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Aldehyde dehydrogenase induction in arsenic-exposed rat bladder epithelium

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ABSTRACT

Arsenic is widely distributed in the environment. Many human cancers, including urothelial carcinoma (UC), show a dose-dependent relationship with arsenic exposure in the south-west coast of Taiwan (also known as the blackfoot disease (BFD) areas). However, the molecular mechanisms of arsenic-mediated UC carcinogenesis has not yet been defined. *In vivo* study, the rat bladder epithelium were exposed with arsenic for 48 h. The proteins were extracted from untreated and arsenic-treated rat bladder cells and utilized two-dimensional gel electrophoresis and mass spectrometry. Selected peptides were extracted from the gel and identified by quadrupole-time of flight (Q-TOF) Ultima-Micromass spectra. The significantly difference expression of proteins in arsenic-treated groups as compared with untreated groups was confirmed by immunohistochemistry (IHC) and western blotting. We found that thirteen proteins were down-regulated and nine proteins were up-regulated in arsenic-treated rat bladder cells when compared with untreated groups. The IHC and western blotting results confirmed that aldehyde dehydrogenase (ALDH) protein was up-regulated in arsenic-treated rat bladder epithelium. Expression of ALDH protein was significantly higher in UC patients from BFD areas than those from non-BFD areas using IHC ($p = 0.018$). In conclusion, the ALDH protein expression could be used as molecular markers for arsenic-induced transformation.

1. Introduction

The purpose of proteome analysis is to identify biomarkers using protein identification, post-translational modifications, levels of relative abundance, and interactions with other proteins. Chemical carcinogenesis biomarkers have be assigned to three categories: internal exposure, early biological effects, and cancer risk (Au, 2007; Toraason et al., 2004). This analysis can be achieved by comparing the two-dimensional electrophoresis (2-DE) proteome patterns in bio-systems exposed to different conditions, including carcinogen exposure. A large range of immobilized pH gradient (IPG) strips and high-throughput 2-DE analysis are used to identify proteins that are significantly changed in cells or animals after exposure to toxic compounds (Chowdhury and Aposhian, 2008; Lau and Chiu, 2007; Taylor-McCabe et al., 2006). The most common proteomic technique is 2-DE polyacrylamide gel electrophoresis (PAGE). The proteins are separated in the first dimension by charge and in the second dimension by molecular weight (MW). The 2-DE technique is followed by an identification

Abbreviations: 2-DE, two-dimensional electrophoresis; ALDH, aldehyde dehydrogenase; BFD, blackfoot disease; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate]; CK-1, type II cytoskeletal 1; CK-10, type I cytoskeletal 10; CSC, cancer stem cell; D.D. water, distilled deionized water; DAB, 3′3-diaminobenzidine; DNA, deoxyribonucleic acid; DTT, dithiothreitol; eEF-1α, elongation factor-1 alpha; hsp27, heat shock protein 27; IACUC, Institutional Animal Care and Use Committee; IEF, isoelectric focusing; IHC, immunohistochemistry; IPG, immobilized pH grading; MS, mass spectrometry; M.W., molecular weight; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NL, non-linear; OS, osteosarcoma; PAGE, polyacrylamide gel electrophoresis; PGK1, phosphoglycerate kinase 1; PVDF, polyvinylidene difluoride; Q-TOF, quadrupole-time of flight; SDS, sodium dodecyl sulfate; UC, urothelial carcinoma.

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step using digital imaging coupled with mass spectrometry (MS) and bioinformatics. However, the new application of toxicoproteomics is uniquely positioned to advance public health and expand our understanding of protein expression in cases of toxicity and environmental disease (Wetmore and Merrick, 2004).

Arsenic has been shown to induce changes in gene transcription in a variety of cell lines. However, arsenic induces oxidative damage by causing single- and double-strand DNA breaks (Hei et al., 1998) and inhibiting DNA repair enzymes (Lynn et al., 1997). Few reports have focused on arsenic-induced alterations in protein patterns *in vivo*. The aim of our study was to investigate the protein profiles in rat bladder cells after arsenic exposure for 48 h using 2-DE and MS. We confirmed the arsenic-induced protein changes using immunohistochemistry (IHC) and western blotting.

2. Material and methods

2.1. Animal experiment design

In this study, we used the animal model detailed by Sessions et al. (2002). Male F344/N (6-8 week-old) rats were purchased from the National Laboratory of Animal Breeding and Research Center (Taipei, Taiwan, R.O.C.). The rats were quarantined for 1 week before the start of the experiment. The rats were housed in stainless steel wire mesh cages and placed in a controlled environment with a 12-h light–dark cycle at $22 \pm 3\,^{\circ}C$ and $60 \pm 10\%$ humidity. The rats were randomly assigned to either the experimental or control group, which each consisted of 7 rats. The experimental group was exposed to 4 μM (approximately 0.52 ppm) sodium arsenite (dissolved in D.D. water) for 48 h (4 times per day at 6 h interval). The control group was given only D.D. water. Next, the rat bladders were dissected, immediately frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for western blotting or fixed in a 10% formalin solution. The expression of aldehyde dehydrogenase (ALDH) protein in the rat bladder epithelium cells of the bladder was detected by IHC and western blotting. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Kaohsiung Medical University (KMU-IACUC-97121).

2.2. Sample preparation

The rat bladder cells lysate was mixed with rehydration buffer (7 M urea, 2 M thiourea, 2% w/v 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 20 mM dithiothreitol (DTT), 0.5% IPG buffer and a trace of bromophenol blue). The protein concentration in the sample was measured using the Bradford method (Huang et al., 2011).

2.3. Two-dimensional (2D) gel electrophoresis

Isoelectric focusing (IEF) was performed using an Ettan IPGphor II system (Amersham Biosciences). Ready-to-use 13-cm Immobiline DryStrips [pH 3–10 non-linear (NL)] (GE Healthcare, Uppsala, Sweden) were rehydrated for 12 h at RT in rehydration buffer. The protein sample was mixed with the sample buffer (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG buffer pH 3–10 NL, 100 mM DeStreak reagent, and a trace of bromophenol blue). After cup-loading using the manufacturer's protocol, protein focusing was achieved using an IPGphor system (GE Healthcare Life Sciences, Piscataway, NJ, USA) with the following IEF parameters: 350 V, step and hold, 3 h; 650 V, gradient, 1 h; 1100 V, gradient, 1 h; 8000 V, gradient, 1.5 V; 8000 V, step and hold, 3 h, giving a total of 15,000 Vh. Next, strips were removed and transferred immediately for a second-dimensional electrophoresis using 10% SDS polyacrylamide (SDS-PAGE) gels (13 cm) and Hoefer SE 600 vertical chambers.

2.4. Image processing

After SDS-PAGE, the analytical and preparative gels were stained with silver or Coomassie blue R-250, respectively. Silver-stained gels were imaged, and the total number of spots on the individual gels was determined using Phoretix 2D Pro imaging software. All 2D electrophoresis data analysis was carried out by ImageMaster™ 2D Platinum 5.0 (GE Healthcare). The spot volumes of all gels were normalized by dividing the raw quantity of each spot by the total quantity of all valid spots within the individual gel. Protein levels that increased or decreased by >15-fold or <0.3-fold were considered to have an altered expression.

2.5. In-gel digestion and protein identification

Each gel slice was cut into small pieces and placed into siliconized tubes. Spots of sliver-stained protein were excised, destained, washed, and dehydrated in acetonitrile. After the gel pieces were completely dried, they were rehydrated with a sequence-grade modified trypsin (freshly prepared) solution at 37 °C for 16 h. The tryptic peptides were extracted in a 50% acetonitrile/5% formic acid solution using sonication. The extracted solutions were evaporated to dryness under vacuum and then redissolved in 0.1% trifluoroacetic acid. All Q-TOF Ultima-Micromass spectra obtained for the parent ions were processed by MassLynx software (Waters, Mifford, MA) to generate peak lists as PKL files. The proteins were identified by comparing experimental data using the MASCOT (Matrix Science) database.

2.6. Western blotting

The rat bladder cells were treated with arsenite for 48 h. The samples were homogenized with lysis buffer and centrifuged for protein analysis. The protein concentration was determined using a Bradford assay (Bio-Rad, Hercules, CA, USA). Samples were subjected to SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane as previously described (Huang et al., 2009). After incubation with 5% skim milk at RT, the membranes were incubated with a primary ALDH antibody (Santa Cruz Biotechnology Inc., CA, USA). Next, the membrane was incubated with a secondary antibody conjugated with horseradish peroxidase. The protein bands were detected using enhanced chemiluminescence (Amersham, Buckinghamshire, England).

2.7. Case selection

We obtained the patient information and specimens for 35 urothelial carcinoma (UC) patients from the archives of the Department of Pathology, Kaohsiung Medical University Hospital dated between 1993 and 2000. The arsenic-contaminated areas, also known as blackfoot disease (BFD) areas of this study were limited to the southwestern townships of Taiwan (Huang et al., 2011). The residents of the BFD areas used high-arsenic artesian well water over a long period. Tissue sections were reviewed by an experienced pathologist, and the histological characteristics were recorded. The clinical profiles of the BFD and non-BFD samples were determined according to the guidelines proposed by the World Health Organization and the TNM system (Eble et al., 2004). All tissue acquisition and this study were approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUH-IRB-970392).

2.8. IHC and evaluation

All slides were prepared from the paraffin-embedded UC tissue from BFD areas, non-BFD areas, and the bladder tissue of rats.