in tobacco smoke and a diet cooked with improper methods, such as polycyclic aromatic hydrocarbons and nitrosamines, are not directly carcinogenic, but are converted to reactive metabolites capable of interacting with cellular DNA (Lin *et al.*, 2002; Kamangar *et al.*, 2005). The risk of environment-related esophageal cancer is suggested to be associated with bioactivation and detoxification of these xenobiotics, which is due to phase I and phase II metabolizing enzymes. Phase I enzymes oxidize a wide range of substrates, resulting in activation of environmental precarcinogens. Phase II enzymes play a central role in the detoxification of carcinogenic electrophilic compounds. The aberrant function of the metabolizing enzyme system may influence the balance

of the metabolizing capacity between bioactivation and detoxification of xenobiotics. Among these metabolizing enzymes, there are four enzymes, cytochrome P450 2E1, glutathione S-transferases P1 (*GSTP1*), NAD(P)H dehydrogenase, quinone 1 (*NQO1*), and methylenetetrahydrofolate reductase (*MTHFR*), which have been associated with susceptibility to cancer by different studies.

Cytochrome P450 2E1 coded by the human *CYP2E1* gene, a member of the cytochrome P450 mixed-function oxidase system, is involved in the metabolism of exogenous substrates, including benzene, carbon tetrachloride, ethylene glycol, and nitrosamines, which are premutagens found in cigarette smoke. It is becoming increasingly evident that *CYP2E1* is expressed in human esophageal tissue (Lechevrel *et al.*, 1999; Wang *et al.*, 2009). Due to its many substrates, the aberrant function of this enzyme may play a role in oncogenesis of esophageal cancer. Glutathione S-transferases (GSTs) are a superfamily of dimeric phase II metabolic enzymes that play an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. *GSTP1* is a major member of the cytosolic GST superfamily expressed in

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esophagus and generally serves as a protector of cellular macromolecules from the damage caused by cytotoxic and carcinogenic agents that are thought to function in the xenobiotic metabolism and play a role in susceptibility to cancer (Brabender *et al.*, 2002). NQO1 is a flavoenzyme that catalyzes obligate two-electron reduction of a wide variety of substrates, including quinones, quinone-imines, nitro- and azo-compounds. The enzyme functions via a hydride transfer mechanism with both NADH and NADPH and generates antioxidant forms of both vitamin E and ubiquinone after a free-radical attack, which precludes generation of reactive oxygen radicals and protects cells from oxidative challenge. NQO1 knockout mice demonstrated increased susceptibility to benzo(a)pyrene- and 7,12-dimethylbenz(a) anthracene-induced skin carcinogenesis (Long *et al.*, 2001), which suggested that NQO1 plays an important role in protection against exogenous compounds. MTHFR, encoded by the *MTHFR* gene, catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene THF) to 5-methyltetrahydrofolate (5-methyl THF), whereas the latter is the primary methyl donor for methionine synthesis. Diminution in the activity of the MTHFR enzyme influences the availability of 5-methyl THF, affecting misincorporation of uracil in the DNA synthesis pathway, which might result in double-strand breaks during uracil excision repair processes, thus increasing the risk of chromosomal aberrations (Stolzenberg-Solomon *et al.*, 2003).

Since the expression of metabolizing enzymes in the esophagus usually is influenced by environmental xenobiotics, we prefer to use peripheral blood mononuclear cells (PBMCs) to evaluate the role of the basal expression level of genes that have not been affected by tissue-specific mutagens or carcinogens in the susceptibility of ESCC. In the present research, we established quantitative real-time reverse transcription (RT)-polymerase chain reaction (PCR) to determine the relative expression levels of the four metabolizing enzyme genes (*CYP2E1*, *GSTP1*, *MTHFR*, and *NQO1*), and we analyzed the association between the expression of candidate metabolizing enzyme genes and ESCC in PBMCs from 100 patients with newly diagnosed, untreated ESCC and 117 healthy controls from the same community.

Material and Methods

Study subjects

The present study included 100 ESCC patients and 117 healthy controls. All cases were from the Huaian County of the Jiangsu province, China. Patients were newly diagnosed with histologically confirmed primary cancer and previously untreated (no radiotherapy or chemotherapy) ESCC from April 2005 to April 2006. Healthy control subjects were recruited from a cancer-screening program for early detection of esophageal cancer in the same area and matched with ESCC patients by age (± 5 years), sex, and residence. The selection criteria included no individual history of cancer and digestive disease. During the period of recruitment, each subject was scheduled for an interview after informed consent was obtained, and a structured questionnaire was administered by the interviewer to collect information about demographic data and risk factors such as smoking status and alcohol use. At the same time, each subject donated 5 mL of peripheral blood in heparinized tubes.

RNA extraction and RT

Peripheral blood samples were obtained from 100 ESCC patients and 117 healthy controls. PBMCs were isolated using Ficoll-Hypaque density-gradient centrifugation. Isolated PBMCs were washed once with phosphate-buffered saline. Total RNA was isolated from cell preparations using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The concentration of RNA was determined spectrophotometrically by monitoring UV absorbance at 260 nm. Purity was assessed by the absorbance ratio 260 nm/280 nm. One to two micrograms of total RNA was used for RT to synthesize cDNA using MMLV reverse transcriptase (Promega). Briefly, 0.5 μ g of oligo dT₁₈ was added to each tube and annealed to RNA by incubating at 70°C for 5 min to melt the secondary structure within the template, and then quick chilled on ice. RT of RNA was performed in a final volume of 25 μ L containing MMLV 5 $\times$ reaction buffer 5 μ L, 10 mM dNTP mixture 1.25 μ L, ribonuclease inhibitor (Sigma) 25 U, MMLV (Promega) 200 U, and RNase-free water to a final volume. The cDNA was synthesized at 42°C for 60 min, heated to 95°C for 5 min, and then stored at -20°C .

Quantitative real-time PCR

The gene expression of *CYP2E1*, *GSTP1*, *MTHFR*, and *NQO1* was determined using a quantitative fluorescent-based dynamic PCR analysis. All real-time PCR reactions were performed in triplicate on the RT products with an ABI 7300 Prism Sequence Detection System (Applied Biosystems), using SYBR Green I dye. The amount of template cDNA was expressed by a threshold cycle (Ct) that was determined by the amplification curve (exponential phase). The parameter Ct is defined as the fractional cycle number at which the fluorescence caused by the binding of SYBR Green dye to double-stranded DNA reaches the detection threshold.

The mRNA levels were compared between subjects by a comparative Ct method with separate tubes, as described elsewhere (Livak and Schmittgen, 2001). Briefly, the individual level of initial target cDNA was expressed as the difference in Ct between the target and an endogenous control (ΔCt). The relative amount of target in a subject was finally given by $2^{-\Delta\text{Ct}}$, which was normalized to an internal control.

Specific primers were designed for each gene containing minimal internal structures (i.e., hairpins and primer dimers) and optimal melting temperatures (i.e., max difference in Tm of two primers as 2°C) using Primer Express software (Version 2.0; Applied Biosystems). Primer's specificity for each gene was tested with the standard nucleotide basic local alignment search tool (BLASTn) covered on the National Center of Biotechnology Information homepage (<http://ncbi.nlm.nih.gov/>). To exclude contamination of cDNA preparations with genomic DNA, primers were designed to amplify regions containing at least one intron in the gene. The sequences and positions of the oligonucleotide sets for each reaction are shown in Table 1. β -Actin was used as an internal control for blood samples.

The PCR reaction was carried out in 96-well plates (Axygen) with a final volume of 20 μ L. The reaction components were 1 μ L of cDNA synthesized as above, 10 μ L of 2 $\times$ SYBR Green PCR Master Mix (Applied Biosystems), and 0.6 μ M of each pair of oligonucleotide primers. Thermal cycling conditions were 50°C for 2 min and 95°C for 10 min, followed by 40

TABLE 1. PRIMER SEQUENCES OF METABOLIZING ENZYME GENES IN THE PRESENT STUDY

Gene name	Primer	Sequence ^a	Amplicon size (bp)	Accession number
CYP2E1	Forward	5'-ACCTGCCCCATGAAGCAACC-3'	246	NM_000773
	Reverse	5'-GAAACAACCTCCATGCGAGCC-3'		
GSTP1	Forward	5'-CCTGTACCAGTCCAATACCATCCT-3'	71	NM_000852
	Reverse	5'-CCTGCTGGTCCTTCCCATAG-3'		
MTHFR	Forward	5'-GGCCATCTGCACAAAGCTAAG-3'	140	NM_005957
	Reverse	5'-AACTCACTTCGGATGTGCTTCAC-3'		
NQO1	Forward	5'-TCATTCTCTGGCCAATTCAGAGT-3'	91	NM_000903
	Reverse	5'-GGAGTGTGCCCAATGCTATATG-3'		
β -actin	Forward	5'-CCTGGCACCCAGCACAAT-3'	70	NM_001101
	Reverse	5'-GCCGATCCACACGGAGTACT-3'		

^aDesigned with Primer Express 2.0 software.

cycles of 95°C for 15 s, optimal annealing temperature for 60 s. A dissociation curve analysis was added after the final PCR cycle to evaluate the presence of nonspecific PCR products and primer dimers. During initial optimization runs, 10-fold serial dilutions were employed to demonstrate a linear amplification range for each gene.

Statistical analysis

Differences in the basal characteristics of patients and control groups were estimated with Fisher's exact test and Student's *t*-test. Analysis of variance statistics was used to estimate differences of mRNA levels between the case and control group at a significance level of 5%. Associations between gene expression levels within the demographics status were explored using Spearman rank correlation coefficients. For calculation of crude odds ratio [OR] and confidence interval [CI], the median of the relative expression level of each gene in the controls was defined as the cut-off point so that the corresponding expression level could be classified as high or low expression. The nonconditional logistical regression models were constructed to calculate adjusted ORs with adjustment for age, sex, smoking status, and alcohol use. All of the statistical analyses were performed using SAS software (Version 8.0).

Results

Demographic characteristics

A summary of selected characteristics, including smoking status of the ESCC patients and controls is shown in Table 2. The mean ages of the patients and controls were 63.67 ± 9.58 and 62.50 ± 9.39 years, respectively. The ratio of male to female was 1.38 and 1.44 in cases and controls, respectively. No statistical differences were observed between cases and controls in the distribution of age and sex, suggesting that the frequency matching was adequate, and the difference was significant in the distribution of cancer family history between cases and controls.

Gene expression in cases and controls

Using the quantitative real-time RT-PCR technique, we examined the expression of four metabolizing enzyme genes, including CYP2E1, GSTP1, MTHFR, and NQO1, in PBMCs from ESCC patients and healthy controls. The relative expression levels of the four metabolism genes in the case group

were consistently lower than that in the control group. As shown in Table 3, the positive frequencies of all four genes showed no statistical differences between patients and controls. The medians of relative expression levels in cases and controls are presented in Table 3. Statistically significant differences were found in the relative expression levels of CYP2E1 ($p=0.036$), GSTP1 ($p=0.0001$), MTHFR ($p=0.009$), and NQO1 ($p=0.002$) between the PBMC samples of patients compared with controls (Student's *t*-test, Table 3). There was no apparent association between gene expression and age, sex, smoking status, and alcohol use.

Association of mRNA levels with ESCC

Using the median of the relative expression level in the controls as the cut-off point for each gene, each gene expression level was defined as high expression or low expression for calculation of crude ORs and 95% CIs as shown in Table 4. Significantly increased risk for esophageal cancer was observed with reduced expression of GSTP1 (OR=3.644, 95%

TABLE 2. CHARACTERISTICS OF ESOPHAGEAL SQUAMOUS CELL CARCINOMA PATIENTS AND CONTROLS

Variable	Cases (n=100)	Controls (n=117)	p Value ^a
Age (in years) mean $\pm$ SD	63.670 $\pm$ 9.580	62.504 $\pm$ 9.389	0.368
Sex			
Male	58	69	0.885
Female	42	48	
Smoking index ^b			
Non-user	47	50	0.753
Ever ^c			
<400	20	29	
$\geq$ 400	33	38	
Alcohol use			
Never	63	77	0.667
Ever ^d	37	40	
Family history			
No	61	103	0.001
Yes	39	14	

^a*t*-Test or χ^2 test.

^bSmoking index=cigarettes/day $\times$ smoking years.

^cSmoked more than 100 cigarettes in lifetime.

^dConsumption of at least one drink a week.

SD, standard deviation.

TABLE 3. mRNA RELATIVE EXPRESSION LEVELS OF METABOLISM GENES ANALYZED IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA PATIENTS AND CONTROLS

Gene	Group (No.)	Mean $\pm$ SD of Δ Ct ^a	t Value	p value ^b
CYP2E1	Cases (97)	10.027 $\pm$ 3.298	2.112	0.036
	Controls (117)	9.024 $\pm$ 3.587		
GSTP1	Cases (100)	6.032 $\pm$ 1.207	5.453	0.0001
	Controls (117)	4.996 $\pm$ 1.586		
MTHFR	Cases (99)	13.945 $\pm$ 3.190	2.643	0.009
	Controls (115)	12.630 $\pm$ 4.082		
NQO1	Cases (100)	9.775 $\pm$ 2.130	3.107	0.002
	Controls (116)	8.720 $\pm$ 2.846		

^a Δ Ct = Ct_{interest gene} - Ct _{β -actin}.

^bt-Test.

CI: 1.947–6.823) and NQO1 (OR = 1.870, 95% CI: 1.046–3.345). The result showed that reduced expression of GSTP1 provided a moderate risk for ESCC.

Logistic regression model for metabolism gene expression levels in ESCC patients and controls

To adjust for potential confounders, we fitted the data into an unconditional logistic regression model, including age, sex, smoking status, alcohol use, and the expression level (Δ Ct) of the four genes. One-way logistic regression analysis indicated that there was a significant association between GSTP1 mRNA expression and the risk for ESCC (OR = 1.768, 95% CI: 1.395–2.242). Stepwise logistic regression analysis was carried out with 14 factors, including the factors as above, and the first-level interaction among the four genes expression. The result (shown in Table 5) explored that the main effect of GSTP1 increased the risk to develop esophageal cancer by 2.64-fold, and the weak combined effect between GSTP1 and CYP2E1 should be examined in a future study.

Discussion

Peripheral blood is commonly easy to obtain and relatively noninvasive compared with tissue. The potential utility of

peripheral blood as surrogate for target tissues to monitor molecular events remains a desirable goal (Borovecki *et al.*, 2005; Sumner *et al.*, 2006; Runne *et al.*, 2007; Gatta *et al.*, 2009; Duzkale *et al.*, 2011; Gui *et al.*, 2011). With the microarray technology, Tang *et al.* (2001) found that mRNA expression profiles in peripheral blood cells were distinct under different disease conditions, which showed the potential of peripheral blood cells for diagnostic, mechanistic, and therapeutic assessment of a wide variety of disease states. Several studies have reported that blood mRNA represents a useful template to investigate specific markers for individual susceptibilities to cancer (Furukawa *et al.*, 2004; Horriike *et al.*, 2005), and many studies proved that mRNA of metabolizing genes had measurable levels in peripheral blood (Krovat *et al.*, 2000; Godoy *et al.*, 2002). In this study, we determined the expression levels of four metabolizing enzyme genes (CYP2E1, GSTP1, MTHFR, and NQO1) with PBMCs from 100 ESCC patients and 117 healthy controls. Interindividual differences in the gene expression are evaluated between patients and healthy controls, and this may contribute to the interindividual susceptibility to environmental carcinogens.

The N-nitrosodimethylamine demethylase, an ethanol-inducible nitrosamine-metabolizing P-450, coded by the human cytochrome P450 2E1 gene, plays an important role in the metabolic transformation of a number of low-molecular-weight organic compounds, including aniline, benzene, as well as N-nitrosamines (Tanaka *et al.*, 2000). Several researches showed that the altered CYP2E1 enzyme activity was associated with the development of some cancers (Oyama *et al.*, 2003; Cai *et al.*, 2005; Lu *et al.*, 2011). Our result appeared a slight decrease of the CYP2E1 mRNA level in the case group compared with that in controls. However, this difference showed no statistical significance after adjusted by age, gender, smoking status, and alcohol use. In contrast to the association of polymorphism on the genomic sequence in the CYP2E1 gene with individual susceptibility to esophageal cancer in our study and others' research, the change of mRNA quantity showed a weak influence on this disease. The inconsistent effect of polymorphisms and mRNA expression in the CYP2E1 gene on esophageal cancer implied that the expressive transcription activity of CYP2E1 might be determined by the combined effect of some coexistent factors

TABLE 4. CRUDE ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR METABOLISM GENE EXPRESSION LEVELS IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA PATIENTS AND CONTROLS

Gene expression level	No. cases (%)	No. controls (%)	χ^2	p Value	OR (95% CI)
CYP2E1					
High	28 (28.9%)	48 (41.0%)	3.424	0.064	1.000
Low	69 (71.1%)	69 (59.0%)			1.714 (0.966–3.041)
GSTP1					
High	18 (18.0%)	52 (44.4%)	17.254	0.001 ^a	1.000
Low	82 (82.0%)	65 (55.6%)			3.644 (1.947–6.823)
MTHFR					
High	28 (28.3%)	47 (40.9%)	3.703	0.054	1.000
Low	71 (71.7%)	68 (59.1%)			1.753 (0.987–3.112)
NQO1					
High	26 (26.0%)	46 (39.7%)	4.506	0.034 ^a	1.000
Low	74 (74.0%)	70 (60.3%)			1.870 (1.046–3.345)

^a $p < 0.05$.

OR, odds ratio; CI, confidence interval.

TABLE 5. ONE-WAY AND MULTIVARIATE LOGISTIC REGRESSION ANALYSIS FOR METABOLISM GENE EXPRESSION LEVELS IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA PATIENTS AND CONTROLS

Logistic regression analysis	Independent variable	OR (95% CI) ^a	p Value
One-way ^b	CYP2E1	1.084 (0.999 ~ 1.177)	0.055
	GSTP1	1.768 (1.395 ~ 2.242)	0.0001
	MTHFR	1.098 (1.018 ~ 1.186)	0.018
	NQO1	1.177 (1.051 ~ 1.319)	0.005
Multivariate ^c	GSTP1	2.640 (1.719 ~ 4.056)	0.0001
	MTHFR	1.244 (1.071 ~ 1.443)	0.004
	CYP2E1*GSTP1 ^d	1.036 (1.009 ~ 1.065)	0.011

^aOR when the unit of Δ Ct equals to 1.

^bAdjusted in a logistic regression model, including age in years, sex, smoking status, and alcohol use.

^cDeviance and Pearson Goodness-of-Fit Statistics: Deviance = 250.9, DF = 207, $p = 0.02$; Pearson = 201.3, DF = 207, $p = 0.599$.

^dThe level of CYP2E1*GSTP1 is defined as the unit of Δ Ct (CYP2E1) increasing with the unit of Δ Ct (GSTP1) decreasing.

in vivo or be disturbed by some confounding factors coming from life habit, which made a different characteristics of mRNA regulation *in vivo* compared with that *in vitro* (Novak and Woodcroft, 2000).

GSTs are phase II metabolizing enzymes that catalyze intracellular detoxification reactions by conjugating of glutathione (GSH) to a variety of electrophilic substrates coming from phase I reaction, including benzo[a]pyrene, nitrosamines, halohydrocarbon, and aflatoxin. (Strange *et al.*, 2001). GSTP1 is widely expressed in normal human epithelial tissue (Terrier *et al.*, 1990). Rasmi *et al.* (2006) reported a higher level of GSTP1 mRNA in esophageal cancer tissues compared with tissues adjacent to cancer. However, some studies suggested different results about the association of GSTP1 mRNA and the encoding protein with esophageal cancer (Brabender *et al.*, 2002; Cobbe *et al.*, 2003). To evaluate whether PBMCs could be used to evaluate the basal expression level of the GSTP1 gene and the relationship between the basal expression level of GSTP1 gene and esophageal cancer, we determined the mRNA level of the GSTP1 gene in PBMCs from patients with ESCC and healthy controls. The result showed that the reduced expression of GSTP1 increased the risk for esophageal cancer by ~3-fold, and there was a weak interaction between reduced GSTP1 mRNA and increased CYP2E1 mRNA. It suggested that the reduced basal expression of GSTP1 resulted in the aberrant metabolizing activity to electrophilic compounds in individuals, which may increase the risk for the development of carcinoma in target tissues that activated carcinogens act on, and the increased expression of CYP2E1 may burden on the detoxification capacity of GSTP1. However, the exact mechanism about the combined effect should be determined by future study.

The NQO1 gene is a member of the NAD(P)H dehydrogenase (quinone) family and encodes a cytoplasmic 2-electron reductase. This FAD-binding protein forms homodimers and reduces quinones to hydroquinones. This protein's enzymatic activity prevents the one-electron reduction of quinones that results in the production of radical species and plays an important role in protection against endogenous and exogenous

quinones (Vasilou *et al.*, 2006). Many epidemiological studies have found that the coding SNPs of NQO1 were associated with the increased risk for some diseases (Wang *et al.*, 2008; Reddy *et al.*, 2009; Sameer *et al.*, 2010). However, few studies have elucidated the effect of expression of the NQO1 gene on the risk of ESCC. In present study, statistically significant differences were found in the relative expression levels of NQO1 between the PBMC samples of patients compared with controls (Student's *t*-test and one-way logistic regression), but the effect did not enter the stepwise regression model, which may explain the weak influence on the disease.

MTHFR is a key enzyme in folate metabolism, catalyzing the irreversible conversion of 5,10-methylene THF, the central metabolite for folate metabolism, to 5-methyl THF, the main circulating form of folate, which serves as both a cofactor and substrate for regeneration of methionine, the precursor of SAM (Choi and Mason, 2000). The aberrant capacity of folate metabolism enzymes was supposed to influence cancer risk through impacting on the DNA methylation pattern or integrity of DNA synthesis (Friso and Choi, 2005). Our result showed that the reduced mRNA level of MTHFR had a weak association with the risk for esophageal cancer (OR = 1.244), which may be explained by the hypothesis that the reduced mRNA level of MTHFR will decrease *de novo* methionine methyl group supply and then leads to hypomethylation of genomic DNA. Some studies *in vitro* and population epidemiological studies supposed that the different folate status has the converse effect on the association of MTHFR with cancer risk (Wang *et al.*, 2005; Stankova *et al.*, 2008). However, because of the absent dietary folate intake data, we cannot evaluate the gene-nutrient interaction in the present study. In future investigation of nutrition, the analysis of the expression of MTHFR with a different folate status will help to understand the complex metabolizing process of folate *in vivo*.

Conclusions

Our study demonstrated that the expression levels of four metabolism genes (CYP2E1, GSTP1, MTHFR, and NQO1) in PBMCs were measurable, and reduced expression of GSTP1 in PBMCs was significantly associated with the risk for ESCC (OR = 2.640), suggesting an important etiology clue to the early progression of ESCC in Huaian of China and could be a useful biomarker of esophageal cancer. In an advanced study, we plan to identify tumor-specific genetic and epigenetic events involved in gene transcription regulation for screening of the high-risk population of ESCC.

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Author Disclosure Statement

No competing financial interests exist.

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