Elevated *ERCC1* Gene Expression in Blood Cells Associated with Exposure to Arsenic from Drinking Water in Inner Mongolia

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Abstract. *Background: Chronic arsenic exposure has been associated with human cancer. The objective of this study was to investigate the effects of arsenic exposure on a DNA nucleotide excision repair gene, ERCC1, expression in human blood cells. Patients and Methods: Water and toenail samples were collected from a total of 327 Inner Mongolian residents for arsenic analysis. Blood samples were collected to determine mRNA expression levels by real-time PCR. Results: The mRNA levels of ERCC1 expression were positively associated with water arsenic concentration (slope=0.313, p=0.0043) and nail arsenic concentration (slope=0.474, p=0.0073). mRNA levels of ERCC1 expression were significantly correlated with those of OGG1, a base pair excision repair gene (r=0.275, p<0.0001). Conclusion: The results showed that mRNA levels of ERCC1 expression were significantly associated with arsenic concentrations in drinking water, implicating the DNA repair response was induced by arsenic exposure.*

Chronic arsenic exposure by ingestion of contaminated drinking water occurs worldwide and has become a major public health concern. Areas of China, Bangladesh, India, Chile and parts of the United States such as New Hampshire, Nevada and California are known for the arsenic-contaminated water in groundwater (1-3). Epidemiological studies show that long-term arsenic exposure is associated with wide range of health effects, including high risk of lung, bladder and skin cancer (4, 5). The molecular mechanisms of arsenic toxicity and carcinogenesis are still poorly understood.

One widely accepted mode of arsenic action is its role in oxidative stress, inducing genomic instability (6). Arsenic is known to generate reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical and superoxide anion, which induce a variety of oxidative DNA adducts and DNA protein cross-links, and single-strand DNA and double-strand DNA breaks (7, 8). Arsenic-induced oxidative stress and oxidative DNA damage are evident in various tissue culture cells, animals and humans chronically exposed to arsenic (9, 10). Elevated levels of oxidative stress DNA damage product 8-hydroxy-2' -deoxyguanosine were found in the skin lesions of arsenic-exposed individuals (9). When these oxidative DNA lesions are not removed by DNA repair mechanisms, in time they can become self-perpetuating mutations that contribute to aging and human degenerative diseases such as cancer and other chronic diseases (11).

DNA damages is repaired by different DNA repair pathways depending on the nature of damage. The nucleotide excision repair (NER) pathway repairs a wide spectrum of structurally unrelated lesions such as UV-induced photoproducts, bulky chemical adducts and certain DNA cross-links (12), whereas base excision repair (BER) typically repairs oxidation and alkylation of nucleobases. Excision repair cross complementation group-1 (*ERCC1*) is an essential gene in the NER pathway encoding a subunit of endonucleases which make the 5' incision of DNA damage in NER (13). *ERCC1* expression has been shown to correlate

Abbreviations: ERCC1, Excision repair cross complementation group-1; NER, nucleotide excision repair; BER, base excision repair; OGG1, 8-oxo-guanine DNA glycosylase; PCR, polymerase chain reaction; ROS, reactive oxygen species.

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with the capacity for repair of DNA damage (14). It has been reported that *ERCC1* expression is significantly elevated by the cisplatin treatment of A2780/CP70 human ovarian cancer cell line (15). *ERCC1* mRNA levels in liver tissue increased 2-fold in mice treated with inorganic arsenicals (16). These studies indicate that *ERCC1* is inducible by DNA-damaging agents such as arsenic.

The present study was conducted in the Bayingnormen (Ba Men) region of Inner Mongolia, China, where the residents have been chronically exposed to arsenic *via* drinking water (17). The residents of Ba Men are mostly farmers and have been exposed to a wide range of arsenic levels up to 1.8 mg/l for more than 20 years. Arsenic exposure has been associated with health effects including dermal, neurological and cardiovascular effects (17, 18). We selected the Ba Men region as our study site to investigate the health effects of arsenic for the following reasons. More than 80% of Ba Men residents have their own wells. Therefore, it is possible to assess arsenic exposure on an individual basis. The Ba Men residents grow their own grain and livestock and have good nutrition. Ba Men is located in an inland area of China. The residents occasionally consume freshwater fish from the Huang He River and very seldom consume seafood from the ocean (19). A previous report showed that the arsenic levels of the water from the Huang He River are below the Chinese arsenic standard for drinking water (<50 μg/l) (17). We previously reported that elevated 8-oxo-guanine DNA glycosylase (*OGG1*) mRNA levels in blood cells of the exposed individuals are associated with arsenic concentrations in well water in this population (19). It is known that *OGG1* and *ERCC1* work in a coordinated fashion and play important roles in maintaining genomic integrity (20). It was reported that the NER pathway is coordinated with the BER pathway to repair DNA damage induced by oxidative stress and sunlight in humans (21). To evaluate the role of NER in arsenic-induced carcinogenesis, we investigated the relationship between arsenic concentrations in well water and *ERCC1* expression in the blood of the Ba Men residents exposed to a wide range of arsenic exposure levels. It has been reported that arsenic concentrations in toenails reflect long-term arsenic exposure up to 12 months (22). In our previous study in the same population, arsenic concentrations in well water and toenails were highly correlated (19). In this study, we also analyzed the relationship between *ERCC1* expression levels and toenail arsenic concentrations. The relationship between the levels of *ERCC1* and *OGG1* gene expression was also investigated.

Materials and Methods

Study participants. A total of 327 residents living in the sub-villages of Wulan, Jianshe, Fengchan and Xinyao in Sha Hai Village, Hangjin Hou County and the sub-villages of Miaohao, Xigelian in Sheng Feng Village, Wu Yuan County participated in this study. The residents in these sub-villages have been exposed to a wide range of arsenic levels in drinking water from the artesian wells for more than 10 years. Prior to participant selection, the well water samples of the homes from these sub-villages were collected and analyzed for arsenic concentrations. The study participants were selected according to the following criteria set for study design focusing on the arsenic effects on *ERCC1* expression at arsenic levels equal to or lower than 200 μg/l. The criteria for selection were: (i) 70% or more of individuals with arsenic exposure levels from non-detectable to 200 μg/l and 30% with arsenic exposure levels >200 μg/L, (ii) approximately equal number of males and females and 30% of smokers and 70% nonsmokers, and (iii) age from 11-65 years with exposure of at least 5 years' duration. Questionnaires were administered to all participants to obtain demographic information, history of well use, diet, smoking, occupation, pesticide use and medical information. All participants gave written informed consent to participate in this study. This study was conducted according to the recommendations of the World Medical Association Declaration of Helsinki for International Health Research (23). The research protocol met the requirements for protection of human subject certification by the U.S. Environmental Protection Agency.

Water and human sample collection and analysis. Water samples were collected from each participant's home well and sent to the United States on ice packs and stored at –80˚C until analysis. Quantification of inorganic arsenic in well water was performed as reported previously (19). Briefly, water samples were acidified to pH 1.4-1.6 with HNO_3 and then diluted with 1% HNO_3 5-10 times for arsenic analysis using inductively coupled plasma mass spectrometry (ICPMS) as described by Gong *et al*. (24). The detection limit for arsenic by ICPMS was 0.1 μg/l.

Toenail samples were analyzed for arsenic content by instrumental neutron activation analysis at the Nuclear Services Department, North Carolina State University, Raleigh, North Carolina, USA (25). The detection limit by INAA was 0.012 μg/g for arsenic.

Real-time quantitative PCR. A 2.5 ml peripheral blood sample was collected into a PAXgene blood RNA tube (Qiagen, Valencia, CA, USA), kept at room temperature for two hours and then stored at –40˚C until shipping. The blood samples were shipped to the USA in dry ice and stored at –80˚C until RNA isolation. Total RNA was isolated from whole blood using PAXgene blood RNA kits according to the manufacturer's instructions (Qiagen). The total amount of RNA was quantified by Beckman spectrophotometer. The ratios of 260/280 of all isolated RNA were between 1.90-2.10. The integrity of RNA was checked by Bioanalyzer 2100 (Agilent Technologies, Walbronn, Germany). There was no degradation of RNA indicated by 18S and 28S RNA in Bioanalyzer analysis. The first stranded-cDNA synthesis was conducted in a reaction mixture of total volume 50 μl containing 1X buffer, 2.5 μM random primers, 5 mM DTT, 500 μM dNTP, 20 unit RNase inhibitor, 200 unit SuperScript III reverse transcriptase (Invitrogen, CA, USA) and 500 ng total RNA. A total of 2 μl of cDNA were used for each real-time PCR measurement in total volume of 50 μl. *ERCC1* and *β-actin* cDNA were PCR amplified in the same tube using ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster, CA, USA). *ERCC1* (assay ID: HS00157415_m1) and *β-actin* (assay ID: 4326315E) primers and probes were obtained from Applied Biosystems. Real-time PCR was performed in duplicate with 2X TaqMan Universal PCR Master Mix. *ERCC1* mRNA levels were determined by a relative standard curve method using HT1080 cell RNA as standard (User Bulletin #2; Applied

Biosystems) and normalized to *β-actin* mRNA amount assayed in the same tube. *β-Actin* was chosen as an endogenous control because *β-actin* mRNA levels showed the least variation amongst 11 candidate genes examined. Pooled blood cDNAs and HT1080 cell cDNA were used as positive control in all assays. RNase-free water and blood cell RNA were used as negative controls in the experiments. A standard curve was included in each real-time PCR assay. To avoid amplification of the contaminating residual genomic DNA, primers and probes were designed around the junction region of two exons so that they are mRNA specific. Ten percent of blood cDNA samples were repeated. The standard deviation on repeated measurements of same blood cDNA in the repeated experiments was <10%, indicating the day to day variation of the assay.

Statistical analysis. Mean *ERCC1* expression was compared in subgroups using *t*-test or F-tests from analysis of variance models (ANOVA). Linear regression models and Spearman and Pearson correlation coefficients were used to evaluate the association between continuous dependent variables (*ERCC1* expression) and independent predictor variables. Logistic regression models were used to evaluate the association between binary dependent variables (skin hyperkeratosis) and independent predictor variables. Interaction terms between key covariates and predictors were included in regression models. Multivariate models were constructed, controlling for variables that were related to the outcome or predictor variable. To test for interaction or effect modification, multiplicative interaction terms were between water and nail arsenic and sex, age category and smoking. Wald tests were conducted to test the significance of the interaction terms. To assess the effect of non-linearity, two- and four-degree fractional polynomials were fitted and compared to a linear model using a deviance difference test (26). Statistical analyses were conducted using Stata version 9.2 (StataCorp 2005, College Station, TX, USA).

Results

Study participants. The information on study participants is shown in Table I. The study participants were mostly farmers (79%). Among the 327 participants, 164 were male and 163 were female, with ages ranged from 11 to 61 years (median age of 38). Thirty three percent were smokers and 67% were nonsmokers. The arsenic concentrations of the well water used by the study participants ranged from 0.34 to 826 μg/l, and with a mean exposure of 13 years. Seventy-six percent of participants had been exposed to arsenic at the concentrations of 0.34 to 200 μg/l and 24% to 201-826 μg/l. Most participants (81%) did not drink alcohol or drank less than twice a week. Forty-eight percent reported having used pesticides in the previous 5 years. Most participants (99%) reported eating meat and dairy products, and most consumed freshwater fish occasionally. Regarding skin abnormalities, 31% of study participants showed skin hyperkeratosis, 12% showed depigmentation and 3% showed hyperpigmentation.

ERCC1 expression versus water and nail arsenic. *ERCC1* gene expression was positively associated with the arsenic level in drinking water (slope=0.313, p=0.0043) (Figure 1). Non-linear fractional polynomial models did not show improvement over the linear model (data not shown). Arsenic concentrations in well water and toenails were highly correlated (Spearman r=0.8817, p<0.0001). As shown in Figure 2, *ERCC1* expression was also positively associated with toenail arsenic concentrations (slope=0.474, p=0.0073). We also evaluated the effects of other factors, including age, sex, smoking, alcohol and pesticide use on *ERCC1* gene expression. *ERCC1* mRNA expression was associated with water arsenic in the age group of 19-50 years (slope=0.366, p=0.0065) and showed borderline significance in the age group of 50 years and older

Table I. *Study participants' characteristics*.

Characteristic	No. (%)
Gender	
Male	164 (50.2)
Female	163 (49.8)
Age (years)	
9-29	97 (29.8)
30-50	176 (54.0)
51-61	53 (16.2)
Tobacco smoking	
Nonsmokers	220 (67.3)
Smokers	107 (32.7)
Education	
None	51 (15.6)
Elementary	99 (30.4)
Jr. high school	154 (47.3)
High school	20 (6.1)
College or above	2 (0.6)
Alcohol	
At least twice a week	61 (18.7)
Less than twice a week	266 (81.3)
Occupation	
Farmer	258 (79.1)
Manufacturing	1 (0.3)
Other	67 (20.6)
Pesticide use in previous 5 years	
Yes	157 (48.0)
No	170 (52.0)
Eat meat or dairy	
Occasionallya	2 (0.6)
Oftenb	323 (99.4)
Eat fresh water fish	
Never	2 (0.6)
Occasionally	306 (94.2)
Often	17 (5.2)
Skin hyperkeratosis	
Yes	99 (30.5)
No	226 (69.5)
Skin hyperpigmentation	
Yes	11(3.4)
No	316 (96.6)
Skin depigmentation	
Yes	38 (11.6)
No	289 (88.4)

[a]1-5 times per month; [b]at least 5 times per month.

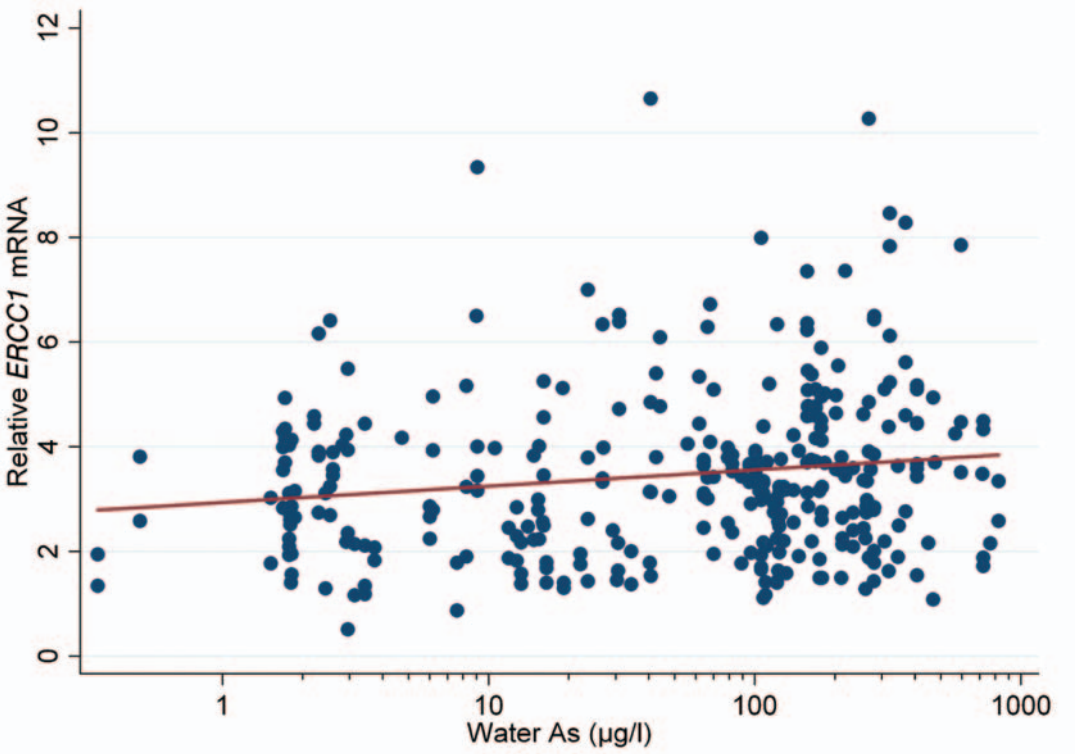

Figure 1. *Association between ERCC1 mRNA levels and water arsenic concentrations. Adjusting for age, sex, smoking and pesticide use, ERCC1 mRNA levels were significantly associated with water arsenic using a linear regression model: n=326, slope=0.313, p=0.0043; Spearman r=0.154, p=0.0054.*

(slope=0.438, p=0.069). The 11-18 years age group did not show statistical significance (p=0.85). There was significant association between *ERCC1* expression and water arsenic exposure (slope=0.38, p=0.0264) in males, and females showed a border-line significant association (slope=0.24, p=0.082). The association was significant for nonsmokers (slope=0.342, p=0.0107). The trend was positive among smokers, although the association was not statistically significant, possibly due in part to lower numbers (N=107; slope=0.242, p=0.203). Alcohol and pesticides use did not affect *ERCC1* expression in the study participants. There were no interactions between the arsenic effect on *ERCC1* mRNA expression and age, gender or smoking.

ERCC1 versus OGG1 expression. *ERCC1* mRNA levels were positively correlated with *OGG1* mRNA levels in the study participants (r=0.275, Spearman r=0.275, p<0.0001) (Figure 3). Data for *OGG1* mRNA levels were obtained from the previous study (19).

Discussion

In this study, we investigated effects of chronic arsenic exposure on *ERCC1* mRNA expression of blood cells in a Chinese population and also linked *ERCC1* gene expression to toenail arsenic concentrations of the study participants. The results showed that *ERCC1* mRNA levels significantly increased with water and toenail arsenic levels. We also investigated other factors that affect *ERCC1* expression. There were no significant differences in arsenic induction of *ERCC1* expression due to tobacco smoke or pesticide use. There were some differences in the relationship between arsenic exposure and induction of *ERCC1* expression by age, showing a stronger effect in two older groups. This may be due to the older groups ingesting larger quantities of the arsenic-contaminated water and thus being exposed to greater oxidative stress and the associated DNA repair responses than the younger groups. It has been reported that sunlight radiation increases *ERCC1* expression in humans (20). In this study, we also found that the male participants showed higher rate of *ERCC1* expression increase associated with arsenic exposure than did females. Males in Ba Men usually engaged in more outdoor physical activity than females and consumed more arsenic-contaminated drinking water, as well as being exposed to more UV radiation from sun. The higher exposures in males may induce greater oxidative stress and DNA repair response than in females.

It has been reported that the protective response to environmental toxicants increases gene transcription, protein levels and enzyme activity of DNA repair and oxidative stress

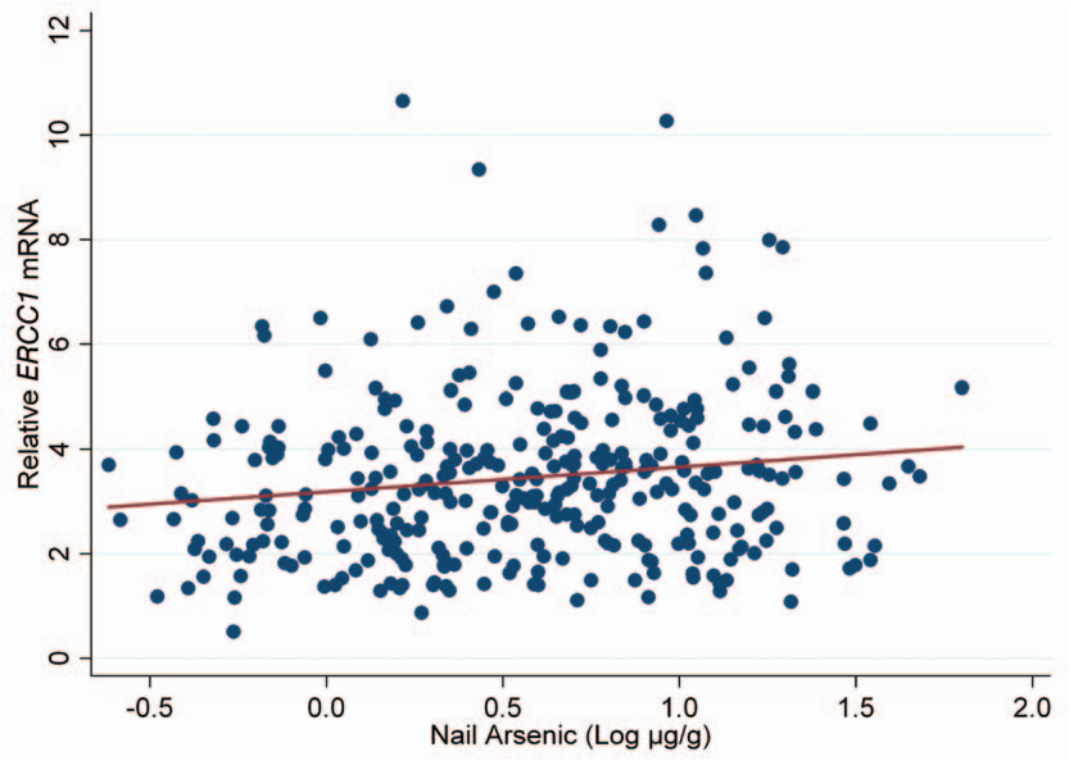

Figure 2. *Association between ERCC1 mRNA levels and nail arsenic concentration. Adjusting for age, sex and smoking, ERCC1 mRNA levels were significantly associated with water arsenic using a linear regression model. n=326, slope=0.474, p=0.0073; Spearman r=0.159, p=0.0040.*

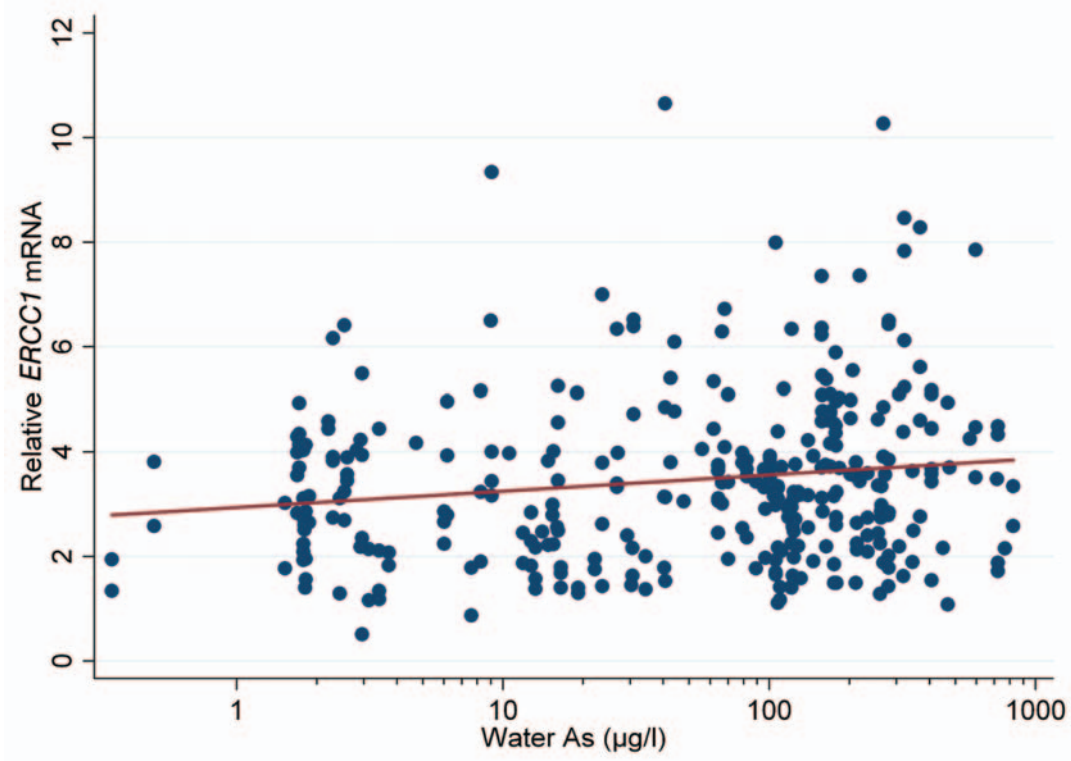

Figure 3. *Correlation between ERCC1 and OGG1 mRNA levels. ERCC1 and OGG1 mRNA levels were positively correlated: Spearman r=0.275, p<0.0001.*

response genes (27). This protective response may be responsible for the increased *ERCC1* mRNA expression due to arsenic exposure in this study. Arsenic in the range of 0.1-2 μM induces a protective response in human cultured cells (28), whereas it induces down-regulation of DNA repair and oxidative stress genes at levels of arsenic exposure >10 μM (29, 30). Mice treated with arsenic showed elevated expression of DNA repair response genes including *ERCC1* mRNA levels (16). Wu *et al*. reported that *ERCC1* mRNA levels were up-regulated in lymphocytes from arsenic-exposed humans with blood arsenic levels between 9.60-46.5 μg/l in Taiwan (31). Andrew *et al*. reported that arsenic exposure was associated with decreased expression of *ERCC1* mRNA and protein in lymphocytes in arsenic-exposed individuals in New Hampshire, U.S.A. and Mexico (29, 32). The disagreement may be due to the differences in water arsenic concentrations in these studies comparing with ours and Taiwanese studies.

The molecular mechanisms of *ERCC1* gene induction in arsenic-exposed individuals are not completely known. The transcription activator responsible for the induction of *ERCC1* gene expression has not been well established. There is evidence showing that the induction of *ERCC1* expression appears to be mediated through transcription factor AP-1, which is a heterodimer of c-Jun and c-Fos protein. AP-1 DNA binding sites are the *cis*-acting elements of *ERCC1*. There is an AP-1-like binding site in the 5'-flanking region of *ERCC1* (33). It has been reported that cisplatin induces an AP-1 mediated increase in *ERCC1* mRNA expression in human ovarian tumor cells. Suppression of c-Jun and c-fos expression by cyclosporine A and herbimycin A block-d the cisplatin-induced increase in *ERCC1* mRNA levels (15). It has been reported that arsenite induced c-Jun and c-fos expression and increased AP-1 transactivation activity in tissue culture cells (34). Arsenic has been reported to induce activation of transcription factor AP-1 *in vitro* and *in vivo* (35). Furthermore, ROS induced by low concentrations of arsenic have been shown to increase the transcription of AP-1, which results in cell signaling, transcription factor binding to DNA, and subsequent stimulation of cell proliferation (36). Therefore, the observed increase of *ERCC1* expression in this study may result from arsenic-induced oxidative stress and followed by increased expression of c-Jun and c-fos and AP-1 activation, which is then followed by enhanced *ERCC1* mRNA expression.

Our previous study showed that arsenic exposure increased mRNA levels of the BER repair gene *OGG1* in blood cells from this population (19). In this study, we found that the mRNA levels of these two DNA repair genes were significantly correlated. NER and mismatch repair in yeast have been shown to play a role in the repairing of 8-hydroxy-2'-deoxyguanosine (8-OHdG), the main oxidation product of oxidative stress (37). The NER and BER up-regulation mechanisms could mediate global repair generated by the oxidative stress-induced DNA damage caused by arsenic and maintain tolerable levels of DNA damage. In concert with *OGG1*, the elevated *ERCC1* mRNA levels could be interpreted as enhanced DNA repair capacity for eliminating the oxidative stress-induced DNA damage in arsenic-exposed individuals. A similar close association has also been shown between *ERCC1* and *OGG1* in healthy humans with solar radiation and patients with colorectal adenomas and carcinomas (20, 38). The coordinated expression of *ERCC1* and *OGG1* indicates that both genes are subjected to common transcription regulation or the same mode of action.

In conclusion, the positive association of *ERCC1* expression with chronic arsenic exposure in a human population was shown in the present study, suggesting that arsenic exposure could induce DNA damage repair response. *ERCC1* mRNA expression was positively correlated with *OGG1* mRNA levels, suggesting a similar mode of action. The present study also demonstrated that the technology of real-time PCR for quantitative gene expression is a valuable tool for human epidemiological studies in assessing health effects of environmental contaminants.

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References

1 Chakraborti D, Sengupta MK, Rahman MM, Ahamed S, Chowdhury UK, Hossain MA, Mukherjee SC, Pati S, Saha KG, Dutta RN and Quamruzzaman Q: Groundwater arsenic contamination and its health effects in the Ganga-Meghna-Brahmaputra plain. J Environ Monit *6*: 74N-83N, 2004.

2 Ferreccio C, Gonzalez C, Milosavjlevic V, Marshall G, Sancha AM and Smith AH: Lung cancer and arsenic concentrations in drinking water in Chile. Epidemiology *11*: 673-679, 2000.

3 Nordstrom DK: Public health. Worldwide occurrences of arsenic in ground water. Science *296*: 2143-2145, 2002.

4 Chen CJ, Hsu LI, Wang CH, Shih WL, Hsu YH, Tseng MP, Lin YC, Chou WL, Chen CY, Lee CY, Wang LH, Cheng YC, Chen CL, Chen SY, Wang YH, Hsueh YM, Chiou HY and Wu MM: Biomarkers of exposure, effect, and susceptibility of arsenic-induced health hazards in Taiwan. Toxicol Appl Pharmacol *206*: 198-206, 2005.

5 Tapio S and Grosche B: Arsenic in the aetiology of cancer. Mutat Res *612*: 215-246, 2006.

6 Kitchin KT and Ahmad S: Oxidative stress as a possible mode of action for arsenic carcinogenesis. Toxicol Lett *137*: 3-13, 2003.

7 Shi H, Hudson LG, Ding W, Wang S, Cooper KL, Liu S, Chen Y, Shi X and Liu KJ: Arsenite causes DNA damage in keratinocytes *via* generation of hydroxyl radicals. Chem Res Toxicol *17*: 871-878, 2004.

8 Bau DT, Wang TS, Chung CH, Wang AS and Jan KY: Oxidative DNA adducts and DNA-protein cross-links are the major DNA lesions induced by arsenite. Environ Health Perspect *110(Suppl 5)*: 753-756, 2002.
9 Liu SX, Athar M, Lippai I, Waldren C and Hei TK: Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. Proc Natl Acad Sci USA *98*: 1643-1648, 2001.
10 Matsui M, Nishigori C, Toyokuni S, Takada J, Akaboshi M, Ishikawa S, Imamura S and Miyachi Y: The role of oxidative DNA damage in human arsenic carcinogenesis: detection of 8-hydroxy-2'-deoxyguanosine in arsenic-related Bowen's disease. J Invest Dermatol *113*: 26-31, 1999.
11 Cooke MS, Evans MD, Dizdaroglu M and Lunec J: Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J *17*: 1195-214, 2003.
12 de Laat WL, Jaspers NG and Hoeijmakers JH: Molecular mechanism of nucleotide excision repair. Genes Dev *13*: 768-785, 1999.
13 Wood RD: DNA damage recognition during nucleotide excision repair in mammalian cells. Biochimie *81*: 39-44, 1999.
14 Dabholkar M, Bostick-Bruton F, Weber C, Egwuagu C, Bohr VA and Reed E: Expression of excision repair genes in non-malignant bone marrow from cancer patients. Mutat Res *293*: 151-160, 1993.
15 Li Q, Tsang B, Bostick-Bruton F and Reed E: Modulation of excision repair cross complementation group 1 (*ERCC-1*) mRNA expression by pharmacological agents in human ovarian carcinoma cells. Biochem Pharmacol *57*: 347-353, 1999.
16 Liu J, Kadiiska MB, Liu Y, Lu T, Qu W and Waalkes MP: Stress-related gene expression in mice treated with inorganic arsenicals. Toxicol Sci *61*: 314-320, 2001.
17 Ma HZ, Xia YJ, Wu KG, Sun TZ and Mumford JL: Human exposure to arsenic and health effects in Bayingnormen, Inner Mongolia. *In*: Arsenic Exposure and Health Effects. Chappell WR, Abernathy CO and Calderon R (eds.). Amsterdam, Elsevier, pp. 127-131, 1999.
18 Mumford JL, Wu K, Xia Y, Kwok R, Yang Z, Foster J and Sanders WE: Chronic arsenic exposure and cardiac repolarization abnormalities with QT interval prolongation in a population-based study. Environ Health Perspect *115*: 690-694, 2007.
19 Mo J, Xia Y, Wade TJ, Schmitt M, Le XC, Dang R and Mumford JL: Chronic arsenic exposure and oxidative stress: *OGG1* expression and arsenic exposure, nail selenium, and skin hyperkeratosis in inner mongolia. Environ Health Perspect *114*: 835-841, 2006.
20 Vogel U, Moller P, Dragsted L, Loft S, Pedersen A and Sandstrom B: Inter-individual variation, seasonal variation and close correlation of *OGG1* and *ERCC1* mRNA levels in full blood from healthy volunteers. Carcinogenesis *23*: 1505-1509, 2002.
21 Risom L, Moller P, Vogel U, Kristjansen PE and Loft S: X-ray-induced oxidative stress: DNA damage and gene expression of *HO-1, ERCC1* and *OGG1* in mouse lung. Free Radic Res *37*: 957-966, 2003.
22 Slotnick MJ and Nriagu JO: Validity of human nails as a biomarker of arsenic and selenium exposure: A review. Environ Res *102*: 125-139, 2006.
23 World Medical Association. 1989. Declaration of Helsinki. Available: http://www.wma.net/e/policy/pdf/17c.pdf (accessed 22 January 2009).
24 Gong Z, Lu X, Watt C, Wen B, He B, Mumford J, Ning Z, Xia Y and Le XC: Speciation analysis of arsenic in groundwater from Inner Mongolia with an emphasis on acid-leachable particulate arsenic. Anal Chim Acta *555*: 181-187, 2006.
25 Heydorn K: Neutron Activation Analysis for Clinical Trace Element Research, Vols 1 and 2. Boca Raton, FL: CRC Press, 1984.
26 Royston P, Ambler G and Sauerbrei W: The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol *28(5)*: 964-974, 1999.
27 Snow ET, Sykora P, Durham TR and Klein CB: Arsenic, mode of action at biologically plausible low doses: What are the implications for low-dose cancer risk? Toxicol Appl Pharmacol *207*: 557-564, 2005.
28 Smith KR, Klei LR and Barchowsky A: Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. Am J Physiol Lung Cell Mol Physiol *280*: L442-449, 2001.
29 Andrew AS, Burgess JL, Meza MM, Demidenko E, Waugh MG, Hamilton JW and Karagas MR: Arsenic exposure is associated with decreased DNA repair *in vitro* and in individuals exposed to drinking water arsenic. Environ Health Perspect *114*: 1193-1198, 2006.
30 Zhang TC, Schmitt MT and Mumford JL: Effects of arsenic on telomerase and telomeres in relation to cell proliferation and apoptosis in human keratinocytes and leukemia cells *in vitro*. Carcinogenesis *24*: 1811-1817, 2003.
31 Wu MM, Chiou HY, Ho IC, Chen CJ and Lee TC: Gene expression of inflammatory molecules in circulating lymphocytes from arsenic-exposed human subjects. Environ Health Perspect *111*: 1429-1438, 2003.
32 Andrew AS, Karagas MR and Hamilton JW: Decreased DNA repair gene expression among individuals exposed to arsenic in United States drinking water. Int J Cancer *104*: 263-268, 2003.
33 van Duin M, Koken MH, van den Tol J, ten Dijke P, Odijk H, Westerveld A, Bootsma D and Hoeijmakers JH: Genomic characterization of the human DNA excision repair gene ERCC-1. Nucleic Acids Res *15*: 9195-9213, 1987.
34 Cavigelli M, Li WW, Lin A, Su B, Yoshioka K and Karin M: The tumor promoter arsenite stimulates AP-1 activity by inhibiting a JNK phosphatase. EMBO J *15*: 6269-6279, 1996.
35 Huang C, Bode AM, Chen NY, Ma WY, Li J, Nomura M and Dong Z: Transactivation of AP-1 in AP-1-luciferase reporter transgenic mice by arsenite and arsenate. Anticancer Res *21*: 261-267, 2001
36 Germolec DR, Yoshida T, Gaido K, Wilmer JL, Simeonova PP, Kayama F, Burleson F, Dong W, Lange RW and Luster MI: Arsenic induces overexpression of growth factors in human keratinocytes. Toxicol Appl Pharmacol *141*: 308-318, 1996.
37 Scott AD, Neishabury M, Jones DH, Reed SH, Boiteux S and Waters R: Spontaneous mutation, oxidative DNA damage, and the roles of base and nucleotide excision repair in the yeast *Saccharomyces cerevisiae*. Yeast *15*: 205-218, 1999.
38 Saebo M, Skjelbred CF, Nexo BA, Wallin H, Hansteen IL, Vogel U and Kure EH: Increased mRNA expression levels of *ERCC1, OGG1* and *RAI* in colorectal adenomas and carcinomas. BMC Cancer *6*: 208-214, 2006.

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