行政院國家科學委員會專題研究計畫　期中進度報告

（子計畫五）膀胱移形細胞癌之個案，健康對照及一等親之慢性砷中毒相關酵素基因多型性之比較（2/3）

計畫類別：整合型計畫
計畫編號：NSC92-3112-B-038-001-
執行期間：92 年 05 月 01 日至 93 年 04 月 30 日
執行單位：臺北醫學大學公共衛生學科

計畫主持人：薛玉梅

報告類型：完整報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 93 年 2 月 23 日

基因體醫學國家型科技計畫

National Research Program for Genomic Medicine
National Science Council, the Executive Yuan, ROC.

砷與移行細胞癌之毒理基因體研究- (子計畫五) 膀胱移行細胞癌之個案，健康對照及一等親之慢性砷中毒相關酵素基因多形性之比較（2/3）

Toxicogenomic Study in Transitional Cell Carcinoma and Arsenic (Project #5) Comparison on Genetic Polymorphism of Arseniasis Related Enzymes among Bladder Transitional Cell Carcinoma Patients, Healthy Controls and First Degree Relatives（2/3）

報告類別：☐ 新進研究計畫 (New Proposal) ☐ 修正後計畫書 (Revised Proposal) ☑ 年度成果報告 (Progress Report)

計畫類別：☐ 個別型計畫 (Individual Project) ☑ 整合型計畫 (Program Project)

計畫編號：**91GMP012-5**

計畫主持人 (Principle Investigator)：薛玉梅

共同主持人 (Co-Principle Investigator)：黃金鼎

執行單位 (Institution)：臺北醫學大學醫學系公共衛生學科

中華民國 92 年 12 月 27 日

National Research Program for Genomic Medicine
National Science Council, the Executive Yuan, ROC.
Progress Report—Research Project

基因體醫學國家型科技計畫

國科會延續性計畫進度報告

整合型計畫 (Program Project Grants)

Program Classification:

☑ **Genomic Medicine** ☐ **Bioinformatics**

☐ **Proteomics & Structural Genomics** ☐ **ELSI**

Project Number: **91GMP012-5** (計畫編號)
NSC Funding Number: **NSC-93-3112-B-038-001** (93 年度國科會預核編號)

Title of Program 總計畫名稱	(in Chinese) 中文 砷與移行細胞癌之毒理基因體研究- (子計畫五) 膀胱移形細胞癌之個案，健康對照及一等親之慢性砷中毒相關酵素基因多形性之比較
	(in English) 英文 Toxicogenomic Study in Transitional Cell Carcinoma and Arsenic- (Project #5) Comparison on Genetic Polymorphism of Arseniasis Related Enzymes among Bladder Transitional Cell Carcinoma Patients, Healthy Controls and First Degree Relatives（2/3）
Institution 研究（執行）單位	(in Chinese) 中文 臺北醫學大學醫學系公共衛生學科
	(in English) 英文 Department of Public Health, School of Medicine, Taipei Medical University
Principle Investigator 計畫主持人	(in Chinese) 中文 薛玉梅
	(in English) 英文 Yu-Mei Hsueh

FY	2002	2003	2004	Total
Budget	2,820,300	2,864,500	3,040,100	

(in NT dollars: 1USD = 34 NTD)

Signature of the PI：_____Date：12/27/2003

Progress Report

B1. Response to previous reviewers' critiques

Please describe the previous reviewers' critiques and how based on the critiques, you made modifications to specific aims, experimental design, or resource allocation etc.

Comment V-1. In the previous summary statement, it was pointed out that there is lack of an analytical plan for addressing the complicated issue of gene-environment interaction.

Response: We will use univariate and multivariate logistic regression model to analyze the relationship between arsenic methylation capability, oxidative and antioxidant enzyme gene polymorphism, homocysteine metabolism related enzymes gene polymorphism and TCC adjusted for age, gender, cigarette smoking and alcohol drinking habit. On the other hand, we also use logistic regression model to explore the interaction among gene polymorphism, chronic arsenic exposure indices and arsenic methylation capability that related to the risk of TCC.

Comment V-2. It is also noted that the consideration of using first-degree relatives as alternative controls is potentially useful, but it was not well conceived and developed. These two issues have not been addressed in this continuation application.

Response: This year we recruited the first-degree relatives as alternative controls, and next year we will continue to recruit enough sample size of first-degree relatives.

Comment V-3. Furthermore, it is noted that one of the six specific aims is to investigate the arsenic exposure and selenium levels of biospecimens between UC patients in arseniasis-endemic and in non-endemic areas. However, the role selenium plays in urothelial carcinoma has not been elucidated.

Response:

Please see the component 5 proposals page 11. Selenium is an essential component of glutathione peroxidase, which is an important enzyme for processes that protect lipids in polyunsaturated membranes from oxidative degradation. Mice on the selenium deficient diet appeared to eliminate arsenate, arsenite, and dimethylarsinic acid in urine more slowly than selenium sufficient mice (55). Selenite abolished the induction of heme oxygenase mRNA in the cells exposed to arsenite (56). Selenium inhibited induction of tetraploidy by DMA may yield clues to the role of selenium in the chemoprevention of carcinogenesis by chemical carcinogens (57). Interaction between selenium and arsenic has been used to protect against the genotoxic effects of sodium arsenite through dietary intervention by an equivalent amount of sodium selenite (58). It is very interesting to investigate the protective role of selenium against arseniasis related TCC.

55. EM Kenyon, MF Hughes, OA Levander. Influence of dietary selenium on the disposition of arsenate in the female B6C3F1mouse. J Tox Env Health 1997;51:279-299.

56.S Taketani, H Kohno, R Tokunaga, T Ishii, S Bannai. Selenium antagonizes the induction of human heme oxygenase by arsenite and cadmium ions. Biochemistry International 1991;23(4):625-632.
57.H Ueda, K Kuroda, G Endo. The inhibitory effect of selenium on induction of tetraploidy by dimethylarsinic acid in Chinese Hamster Cells. Anticancer Research 1997;17:1939-1944.
58.S Biswas, G Talukder, A Sharma. Prevention of cytotoxic effects of arsenic by short-term dietary supplementation with selenium in mice in vivo. Mutation Res 1999;441:155-160.

Component Project No. 91GMP012-5 (請填入子計畫編號)

B2. Specific Aims

Please state the overall goals of the project, and specific aims, as reviewed and approved by the Study Section and actually awarded. If these specific aims as actually funded did not differ in scope from those actually pursued during the grant period, and if the aims have not been modified, state this. If they have been modified, give the revised aims.

This component project was established as a case control study, and we want to understand the arsenic methylation capability profile of urothelial carcinoma (UC) patients in the arseniasis endemic area and non-endemic area and healthy controls; and to understand the involvement of genetic polymorphism of susceptibility genes in arsenic metabolism, oxidant production, and antioxidant production. The specific aims are

1. To compare arsenic methylation capability profile among bladder transitional cell carcinoma (TCC) patients, healthy controls and first-degree relatives.
2. To investigate the arsenic exposure and selenium level of biospecimens between TCC cases in arseniasis-endemic and in non-endemic areas.
3. To use single nucleotide polymorphism (SNP) to analyze the polymorphic sites of arseniasis-related genes including oxidative enzyme (NADPH oxidase and nitric oxide synthase), reactive oxygen species defense enzyme (glutathion peroxidase, heme oxygenase-1, superoxide dismutase and catalase), and homocysteine metabolism related enzyme (cystathionine synthase,5,10;CBS, methylene-tetrahydrofolate reductase;MTHFR, and methionine synthase;MS) among bladder TCC patients, healthy controls and first-degree relatives.
4. To examine gene-environment interaction on the risk of bladder TCC in arseniasis-endemic and non-endemic areas.

Component Project No. 91GMP012-5 （請填入子計畫編號）

B3. Progress Summary

Summarize concisely the results obtained for **each specific aim during the past year (or reporting period)**. Negative results, if any, should also be included and approaches taken to improve the prospects of the project discussed. (Do not exceed **5 pages**, not including figures and references.)

A. We submitted the interesting result about the relationship between HO-1 and TCC. The title is "Males with Longer GT Repeats in the HO-1 Promoter is Associated with Higher Urothelial Carcinoma Risk". Unfortunately, this manuscript rejected by Cancer Research because the statistical power is not sufficient, we will continue to recruit more samples. The manuscript is shown below.

Introduction

Urinary bladder cancer is the sixth most common malignancy in developed countries, and was ranked as the 9th most common cancer for males in Taiwan in 2000. Age-specific bladder cancer (ICD-9 188) incidence rates for males and females in Taiwan were 9.14 and 3.70 per 100,000 people, respectively in 1999 [1]. These are lower rates than for the US with about 30 per 100,000 males from 1973 to1999. Other than environmental factors, the different incidence of bladder cancer may imply the polymorphism in bladder cancer-associated genes. Most bladder tumors are transitional cell carcinomas (TCCs) [2]. TCC is the most frequent histotype, although the distribution of histotypes varies in different populations.

Heme oxygenase (HO) is the rate-limiting enzyme in heme degradation. HO cleaves the α-meso carbon bridge of heme, yielding equimolar quantities of carbon monoxide (CO), biliverdin, and free iron [3]. Three distinct isoforms of HO (HO-1, HO-2, and HO-3) have been identified. HO-2 and HO-3 are constitutively expressed proteins, while in contrast, HO-1 is an inducible protein [4]. HO-1 is a stress-response protein that can be induced by various oxidative agents, including heavy metals, inflammatory mediators, ultraviolet radiation, endotoxin, and heme/hemoglobin [3,5]. In addition, atherosclerosis, hypertension, Alzheimer's disease, and asthma have been associated with increased expression of HO-1 [6-10]. HO-1 expression in solid tumor may confer resistance by tumor cells to hypoxic stress [11].

Human HO-1 gene has been localized to the human chromosome, 22q12 [12]. A $(GT)_n$ dinucleotide repeat in the 5' flanking region of human HO-1 gene shows length polymorphism and can modulate the levels of gene transcription[13]. Cultured cell lines with different HO-1 promoter $(GT)_n$ repeats were analyzed for the expression of HO-1. The alleles with shorter $(GT)_n$ repeats showed a greater up-regulation of transcriptional activity upon H_2O_2 exposure [13]. The genetic polymorphism allows the study of possible involvement of HO-1 in certain human diseases. The microsatellite polymorphism in the human HO-1 promoter was associated with chronic pulmonary emphysema [13], abdominal aortic aneurysm [14], and coronary artery disease [15,16]. However, although several reports have documented the observation of HO-1 in various animal tumor model and human tumor cells [11,17-20], the relationship between HO-1 $(GT)_n$ repeat polymorphism and cancer is unclear. It is important to elucidate whether or not the long-class $(GT)_n$ repeat polymorphism genotype is associated with a greater cancer risk. This study is by far the first to report the microsatellite polymorphism in the HO-1 gene promoter in UC cases and controls.

Material and Methods

Study Population

This was a case-control study at Taiwan University Hospital and Chi-Mei Medical Center in Taiwan from May 2001 to May 2002. In total, 106 cases, 24~93 years of age, were hospitalized patients who had a diagnosis of UC (ICD9-188). There were 106 controls, 24~89 years of age, selected from both hospital outpatient departments. Of the total, 65 cases and 60 controls came from Taiwan University Hospital and 41 cases and 46 controls from Chi-Mei Medical Center. Controls had neither UC nor hematuria and were frequency-matched to cases by area and gender.

Well-trained assistants carried out the standardized personal interview based on a structured questionnaire. Information obtained from the interview included socioeconomic and demographic characteristics, risk factors for UC such as the lifestyle of alcohol consumption, cigarette smoking, dietary consumption frequency, and exposure to dyes; as well as personal and family histories.

Molecular Genetics

Blood samples were collected from study subjects. DNA was extracted from the buffy coat using previous protocol [21] and sent blinded to the laboratory for genotyping, with each plate containing a mixture of cases and control, unknown to the laboratory technicians. DNA extraction is described below. Buffy coats were lysed with 3 volumes of RBC buffer (0.15 M NH_4Cl, 0.1 mM EDTA, 10 mM $KHCO_3$, pH 7.5). After a 10-min incubation at room temperature, the mixture was spun at 2,000 g for 10 min. The supernatant was discarded. An equal volume of cell lysis buffer (200 mM Tris-HCl, pH 8.5, 100 mM EDTA, 35 mM SDS) was added to the mixture and mixed gently several times until a homogeneous phase was obtained. The homogeneous solution was then added with 1/3 volume of the protein precipitation solution (10 M NH_4 acetate), and gently shaken for 20 s to achieve uniformity. The mixture was centrifuged at 2,000 g for 15 min, and the supernatant was transferred to a clean tube for DNA precipitation by adding 4/5 volume of isopropanol. DNA of 212 subjects was available for HO-1 $(GT)_n$ repeats polymorphism analysis.

The poly $(GT)_n$ repeat of HO-1 gene was amplified by PCR with a forward primer, 5'-CCA GGC TTT TGC TCT GAG CA, and a reverse primer, 5'-ACC GCA TAC CAG GGT GCC. Thermocycler conditions for amplification consisted of one cycle of 95℃ for 5 min, 35 cycles of 94℃ for 30s, 60℃ for 30s, and 72℃ for 60s, and one cycle of 72℃ for 10 min followed by holding at 4℃. The number of repeats was observed by direct sequencing (ABI 3100 automated DNA sequencer). All sequencing reactions used ABI standard reagents and protocols for the ABI 3100 automated DNA sequencer.

Statistical Analysis

Student's *t* test was used to compare the average age between UC cases and controls. Univariate logistic regression was performed to compare the distributions of gender, age, cigarette smoking history, the frequencies of alleles and genotypes between cases and controls. Multivariate logistic regression analysis to adjust for age and cigarette smoking history was performed to calculate odds ratio (OR), and 95% confident interval (CI). SAS Version 8.2 was used for all statistical analyses.

Results

Table 1 shows the sociodemographic characteristics of UC patients and matched controls. Cases and controls did not differ significantly in gender. The average age of cases significantly differed from the controls. The risk of UC significantly increased in older age. The ORs for the 50~70- and ≥70-years-older groups compared to <50-years-old group were 2.7 (95% CI 1.2-62) and 2.9 (95% CI 1.3-6.5), respectively. Current or former cigarette smokers had a

borderline risk of UC (OR=1.9, 95% CI 0.9-3.6) compared with subjects who had never smoked. The *p* value of the χ^2 test determining the distribution of UC cases and controls with a cigarette smoking history was 0.07.

The frequency distribution of the HO-1 $(GT)_n$ repeats in UC cases and controls is shown in Figure 1. The range of the number of HO-1 $(GT)_n$ repeats was 14 to 37 in UC cases and 15 to 35 in the controls. The peak number of HO-1 $(GT)_n$ repeats in both cases and controls was 23 and 29 repeats. The frequency of the number of HO-1 repeats for UC cases and controls did not significantly differ (p= 0.34). Figure 2 examines whether or not the length polymorphism in the HO-1 gene promoter is associated with gender. The frequency of HO-1 $(GT)_n$ repeats did not significantly differ (p= 0.13) between males and females by the χ^2 test. The $(GT)_{23}$ and $(GT)_{29}$ repeat number were the two most common alleles in this study.

Table 2 shows the allelic frequencies and risks associated with HO-1 $(GT)_n$ repeats length polymorphism among UC cases and healthy controls. The number of HO-1 $(GT)_n$ repeats was categorized into three strata according to previous study [13]. HO-1 $(GT)_n$ repeats lengths of < 25 were classified as the short (S) class, those with 25~29 repeat lengths as the middle (M) and those of ≥30 as the long (L) class. The UC risk for subjects who were carrier of the M/L or L/L genotype was higher than for those who carried the S/S or S/M genotype, with crude OR values of 2.8 and 2.1, respectively. The same result was obtained using the multivariate mode. The proportion of L allele frequency in UC cases was significantly higher than that in matched controls; the subjects with the L allele had 2.0 (95% CI 1.0-4.0)- and 2.1 (95% CI 1.0-4.2)- fold increased risk than those who had the S allele by the univariate and multivariate models, respectively.

Subjects with the five allele-subclasses were combined into two groups, group I without the L allele (S/S, S/M, and M/M), and group II with at least one L allele (M/L and L/L), in order to further explore the relationship between HO-1 $(GT)_n$ repeats and UC risk in both gender. Table 3 shows HO-1 $(GT)_n$ repeat genotypes and allele frequencies between UC cases and controls by gender. There were no current cigarette smokers among females so the logistic regression model was only adjusted for age. The risk of UC risk of Group II did not differ from that of Group I in females. For males, the UC odds ratio for group II was 4.2 (95% CI 1.1-15.9) compared to in group I. After adjusted for age and cigarette smoking status, the UC risk of group II was significantly higher (OR=4.1, 95% CI 1.1-15.7) than that of group I. The risk of HO-1 $(GT)_n$ repeats allele frequency in males who carried the L allele was significantly higher (by 3.9 fold) than that of males who had the S allele. A strong relationship was shown between UC and HO-1 $(GT)_n$ repeat length polymorphism in males.

Discussion

Oxidative stress is associated with the pathogenesis of various diseases such as cancer, pulmonary disease and vascular disease [22-24]. In addition to environmental factors, genetic factors may be involved in determining a person's susceptibility to diseases induced by oxidative stress. HO oxidatively degrades heme to bilirubin, an efficient scavenger of reactive oxygen species, carbon monoxide and iron [25]. The $(GT)_n$ repeat of the human HO-1 gene promoter is polymorphic [26], and modulates human HO-1 gene transcription [27]. Different cell lines cloned with different $(GT)_n$ repeats of HO-1 promoter had different HO-1 activity. The shorter GT repeats, the higher HO-1 activity was observed in Hep3B and smooth muscle cell [13,15]. Furthermore, a higher magnitude of HO-1 induction was observed in clones with shorter GT repeats than clones with longer repeat during oxidative stress stimulation [13]. Subjects who had a short GT repeat are therefore expected to have a greater increase HO-1 expression. There is no information about what role HO-1 $(GT)_n$ repeats plays in the risk of UC. This study found that subjects carrying long $(GT)_n$ repeat lengths were associated with a significantly increased risk of UC. Peaks of HO-1 $(GT)_n$ repeats were at 23 and 29 repeats in this study. Chinese people seemed to have a lower number of $(GT)_n$ repeats compared with Japanese or Austrian

counterparts [16,26,28].

The age-adjusted prevalence of UC for males is higher than that for female in both Asian and Caucasian populations [1,2]. The age-standardized incidence rate of bladder cancer in males shows a 2.5~6.9-fold greater risk than women in most population [29]. Yamaya et al. noted that the frequency of long HO-1 $(GT)_n$ repeat allele decreased with age increment in male Japanese subjects, but not in female ones.[14] The large $(GT)_n$ repeat in HO-1 gene promoter may be a genetic factor that prevents the attainment of old age in Japanese males [13]. In this study, male with HO-1 genotypes of ≥30 GT repeats had 3.9 times risk of UC compared to males with < 25 GT repeats.

The association between cigarette smoking and bladder cancer has been reported for several decades [30]. The incidence of bladder cancer was significantly decreased (0.5% per year) with the reduction of cigarette smoking amount [31]. The relative risks of bladder cancer in current cigarette smokers and former smokers compared to non-smoker were 2.86 and 1.90, respectively. The risk of bladder cancer in smokers was 2 to 4 times that of non-smokers, and it was increased with the amount and duration of cigarette smoking [32]. The pattern of cigarette smoker in Taiwan differs from that of Western countries; we found only a 1.9-fold borderline risk of UC in smokers compared to non-smokers. Whether or not the bladder cancer risk is influenced by patterns of cigarette smoking requires further study.

HO-1 was induced by metals [33,34], heat shock [34], cytokines [35,36], hypoxia [37,38], glutathione depletion [39], and angiogenesis [40,41]. HO-1 may augment placental vascularization by inducing the expression of vascular endothelial growth factor, a potent angiogenic agent [42]. Angiogenesis is necessary for the successful growth of solid tumors, as well as their malignant growth and metastasis livelihood [43]. The level of vascular endothelial growth factor has been identified in tissue and urinary bladder cancer of canine and has also been associated with disease progression [44-46]. HO-1 expression may be related to progression of UC. Few data exist regarding prognostic or predictive markers in patients with metastasis disease.

HO-1 expression increased in human prostate tumor cells and benign prostatic hyperplasia basal cell, but not in normal prostate tissue [19]. HO-1 RNA and activity of human kidney tumors were higher than those of juxtatumor tissue (3 mm from tumor cell) and normal cells [18]. HO-1 mRNA increased with increments in the human glioma vascular density [41]. Higher HO-1 expression was observed in well-differentiated oral squamous cell carcinoma than in moderately differentiated carcinoma [20]. Deininger et al. 2000 noted that higher HO-1 expression was observed in growth areas of infiltrative oligodendroglioma than in those of solid tumor [17]. Based on these findings, HO-1 expression is postulated to increase in tumor tissues. It is unclear whether HO-1 expression increases before or as the result of tumorgenesis or carcinogenesis.

Molecular changes in bladder cancer can be categorized into the following steps: chromosomal alteration leading to carcinogenesis; cellular proliferation as a result of dysregulation of cell cycle control; and growth control processes leading to metastasis [43]. This study showed that subjects with longer GT repeats (L class alleles) had significantly higher UC risk than subjects carrying the shorter GT repeats (S class alleles). This may suggest that subjects carrying the short repeats produce greater HO-1 expression and are able to prevent the damage from stimulation, while subjects carrying long GT repeats alleviate HO-1 activity, resulting in bladder carcinogenesis, as well as decreases in micronutrients leading to bladder cancer [47] . The HO-1 $(GT)_n$ polymorphism may be an important marker in male UC. Further study of $(GT)_n$ repeats of HO-1 promoter and HO-1 expression in the human cancer process is necessary.

Acknowledgments:

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Republic of China (Taipei).

Reference List

1. Cancer Registry Annual Report Republic of China, 1999. Department of Health, ROC. pp. 102, Bureau of Health Promotion, Department of Health, Executive Yuan Taipei, Taiwan. 2002.
2. Jemal, A., Murray, T., Samuels, A., Ghafoor, A., Ward, E., and Thun, M. J. Cancer statistics, 2003. CA Cancer J. Clin., *53*: 5-26, 2003.
3. Maines, M. D. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J., *2*: 2557-2568, 1988.
4. Durante, W. Heme oxygenase-1 in growth control and its clinical application to vascular disease. J. Cell Physiol., *195*: 373-382, 2003.
5. Abraham, N. G., Drummond, G. S., Lutton, J. D., and Kappas, A. The biological significance and physiological role of heme oxygenase. Cell. Physiol. Biochem., *6*: 129-168, 1996.
6. Dore, S. Decreased activity of the antioxidant heme oxygenase enzyme: implications in ischemia and in Alzheimer's disease. Free Radic. Biol. Med., *32*: 1276-1282, 2002.
7. Ishizaka, N., de Leon, H., Laursen, J. B., Fukui, T., Wilcox, J. N., De Keulenaer, G., Griendling, K. K., and Alexander, R. W. Angiotensin II-induced hypertension increases heme oxygenase-1 expression in rat aorta. Circulation, *96*: 1923-1929, 1997.
8. Lim, S., Groneberg, D., Fischer, A., Oates, T., Caramori, G., Mattos, W., Adcock, I., Barnes, P. J., and Chung, K. F. Expression of heme oxygenase isoenzymes 1 and 2 in normal and asthmatic airways: effect of inhaled corticosteroids. Am. J. Respir. Crit. Care Med., *162*: 1912-1918, 2000.
9. Nakayama, M., Takahashi, K., Komaru, T., Fukuchi, M., Shioiri, H., Sato, K., Kitamuro, T., Shirato, K., Yamaguchi, T., Suematsu, M., and Shibahara, S. Increased expression of heme oxygenase-1 and bilirubin accumulation in foam cells of rabbit atherosclerotic lesions. Arterioscler. Thromb. Vasc. Biol., *21*: 1373-1377, 2001.
10. Ndisang, J. F., Zhao, W., and Wang, R. Selective regulation of blood pressure by heme oxygenase-1 in hypertension. Hypertension, *40*: 315-321, 2002.
11. Doi, K., Akaike, T., Fujii, S., Tanaka, S., Ikebe, N., Beppu, T., Shibahara, S., Ogawa, M., and Maeda, H. Induction of haem oxygenase-1 nitric oxide and ischaemia in experimental solid tumours and implications for tumour growth. Br. J. Cancer, *80*: 1945-1954, 1999.
12. Kutty, R. K., Kutty, G., Rodriguez, I. R., Chader, G. J., and Wiggert, B. Chromosomal localization of the human heme oxygenase genes: heme oxygenase-1 (HMOX1) maps to chromosome 22q12 and heme oxygenase-2 (HMOX2) maps to chromosome 16p13.3. Genomics, *20*: 513-516, 1994.
13. Yamada, N., Yamaya, M., Okinaga, S., Nakayama, K., Sekizawa, K., Shibahara, S., and Sasaki, H. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am. J. Hum. Genet., *66*: 187-195, 2000.
14. Schillinger, M., Exner, M., Mlekusch, W., Domanovits, H., Huber, K., Mannhalter, C., Wagner, O., and Minar, E. Heme oxygenase-1 gene promoter polymorphism is associated with abdominal aortic aneurysm. Thromb. Res., *106*: 131-136, 2002.
15. Chen, Y. H., Lin, S. J., Lin, M. W., Tsai, H. L., Kuo, S. S., Chen, J. W., Charng, M. J., Wu, T. C., Chen, L. C., Ding, Y. A., Pan, W. H., Jou, Y. S., and Chau, L. Y. Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. Hum. Genet., *111*: 1-8, 2002.
16. Kaneda, H., Ohno, M., Taguchi, J., Togo, M., Hashimoto, H., Ogasawara, K., Aizawa, T., Ishizaka, N., and Nagai, R. Heme oxygenase-1 gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors.

Arterioscler. Thromb. Vasc. Biol., *22*: 1680-1685, 2002.
17. Deininger, M. H., Meyermann, R., Trautmann, K., Duffner, F., Grote, E. H., Wickboldt, J., and Schluesener, H. J. Heme oxygenase (HO)-1 expressing macrophages/microglial cells accumulate during oligodendroglioma progression. Brain Res., *882*: 1-8, 2000.
18. Goodman, A. I., Choudhury, M., da Silva, J. L., Schwartzman, M. L., and Abraham, N. G. Overexpression of the heme oxygenase gene in renal cell carcinoma. Proc. Soc. Exp. Biol. Med., *214*: 54-61, 1997.
19. Maines, M. D. and Abrahamsson, P. A. Expression of heme oxygenase-1 (HSP32) in human prostate: normal, hyperplastic, and tumor tissue distribution. Urology, *47*: 727-733, 1996.
20. Tsuji, M. H., Yanagawa, T., Iwasa, S., Tabuchi, K., Onizawa, K., Bannai, S., Toyooka, H., and Yoshida, H. Heme oxygenase-1 expression in oral squamous cell carcinoma as involved in lymph node metastasis. Cancer Lett., *138*: 53-59, 1999.
21. Lee, H. H., Chao, H. T., Ng, H. T., and Choo, K. B. Direct molecular diagnosis of CYP21 mutations in congenital adrenal hyperplasia. J. Med. Genet., *33*: 371-375, 1996.
22. Diaz, M. N., Frei, B., Vita, J. A., and Keaney, J. F., Jr. Antioxidants and atherosclerotic heart disease. N. Engl. J. Med., *337*: 408-416, 1997.
23. Loeb, L. A., Ernster, V. L., Warner, K. E., Abbotts, J., and Laszlo, J. Smoking and lung cancer: an overview. Cancer Res., *44*: 5940-5958, 1984.
24. Robertson, F. W. and Cumming, A. M. Genetic and environmental variation in serum lipoproteins in relation to coronary heart disease. J. Med. Genet., *16*: 85-100, 1979.
25. Tenhunen, R., Marver, H. S., and Schmid, R. Microsomal heme oxygenase. Characterization of the enzyme. J. Biol. Chem., *244*: 6388-6394, 1969.
26. Kimpara, T., Takeda, A., Watanabe, K., Itoyama, Y., Ikawa, S., Watanabe, M., Arai, H., Sasaki, H., Higuchi, S., Okita, N., Takase, S., Saito, H., Takahashi, K., and Shibahara, S. Microsatellite polymorphism in the human heme oxygenase-1 gene promoter and its application in association studies with Alzheimer and Parkinson disease. Hum. Genet., *100*: 145-147, 1997.
27. Okinaga, S., Takahashi, K., Takeda, K., Yoshizawa, M., Fujita, H., Sasaki, H., and Shibahara, S. Regulation of human heme oxygenase-1 gene expression under thermal stress. Blood, *87*: 5074-5084, 1996.
28. Exner, M., Schillinger, M., Minar, E., Mlekusch, W., Schlerka, G., Haumer, M., Mannhalter, C., and Wagner, O. Heme oxygenase-1 gene promoter microsatellite polymorphism is associated with restenosis after percutaneous transluminal angioplasty. J. Endovasc. Ther., *8*: 433-440, 2001.
29. Parkin, D. M., Pisani, P., and Ferlay, J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int. J. Cancer, *80*: 827-841, 1999.
30. Shopland, D. R. Tobacco use and its contribution to early cancer mortality with a special emphasis on cigarette smoking. Environ. Health Perspect., *103 Suppl 8*: 131-142, 1995.
31. Moyad, M. A. Bladder cancer prevention. Part I: what do I tell my patients about lifestyle changes and dietary supplements? Curr. Opin. Urol., *13*: 363-378, 2003.
32. Negri, E. and La Vecchia, C. Epidemiology and prevention of bladder cancer. Eur. J. Cancer Prev., *10*: 7-14, 2001.
33. Elbirt, K. K., Whitmarsh, A. J., Davis, R. J., and Bonkovsky, H. L. Mechanism of sodium arsenite-mediated induction of heme oxygenase-1 in hepatoma cells. Role of mitogen-activated protein kinases. J. Biol. Chem., *273*: 8922-8931, 1998.
34. Eyssen-Hernandez, R., Ladoux, A., and Frelin, C. Differential regulation of cardiac heme oxygenase-1 and vascular endothelial growth factor mRNA expressions by hemin, heavy metals, heat shock and anoxia. FEBS Lett., *382*: 229-233, 1996.
35. Hellmuth, M., Wetzler, C., Nold, M., Chang, J. H., Frank, S., Pfeilschifter, J., and Muhl, H.

Expression of interleukin-8, heme oxygenase-1 and vascular endothelial growth factor in DLD-1 colon carcinoma cells exposed to pyrrolidine dithiocarbamate. Carcinogenesis, *23*: 1273-1279, 2002.
36. Lee, T. S. and Chau, L. Y. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nat. Med., *8*: 240-246, 2002.
37. Lee, P. J., Jiang, B. H., Chin, B. Y., Iyer, N. V., Alam, J., Semenza, G. L., and Choi, A. M. Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. J. Biol. Chem., *272*: 5375-5381, 1997.
38. Motterlini, R., Foresti, R., Bassi, R., Calabrese, V., Clark, J. E., and Green, C. J. Endothelial heme oxygenase-1 induction by hypoxia. Modulation by inducible nitric-oxide synthase and S-nitrosothiols. J. Biol. Chem., *275*: 13613-13620, 2000.
39. Oguro, T., Hayashi, M., Numazawa, S., Asakawa, K., and Yoshida, T. Heme oxygenase-1 gene expression by a glutathione depletor, phorone, mediated through AP-1 activation in rats. Biochem. Biophys. Res. Commun., *221*: 259-265, 1996.
40. Deramaudt, B. M., Braunstein, S., Remy, P., and Abraham, N. G. Gene transfer of human heme oxygenase into coronary endothelial cells potentially promotes angiogenesis. J. Cell Biochem., *68*: 121-127, 1998.
41. Nishie, A., Ono, M., Shono, T., Fukushi, J., Otsubo, M., Onoue, H., Ito, Y., Inamura, T., Ikezaki, K., Fukui, M., Iwaki, T., and Kuwano, M. Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. Clin. Cancer Res., *5*: 1107-1113, 1999.
42. Kreiser, D., Nguyen, X., Wong, R., Seidman, D., Stevenson, D., Quan, S., Abraham, N., and Dennery, P. A. Heme oxygenase-1 modulates fetal growth in the rat. Lab. Invest, *82*: 687-692, 2002.
43. Quek, M. L., Quinn, D. I., Daneshmand, S., and Stein, J. P. Molecular prognostication in bladder cancer--a current perspective. Eur. J. Cancer, *39*: 1501-1510, 2003.
44. Inoue, K., Slaton, J. W., Karashima, T., Yoshikawa, C., Shuin, T., Sweeney, P., Millikan, R., and Dinney, C. P. The prognostic value of angiogenesis factor expression for predicting recurrence and metastasis of bladder cancer after neoadjuvant chemotherapy and radical cystectomy. Clin. Cancer Res., *6*: 4866-4873, 2000.
45. Inoue, K., Kamada, M., Slaton, J. W., Fukata, S., Yoshikawa, C., Tamboli, P., Dinney, C. P., and Shuin, T. The prognostic value of angiogenesis and metastasis-related genes for progression of transitional cell carcinoma of the renal pelvis and ureter. Clin. Cancer Res., *8*: 1863-1870, 2002.
46. Mohammed, S. I., Bennett, P. F., Craig, B. A., Glickman, N. W., Mutsaers, A. J., Snyder, P. W., Widmer, W. R., DeGortari, A. E., Bonney, P. L., and Knapp, D. W. Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. Cancer Res., *62*: 356-358, 2002.
47. La Vecchia, C. and Negri, E. Nutrition and bladder cancer. Cancer Causes Control, *7*: 95-100, 1996.

Table 1 Sociodemographic characteristics of urothelial carcinoma patients and matched controls

	Cases (N=106)		Controls (N=106)		OR (95% CI)
	N	%	N	%	
Gender					
Female	42	39.6	42	39.6	1.0[a]
Male	64	60.4	64	60.4	1.0 (0.6-1.7)
Age					
<50	11	10.4	26	24.5	1.0[a,*]
50-70	41	38.6	36	34.0	2.7 (1.2-6.2)[*]
70+	54	51.0	45	41.5	2.9 (1.3-6.5)[**]
Average age	66.7±1.9		61.4±1.9[b,*]		
Cigarette smoking history					
Never	47	44.3	57	53.8	1.0[a]
Former	0	0.0	4	3.8	
Current	30	28.3	21	19.8	1.9 (0.9-3.6)[+]
Missing	29	27.4	24	22.6	1.6 (0.8-3.0)

[a] Trend test
[b] Student's *t*-test
[+] $0.1<p<0.05$. [*] $p<0.05$. [**] $p<0.01$

Table 2 Allelic frequencies and risk associated with HO-1 $(GT)_n$ repeats length polymorphism among urothelial carcinoma cases and matched controls

	Cases N (%)	Controls N (%)	Crude OR (95% CI)	Multivariate [a] OR (95% CI)
Genotype				
S/S	59 (55.7)	57 (53.8)		
S/M	0 (0.0)	10 (9.4)		
S/S S/M	59	67	1.0	1.0
M/M	31 (29.2)	31 (29.2)	1.1 (0.6-2.1)	1.2 (0.6-2.2)
M/L	5 (4.7)	2 (1.9)	2.8 (0.5-15.2)	2.3 (0.4-12.6)
L/L	11 (10.4)	6 (5.7)	2.1 (0.7-5.9)	2.2 (0.8-6.6)
Alleles class				
S	118 (55.7)	124 (58.5)	1.0	1.0
M	67 (31.6)	74 (34.9)	0.9 (0.6-1.4)	1.0 (0.7-1.5)
L	27 (12.7)	14 (6.6)	2.0 (1.0-4.0)*	2.1 (1.0-4.2)*

[a] Age and cigarette smoking history were adjusted in logistic regression model.
S: Allele repeats length less than 25
M: Allele repeats length between 25 or longer and less than 30
L: Allele repeats length 30 or longer
[*] $p<0.05$.

Table 3: HO-1 $(GT)_n$ repeats genotype and allele frequency between urothelial carcinoma cases and matched controls by gender

	Female				Male			
	Case (N=42)	Control (N=42)	Crude OR (95% CI)	Multivariate[a] OR (95% CI)	Case (N=64)	Control (N=64)	Crude OR (95% CI)	Multivariate OR [b] (95% CI)
Genotype	N (%)				N (%)			
Group I	37 (88.1)	37 (88.1)	1.0	1.0	53 (82.8)	61 (95.3)	1.0	1.0
Group II	5 (11.9)	5 (11.9)	1.0 (0.3-3.7)	0.7 (0.2-2.7)	11 (17.2)	3 (4.69)	4.2 (1.1-15.9)*	4.1 (1.1-15.7)*
Allele class								
S	50 (59.5)	49 (58.3)	1.0	1.0	68 (53.1)	74 (57.8)	1.0	1.0
M	25 (29.8)	26 (31.0)	0.9 (0.5-1.9)	1.2 (0.6-2.7)	42 (32.8)	49 (38.3)	0.9 (0.6-1.5)	1.0 (0.6-1.7)
L	9 (10.7)	9 (10.7)	1.0 (0.4-2.7)	0.8 (0.3-2.3)	18 (14.1)	5 (3.9)	3.9 (1.4-11.1)**	3.9 (1.4-11.2)**

[a] age was adjusted in logistic regression model.

[b] age and cigarette smoking history were adjusted in logistic regression model.

S: Allele repeats length less than 25

M: Allele repeats length between 25 or longer and less than 30

L: Allele repeats length 30 or longer

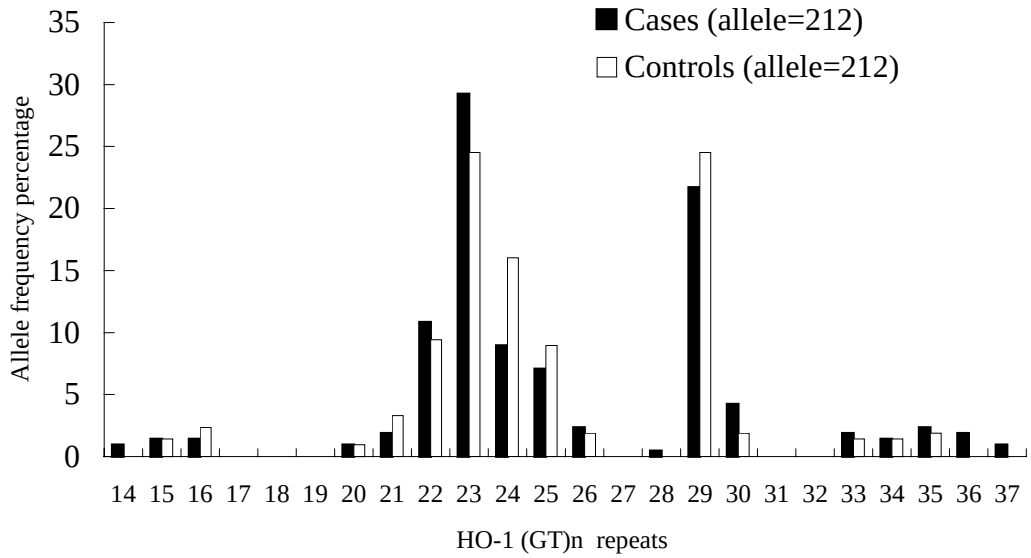

Figure 1: Frequency distribution of the HO-1 $(GT)_n$ repeats in urothelial carcinoma cases and controls

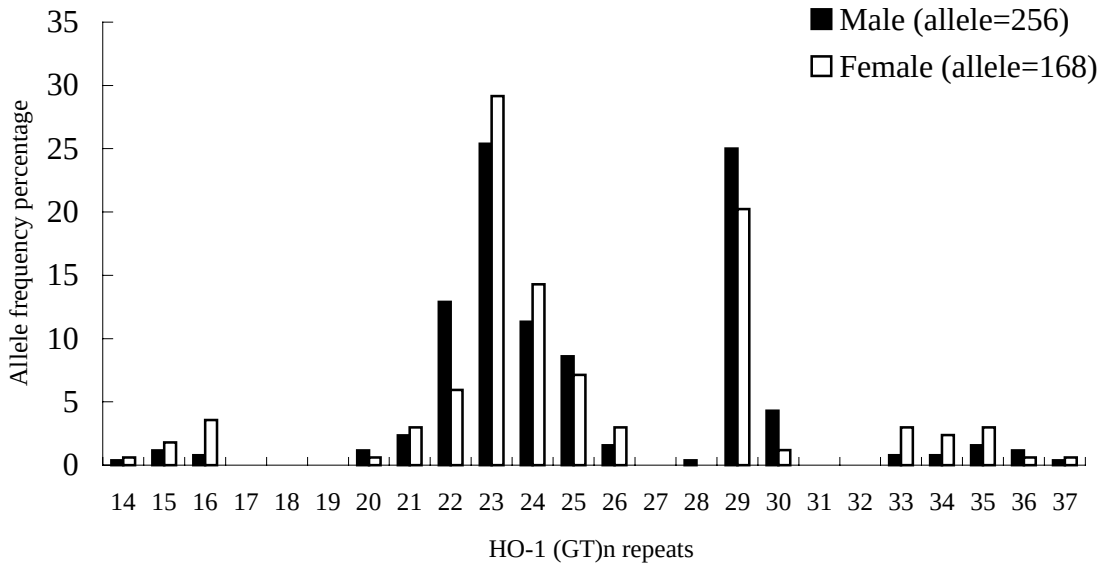

Figure 2: Frequency distribution of the HO-1 $(GT)_n$ repeats in both gender

B. The preliminary result of the ***MTHFR, MS, CBS, MnSOD,*** and ***NADPH oxidase*** gene polymorphisms among UC patients and controls from arseniasis-endemic area and non-endemic area were shown below. The distributions of DD and DI for ***CBS***, UC patients were 100 and 0%, and controls were 98.9 and 1.1%. The distributions of CC, CT or TT for ***MTHFR* (C677T)** UC patients were 94.3, and 5.7%, and controls were 89.4 and 10.6%, the age-sex adjusted OR (95% CI) was 0.57 (0.24-1.33) for CT or TT genotype. The distributions of AA, AG or GG for ***MS* (A2756G)** UC patients were 81.2% and 18.8% and controls were 80.6, and 19.4%, the age-sex adjusted OR (95% CI) was 1.04 (0.61-1.78) for AG or GG genotype. The distribution of CC, CT or TT for ***NADPH oxidase***, UC patients was 18.7 and 81.3% and controls were 14.6 and 85.4% respectively, the age-sex adjusted OR (95% CI) was 1.37 (0.74-2.54) for CT or TT genotype. The distribution of TT, TC or CC for ***Mn SOD***, UC patients was 69.6 and 30.4%, and control was 73.9 and 26.1 respectively, the age-sex adjusted OR (95% CI) was 1.23 (0.76-2.23) for TC or CC genotype (Table 1).

Table 1. The distribution and odds ratio of ***MTHFR, MS, CBS, MnSOD,*** and ***NADPH oxidase*** gene polymorphisms among UC patients from arseniasis- endemic area and non-endemic area

	Control	Case	Crude OR (95 % CI)	Age and sex adjusted OR (95 % CI)
	No (%)			
CBS				
D	268 (98.89)	138 (100.00)		
DI	3 (1.11)	0 (0.00)		
MTHFR				
CC	194 (89.40)	132 (94.29)	1.0	1.0
CT+TT	23 (10.60)	8 (5.71)	0.51 (0.22-1.17)	0.57 (0.24-1.33)
MS				
AA	212 (80.61)	112 (81.16)	1.0	1.0
AG+GG	51 (19.39)	26 (18.84)	0.97 (0.57-1.63)	1.04 (0.61-1.78)
NADPH				
CC	26 (14.61)	26 (18.71)	1.0	1.0
CT+TT	152 (85.39)	113 (81.29)	1.35 (0.74-2.44)	1.37 (0.74-2.54)
SOD				
TT	82 (73.87)	80 (69.57)	1.0	1.0
TC+CC	29 (26.13)	35 (30.43)	1.24 (0.70-2.21)	1.23 (0.76-2.23)

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B4. Projected Timeline & Brief Summary of Plans for Next Year

Provide a short paragraph to describe the plans for next year including a realistic timetable and appropriate milestones for the project, based on the progress reached so far. (Do not exceed **1 page**.)

1. **The third year of this component project, we will continue to recruit UC patients and healthy controls from Chi Mei Hospital and National Taiwan University Hospital (arseniasis-endemic and non-endemic areas), respectively.**
2. **We will continue to analyze gene polymorphism of remethylation related enzymes (CBS, MTHFR, and MS), oxidative enzyme (NADPH oxidase and nitric oxide synthase) and ROS related enzymes (superoxide dismutase, glutathion peroxidase, heme oxygenase-1 and catalase), and estimate the relative risk of TCC about gene polymorphism.**
3. **We will continue to analyze urinary arsenic species and selenium to explore the relationship between arsenic methylation capability and TCC. In addition, we will to evaluate whether differing urinary levels of selenium alter the disposition and methylation of exposed inorganic arsenic between TCC and healthy control.**
4. **We will examine gene-environment interaction on the risk of bladder TCC in arseniasis-endemic and non-endemic areas.**

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B5. Personnel

Summarize the **personnel involved in the project during the grant period**. List the personnel in accordance to the following categories: (1) senior investigators, including visitors; (2) postdoctoral fellows; (3) graduate students; (4) technicians or research assistants. Specify for each individual the period of involvement and the percentage commitment of effort.

Name		Position Title	Education Degree	% of personal effort on this project	Job Description or Responsibilities
In Chinese	In English				
蔡蕙如	Hui-Ju Tsai	Research assistant	Graduate student	100%	Study subjects recruitment, questionnaire interview, urinary arsenic species assay and data analysis
黃詠愷	Yung-Kai Huang	Part time research assistant	Doctor student	50%	DNA extraction, gene polymorphism assay and urinary arsenic species assay
楊淑媄	Shu-Mei Yang	Part time research assistant	Master student	50%	DNA extraction and gene polymorphism assay
蔡曉雯	Hsiao-Wen Tsai	Part time research assistant	Master student	50%	Study subjects recruitment and questionnaire interview
廖敏倫	Min-Lun Liao	Part time research assistant	Undergraduate student	30%	Study subjects recruitment and questionnaire interview
吳凌巧	Ling-Chiao Wu	Part time research assistant	Undergraduate student	30%	Study subjects recruitment and questionnaire interview
史琬菁	Wan-Ching Shin	Part time research assistant	Undergraduate student	30%	Study subjects recruitment and questionnaire interview
張倖瑋	Hsing-Wei Chang	Part time research assistant	Undergraduate student	30%	Study subjects recruitment and questionnaire interview
吳家瑜	Chia-Yu Wu	Part time research assistant	Undergraduate student	30%	Study subjects recruitment and questionnaire interview

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B6. Publications and/or Patents

B6a. Publications

List the title and complete references (author(s), journal or book, year, page number) of all publications **directly resulting from studies supported by the project (i.e., with citation of this grant in the acknowledgement section)**. List the publications for the project in accordance to the following categories: (1) manuscripts published and accepted for publications; (2) manuscripts submitted; and (3) conference proceedings. Provide one copy of each publication not previously reported to the National Science Council in the Appendix.

We submitted the abstract and it was accepted by the American Associate Cancer Research (AACR) annual conference meeting (2003), but because SARS we did not attend the AACR conference meeting. We submitted the new abstract again, and we will participate the AACR annual conference meeting on March, 2004. The study's title and abstract is shown below.

"Comparison of Arsenic Methylation Capability in Transitional Cell Carcinoma Patients between Arseniasis Endemic Area and Non-endemic Area in Taiwan."

Y.M. Hsueh[1], Y.K. Huang[2], H.Y. Chiou[3], Y.S. Pu[4], C.J. Chen[5].

[1]Department of Public Health, School of Medicine, Taipei Medical University, Taipei, Taiwan, [2]Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan, [3]School of Public Health, Taipei Medical University, Taipei, Taiwan, [4]Department of Internal Medicine, National Taiwan University, Taipei, Taiwan, and College of Medicine and [5]Graduate Institute of Epeidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan.

The purpose of this research is to test whether transitional cell carcinoma (TCC) from arseniasis endemic is related to chronic arsenic indices, and to check the difference of arsenic methylation capability between TCC patients from arseniasis endemic and non-endemic areas. Chronic arsenic indices are measured as the duration of living in blackfoot disease endemic area, duration of drinking artesian well water, and cumulative arsenic exposure. Arsenic methylation capability indices are measured as the percentage of inorganic arsenic (arsenite and arsenate /total arsenic), monomethylarsonic acid (MMA) percentage (MMA/total arsenic), and dimethylarsinic acid (DMA) percentage (DMA/total arsenic). A total of 1563 residents, aged 30 or older, were recruited from three arseniasis endemic villages from January to February 1993. By year 2000, cross-examination of household registration and cancer registry profile revealed 32 diagnosed TCC cases. Another 63 TCC cases were recruited from Taiwan University Hospital as the comparison group. We also recruited healthy controls from arseniasis endemic area and non-endemic area. Urinary arsenic was examined by high performance liquid chromatography (HPLC) to specify arsenite, arsenate, MMA, DMA and then quantified by atomic absorption spectrometry. TCC patients from arseniasis endemic area had significantly higher urinary total arsenic than patients from non-endemic area, and healthy controls from arseniasis endemic area. TCC risk of subjects from arseniasis endemic area was significantly associated with increasing cumulative arsenic exposure. After age and gender adjusted, higher proportion of urinary MMA percentage, and lower level of DMA percentage were significantly related to higher risk of TCC. It suggested that TCC patients had poor arsenic methylation capability. In addition, we will also analyze the association between 5,10 methylene- tetraahydrofolate reductase (MTHFR),

cystathionine synthase (CBS) and methionine synthase (MS) gene polymorphism of arsenic methylation related enzymes and risk of TCC and report the results during the conference. Further research is needed to identify other risk factors of TCC.