日本財団 助成事業
The Nippon Foundation

財団法人日中医学協会
2004年度共同研究等助成金－在留中国人研究者－報告書

2005 年 3 月 15 日

財団法人 日中医学協会 御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

中国人研究者名：王 静文 王靜文 印

指導責任者名：徳留 信寛 職名：教授

所属機関名：名古屋市立大学

〒467-8601
所在地：名古屋市瑞穂区瑞穂町字川澄1

電話：853-8176 内線：8176

1．助成金額：600,000 円

2．研究テーマ

中国人の肺腺がんの感受性とGSTZ1遺伝子多型の関連

3．成果の概要（100字程度）

この研究において、Ⅱ相代謝酵素ＧＳＴＺ1の遺伝子多型と肺腺がんの関連を明らかにするため中国人の肺腺がんの症例対照研究を行った。遺伝子型の分布についてGSTZ1 Lys32Glu AA、AG、GG genotypeの頻度は症例群のなかでそれぞれに17.0、51.8、31.2%、対照群のなかで26.1、40.3、33.6%であった。変異したGSTZ1Lys32Glu G 遺伝子は有意に肺腺がんのリスクと関連がなかったが（OR=1.64、95%CI 0.86-3.19)、 GSTM1 null genotypeと またはGSTP1 Val allele との共存が有意に肺腺がんのリスクと関連していた (OR=2.91、95%CI 1.06-8.91; OR=3.95、95%CI 1.46-11.47) 。

4．研究業績

(1) 学会における発表 ☑無 ・ □有（学会名・演題）

(2) 発表した論文 ☑無 ・ □有（雑誌名・題名）

－日中医学協会助成事業－

中国人の肺腺がんの感受性とGSTZ1遺伝子多型の関連

研究者氏名　王　静文
中国所属機関　開蘭範各荘鉱病院内科
日本研究機関　名古屋市立大学健康増進・予防医学
指導責任者　教授　徳留　信寛
共同研究者名　とう　一夫，　丁　建民，　姜　晶

Abstract

The glutathione transferases (GST) zeta class is recently identified and found in a range of species from plants to humans. GSTZ1 catalyze the metabolism of a series of alpha-haloacids including dichloroacetate (DCA) and fluroacetate. GSTZ1 also plays an important role in the catabolism pathway of phenlalanine and tyrosine. The human GSTZ1 locus has some polymorphic sites, which were located at nucleotides 23, 94, 124 and 245. Some allelic variants showed significant functional variation with a number of substrate. In our previous studies, the associations between GSTM1, GSTT1 and GSTP1 genetic polymorphisms and lung adenocarcinoma (lung AC) in a Chinese population have been evaluated. In order to assess the effects of GSTZ1 genetic polymorphisms on the development of lung AC, we conducted this case-control study. The frequencies of GSTZ1 Lys32Glu AA, AG and GG genotype were 17.0, 51.8 and 31.2% among cases and 26.1, 40.3 and 33.6% among controls. GSTZ1 Lys32Glu G allele was found to be associated with an elevated lung AC risk (OR=1.64, 95% CI 0.86-3.19). Moreover, GSTZ1 Lys32Glu G allele showed significantly increased lung AC risk when in combination with either GSTM1 null genotype or mutant GSTP1 Val allele (OR=2.91, 95% CI 1.06-8.91; OR=3.95, 95% CI 1.46-11.47, respectively). GSTZ1 Lys32Glu G allele demonstrated a 5.9-fold increased lung AC risk among heavy smokers compared with non-smokers with GSTZ1 Lys32Glu AA genotype.

Key Words

GSTZ1, Genetic Polymorphisms, Dichloroacetate (DCA), Lung Adenocarcinoma (lung AC)

Introduction

It has been well established that glutathione transferases (GSTs), as a large multigene family encoding phase II drug-metabolizing enzymes, catalyze the conjugation of glutathione with a wide variety of hydrophilic and electrophilic substrates.[1] The GSTs are categorized into Alpha, Mu, Pi, Theta and Zeta classes.[2-4] The Zeta class is recently identified, and found in a range of species, including plants, animals and microorganisms.[4] Compared with other GSTs, members of the Zeta class have a distinct range of substrates and functions, which catalyse the metabolism of a series of alpha-haloacids, including dichloroacetate (DCA) and fluoroacetate.[5, 6] DCA is a common contaminant of chlorinated drinking water,[7] and is hepatocarcinogenic in rodents.[8-11] It has been observed that maleylacetoacetate isomerase (MAAI), which converts maleylacetoacetate (MAA) to fumarylacetoacetate (FAA), the penultimate step in the phenylalanine and tyrosine catabolism pathway, has an identical sequence to that of GSTZ1, therefore GSTZ1 also plays a significant role in the pathway of the catabolism of phenylalanine and tyrosine.[12] Several polymorphic alleles of the human GSTZ1 locus have been reported, and some allelic variants showed significant functional variation with a number of substrates.[13] Polymorphic sites of GSTZ1 were located at nucleotides 23, 94, 124 and 245.[14] The T-to-C transition at nucleotide 23 results in a Leu8Pro substitution. A or G

was variably found at nucleotide 94, and A or G was also variably noted at nucleotide 124, these nucleotide changes lead to Lys32Glu and Arg42Gly substitution, respectively. The C-to-T transition at nucleotide 245 causes Thr82Met substitution. These amino acid alterations are known to affect the activities of the GSTZ1 enzyme for different substrates, and affecting the efficiency of removal for these substances.

In our previous studies in a Chinese population, the associations between GSTM1, GSTT1 and GSTP1 genetic polymorphisms and lung adenocarcinoma (lung AC) have been evaluated.[15,16] But no relationship between lung AC and the GSTT1 genotypes was observed, either separately or in combination with the GSTM1 or GSTP1 genotypes. Although separate GSTM1 and GSTP1 polymorphisms were not statistically related to lung AC risk, the coexistence of GSTM1 null genotype and GSTP1 Val allele was significantly associated with an elevated lung AC risk (OR=2.4, 95% CI 1.1-5.1). To date, the role of GSTZ1 in the development of lung AC has not been investigated in our study, and studies about GSTZ1 genetic polymorphisms with susceptibility to lung cancer are limited. In order to assess the effects of GSTZ1 polymorphisms on lung AC, we conducted this case-control study.

Subjects and methods

This case-control study included 112 cases with lung ACs and 119 cancer-free healthy controls. Detailed descriptions of the subjects have been presented previously.[16]

The GSTZ1 Lys32Glu and Arg42Gly polymorphisms were detected by PCR-RFLP using primers 5 -TTCCTTACTGGTCCTCACATCTC-3 and 5 -TCACCGGATCATGGCCA GCA-3 , the PCR product was digested with BsmAI for Lys32Glu and FokI for Arg42Gly, respectively.[4] But the Arg42Gly genotypes failed to be detected, because bands could not be identified after digested by FokI.

The difference in distribution of genotypes between cases and controls was examined with the χ^2 test. For measuring association between GSTZ1 genotypes and lung AC, ORs and 95% CIs were estimated from unconditional logistic regression using the software package SAS (version 8.2) and adjusted for age, sex and smoked pack-years. The combined effects of GSTZ1 genotypes with GSTM1, GSTT1 and GSTP1 polymorphisms were estimated, and gene-environment interactions in terms of cigarette smoking were also evaluated.

Results

The distribution of GSTZ1 Lys32Glu genotypes is presented in Table 1. The frequency of GSTZ1 Lys32Glu AA genotype was elevated among controls (26.1%) than among cases (17.0%), while heterozygote AG was less in controls as compared to the cases (40.3 and 51.8%, respectively), the frequency of GSTZ1 Lys32Glu GG genotype among cases (31.2%) was similar to controls (33.6%). No significant difference in the distribution of GSTZ1 Lys32Glu genotypes was found between cases and controls (P=0.14). The frequencies of three GSTZ1 Lys32Glu genotypes among controls were not different from those expected from the Hardy-Weinberg equilibrium.

The ORs for lung AC by GSTZ1 polymorphism separately and in combination with GSTM1, GSTT1 and GSTP1 polymorphisms are summarized in Table 2. Either GSTZ1 Lys32Glu AG or GG genotype was associated with an elevated risk of Lung AC (OR=1.84, 1.40; respectively), but not statistically significant. With respect to combined genotypes, although respective polymorphism of GSTM1, GSTP1 or GSTZ1 was not statistically associated with lung AC, when the allele G of GSTZ1 Lys32Glu was combined with GSTM1 null genotype, GSTT1 null genotype and GSTP1 Val allele, significantly increased risks were found for combined GSTZ1 Lys32Glu G allele and GSTM1 null genotype (OR=2.91, 95%CI 1.06-8.91) or mutant GSTP1 Val allele (OR=3.95, 95%CI 1.46-11.47).

Interactions of gene and cigarette smoking are presented in Table 3. The combination of smokers and GSTZ1 Lys32Glu G allele was not found significantly increased lung AC risk (OR=1.88, 95% CI 0.80-4.59) as compared to combined non-smokers

and GSTZ1 Lys32Glu AA genotype. However, when smoked pack-years were stratified, ORs of light smokers (0<pack-years<20), moderate smokers (20<=pack-years<40) and heavy smokers (pack-years>=40) were respectively 0.70 (95% CI 0.29-1.65), 1.04 (95% CI 0.50-2.15) and 4.04 (95% CI 1.42-13.41) as compared to non-smokers. Moreover, among heavy smokers, GSTZ1 Lys32Glu G allele was found to be associated with a 5.9-fold increased lung AC risk (OR=5.93, 95% CI 1.65-25.41).

Discussion

GSTs are widely distributed and expressed in many mammalian tissues, the expression of GSTs is regulated in a tissue-specific manner resulting in quantitatively different protein products in different tissues.[17-19] The highest amount of total GSTs protein has been found to express in liver, followed by brain, pancreas, adrenals, heart and lung,[20, 21] where are the major sites for drug and chemical metabolism. GSTZ1 is expressed in multiple tissues in human.[4] GSTZ1 is mainly expressed in liver, testis and prostate, and moderately expressed in brain, heart, pancreatic islets, adrenal medulla, and the epithelial lining of the gastrointestinal tract, airway, and bladder in rats.[22]

As described above, GSTZ1 catalyse the metabolism of a series of alpha-haloacids, including DCA and fluoroacetate.[5, 6] GSTZ1 also plays an important role in the pathway of the catabolism of phenylalanine and tyrosine.[12] Humans are exposed to DCA by environmental and medical ways. DCA is a by-product of chlorination of drinking water, may be consumed every day.[23, 24] DCA is also a metabolite of trichloroethylene and chloral hydrate, the former is found in industrial solvents and degreasing agents, and the latter is a sedative.[25, 26] DCA is also used clinically for the treatment of congenital lactic acidosis.[27, 28] Although the mechanism by which DCA exerts its toxicity has been unclear, studies have shown that DCA perhaps induce GSTZ1 inactivate,[29, 30] and the DCA-induced inactivation of GSTZ1 perturbs tyrosine metabolism in rats,[31] and these perturbations may result in the multiorgan toxicity of DCA, in accordance with the multiple tissues expression of GSTZ1. In the present study, the varied GSTZ1 Lys32Glu G allele showed elevated lung AC risk (OR=1.64, 95% CI 0.86-3.19). Moreover, coexistence of GSTZ1 Lys32Glu G allele with either GSTM1 null genotype or mutant GSTP1 Val allele was found significantly increased lung AC risk (OR=2.91, 95% CI 1.06-8.91; OR=3.95, 95% CI 1.46-11.47, respectively), which also explained the coordinated effects of GSTs detoxifying enzymes.

Tobacco exposure is clearly associated with the development of lung cancer, and individual susceptibility has been investigated in terms of the ability to activate and detoxify carcinogens. Many studies have analyzed the influence of tobacco smoking on lung cancer risk with association with GSTs polymorphisms, but results are contradictory. Some studies have reported a stronger association between lung cancer risk and GSTM1 null genotype or GSTP1 Val allele among heavy smokers,[32, 33] and several studies showed similar associations for low or moderate doses of smoking.[34, 35] In our previous study, GSTM1 null genotype was found to be associated with a 5.9-fold increased lung AC risk among light smokers,[15] and GSTP1 Val allele was found no significantly elevated risk among smokers.[16] In this study, heavy smokers were associated with an increased lung AC risk (OR=4.04, 95% CI 1.42-13.41) as compared with non-smokers. Furthermore, GSTZ1 Lys32Glu G allele showed significantly increased lung AC risk among heavy smokers (OR=5.93, 95% CI 1.65-25.41), even though interactions between GSTZ1 Lys32Glu G allele and smokers were not obvious.

In conclusion, although the distribution of GSTZ1 Lys32Glu genotypes among lung AC cases was not significantly different from controls, GSTZ1 Lys32Glu G allele was found to be associated with an elevated lung AC risk. Moreover, GSTZ1 Lys32Glu G allele showed significantly increased lung AC risk when in combination with either GSTM1 null genotype or mutant GSTP1 Val allele. Among heavy smokers, GSTZ1 Lys32Glu G allele was found to be associated with a 5.9-fold increased lung AC risk.

References

1. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995; **30**: 445-600.

2. Mannervik B, Alin P, Guthenberg C, Jensson H, Tahir MK, Warholm M, Warholm H. Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. *Proc Natl Acad Sci U S A* 1985; **82**: 7202-6.

3. Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B. Theta, a new class of glutathione transferases purified from rat and man. *Biochem J* 1991; **274**: 409-14.

4. Board PG, Baker RT, Chelvanayagam G, Jermiin LS. Zeta, a novel class of glutathione transferases in a range of species from plants to humans. *Biochem J* 1997; **328**: 929-35.

5. Tong Z, Board PG, Anders MW. Glutathione transferase zeta catalyses the oxygenation of the carcinogen dichloroacetic acid to glyoxylic acid. *Biochem J* 1998; **331**: 371-4.

6. Tong Z, Board PG, Anders MW. Glutathione transferase zeta-catalyzed biotransformation of dichloroacetic acid and other alpha-haloacids. *Chem Res Toxicol* 1998; **11**: 1332-8.

7. Krasner SW, McGuire MJ, Jacangelo JG, Patania NL, Reagan KM, Aieta EM. The occurrence of disinfection by-products in US drinking water. *J Am Water Works Assoc* 1989; **81**: 41-53.

8. Bull RJ, Sanchez IM, Nelson MA, Larson JL, Lansing AJ. Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate. *Toxicology* 1990; **63**: 341-59.

9. DeAngelo AB, Daniel FB, Most BM, Olson GR. The carcinogenicity of dichloroacetic acid in the male Fischer 344 rat. *Toxicology* 1996; **114**: 207-21.

10. Harrington-Brock K, Doerr CL, Moore MM. Mutagenicity of three disinfection by-products: di-and trichloroacetic acid and chloral hydrate in L5178Y/TK +/-(-)3.7.2C mouse lymphoma cells. *Mutat Res* 1998; **413**: 265-76.

11. Tao L, Kramer PM, Ge R, Pereira MA. Effect of dichloroacetic acid and trichloroacetic acid on DNA methylation in liver and tumors of female B6C3F1 mice. *Toxicol Sci* 1998; **43**: 139-44.

12. Fernandez-Canon JM, Penalva MA. Characterization of a fungal maleylacetoacetate isomerase gene and identification of its human homologue. *J Biol Chem* 1998; **273**: 329-37.

13. Blackburn AC, Tzeng HF, Anders MW, Board PG. Discovery of a functional polymorphism in human glutathione transferase zeta by expressed sequence tag database analysis. *Pharmacogenetics* 2000; **10**: 49-57.

14. Blackburn AC, Coggan M, Tzeng HF, Lantum H, Polekhina G, Parker MW, Anders MW, Board PG.. GSTZ1d: a new allele of glutathione transferase zeta and maleylacetoacetate isomerase. *Pharmacogenetics* 2001; **11**: 671-8.

15. Wang J, Deng Y, Li L, Kuriki K, Ding J, Pan X, Zhuge X, Jiang J, Luo C, Lin P, Tokudome S. Association of GSTM1, CYP1A1 and CYP2E1 genetic polymorphisms with susceptibility to lung adenocarcinoma: a case-control study in Chinese population. *Cancer Sci* 2003; **94**: 448-52.

16. Wang J, Deng Y, Cheng J, Ding J, Tokudome S. GST genetic polymorphisms and lung adenocarcinoma susceptibility in a Chinese population. *Cancer Lett* 2003; **201**: 185-93.

17. Tu CP, Weiss MJ, Li NQ, Reddy CC. Tissue-specific expression of the rat glutathione S-transferases. *J Biol Chem* 1983; **258**: 4659-62.

18. Rozell B, Hansson HA, Guthenberg C, Tahir MK, Mannervik B. Glutathione transferases of classes alpha, mu and pi show selective expression in different regions of rat kidney. *Xenobiotica* 1993; **23**: 835-49.

19. Rowe JD, Nieves E, Listowsky I. Subunit diversity and tissue distribution of human glutathione S-transferases:

interpretations based on electrospray ionization-MS and peptide sequence-specific antisera. *Biochem J* 1997; **325**: 481-6.

20. DePierre JW, Morgenstern R. Comparison of the distribution of microsomal and cytosolic glutathione S-transferase activities in different organs of the rat. *Biochem Pharmacol* 1983; **32**: 721-3.

21. Listowsky I, Rowe JD, Patskovsky YV, Tchaikovskaya T, Shintani N, Novikova E, Nieves E. Human testicular glutathione S-transferases: insights into tissue-specific expression of the diverse subunit classes. *Chem Biol Interact* 1998; **111-112**: 103-12.

22. Lantum HB, Baggs RB, Krenitsky DM, Board PG, Anders MW. Immunohistochemical localization and activity of glutathione transferase zeta (GSTZ1-1) in rat tissues. *Drug Metab Dispos* 2002; **30**: 616-25.

23. Uden PC, Miller JW. Chlorinated acids and chloral in drinking wanter. *J Am Water Wouks Assoc* 1983; **75**: 524-7.

24. Weisel CP, Kim H, Haltmeier P, Klotz JB. Exposure estimates to disinfection by-products of chlorinated drinking water. *Environ Health Perspect* 1998; **107**: 103-10.

25. Henderson GN, Yan Z, James MO, Davydova N, Stacpoole PW. Kinetics and metabolism of chloral hydrate in children: identification of dichloroacetate as a metabolite. *Biochem Biophys Res Commun* 1997; **235**: 695-8.

26. Merdink JL, Gonzalez-Leon A, Bull RJ, Schultz IR. The extent of dichloroacetate formation from trichloroethylene, chloral hydrate, trichloroacetate, and trichloroethanol in B6C3F1 mice. *Toxicol Sci* 1998; **45**: 33-41.

27. Stacpoole PW, Harman EM, Curry SH, Baumgartner TG, Misbin RI. Treatment of lactic acidosis with dichloroacetate. *N Engl J Med* 1983; **309**: 390-6.

28. Stacpoole PW, Henderson GN, Yan Z, Cornett R, James MO. Pharmacokinetics, metabolism and toxicology of dichloroacetate. *Drug Metab Rev* 1998; **30**: 499-539.

29. Tzeng HF, Blackburn AC, Board PG, Anders MW. Polymorphism-and species-dependent inactivation of glutathione transferase zeta by dichloroacetate. *Chem Res Toxicol* 2000; **13**: 231-6.

30. Anderson WB, Board PG, Gargano B, Anders MW. Inactivation of glutathione transferase zeta by dichloroacetic acid and other fluorine-lacking alpha-haloalkanoic acids. *Chem Res Toxicol* 1999; **12**: 1144-9.

31. Cornett R, James MO, Henderson GN, Cheung J, Shroads AL, Stacpoole PW. Inhibition of glutathione S-transferase zeta and tyrosine metabolism by dichloroacetate: a potential unifying mechanism for its altered biotransformation and toxicity. *Biochem Biophys Res Commun* 1999; **262**: 752-6.

32. Persson I, Johansson I, Lou YC, Yue QY, Duan LS, Bertilsson L, Ingelman-sundberg M. Genetic polymorphism of xenobiotic metabolizing enzymes among Chinese lung cancer patients. *Int J Cancer* 1999; **81**: 325-9.

33. Hirvonen A, Husgafvel-Pursiainen K, Anttila S, Vainio H. The GSTM1 null genotype as a potential risk modifier for squamous cell carcinoma of the lung. *Carcinogenesis* 1993; **14**: 1479-81.

34. Ryberg D, Skaug V, Hewer A, Phillips DH, Harries LW, Wolf CR, Ogreid D, Ulvik A, Vu P, Haugen A. Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. *Carcinogenesis* 1997; **18**: 1285-9.

35. Garcia-Closas M, Kelsey KT, Wiencke JK, Xu X, Wain JC, Christiani DC. A case-control study of cytochrome P450 1A1, glutathione S-transferase M1, cigarette smoking and lung cancer susceptibility (Massachusetts, United States). *Cancer Causes Control* 1997; **8**: 544-53.

Table 1. Distribution of GSTZ1 genotypes in cases and controls

Genotype	Cases (%)	Controls (%)	*P* value [1]
GSTZ1 Lys32Glu			
AA	19 (17.0)	31 (26.1)	
AG	58 (51.8)	48 (40.3)	
GG	35 (31.2)	40 (33.6)	0.14

[1] *P* value by χ^2 text

Table 2. ORs for lung AC by GSTZ1polymorphism, separately and in combination with GSTM1, GSTT1 and GSTP1 polymorphisms

Genotypes		Cases/Controls [1]	ORs (95% CI) [2]
GSTZ1 genotypes			
AA		19/31	1.00 (ref)
AG		58/48	1.84 (0.92–3.74)
GG		35/40	1.40 (0.67–2.97)
AG+GG		93/88	1.64 (0.86–3.19)
Combined genotypes			
GSTZ1	GSTM1		
AA	present	6/14	1.00 (ref)
AG or GG	present	37/45	1.79 (0.64–5.56)
AA	null	13/17	1.78 (0.54–6.36)
AG or GG	null	56/43	2.91 (1.06–8.91)
GSTZ1	GSTT1		
AA	present	7/16	1.00 (ref)
AG or GG	present	52/49	2.23 (0.86–6.35)
AA	null	12/15	1.76 (0.54–5.97)
AG or GG	null	41/39	2.28 (0.86–6.61)
GSTZ1	GSTP1		
AA	Ile/Ile	8/19	1.00 (ref)
AG or GG	Ile/Ile	59/65	2.22 (0.91–5.91)
AA	Ile/Val or Val/Val	11/12	2.69 (0.82–9.20)
AG or GG	Ile/Val or Val/Val	34/23	3.95 (1.46–11.47)

[1] Numbers of Cases/Controls.

[2] Adjusted for age, sex and smoked pack-years.

Table 3. Assessments of interaction of GSTZ1 genotypes and smoking status

Smoking status	GSTZ1 Lys32Glu	Cases/Controls [1]	OR (95%CI) [2]
Non-smokers	AA	12/19	1.00 (ref)
Non-smokers	AG or GG	52/52	1.55 (0.69–3.62)
Smokers	AA	7/12	0.95 (0.28–3.13)
Smokers	AG or GG	41/36	1.88 (0.80–4.59)
Pack-years[3]			
>0, <20	AA	1/5	0.31 (0.02–2.24)
>0, <20	AG or GG	9/12	1.28 (0.40–4.05)
>=20, <40	AA	4/6	1.10 (0.24–4.78)
>=20, <40	AG or GG	18/20	1.53 (0.57–4.19)
>=40	AA	2/1	4.12 (0.33–104.45)
>=40	AG or GG	14/4	5.93 (1.65–25.41)

[1] Numbers of Cases/Controls.

[2] Adjusted for age and sex.

[3] Also referenced to non-smokers with GSTZ1 Lys32Glu AA genotype.