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Does and duration of exposure time dependent regulation of proteins in the urinary bladder of hamsters exposed to sodium arsenite

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ABSTRACT

Differential in Gel Electrophoresis (DIGE) coupled with Mass Spectrometry (MS) has been used to study the proteomic changes in the urinary bladder of hamsters exposed to sodium arsenite in their drinking water.

Hamsters were exposed to sodium arsenite (173 mg, 57 mg, or 1 mg As/L) in drinking water for 6, 21, or 10 days, respectively, and the control hamsters were given tap water. Several protein spots were down-regulated and several were up-regulated in the urinary bladder of hamsters exposed to sodium arsenite (173 mg As/L) for 6 days, as compared to controls. The volume ratio changes of these proteins in the bladder of hamsters exposed to arsenite were significantly different than that of control hamsters.

Most of the protein spots were unchanged in the urinary bladder sample of hamsters exposed to sodium arsenite (57 mg As/L or 1 mg As/L) for 21 days or 10 days, respectively, as compared with the urinary bladder control samples. Perhaps, the low doses of sodium arsenite and short time exposure were not sufficient to up-regulate or down-regulate the proteins in the bladders of hamsters.

Our results indicate that DIGE can provide new and valuables information as to the specific properties improved in mechanisms of inorganic arsenic toxicity and carcinogenicity.

Abbreviations: DIGE, Differential in Gel Electrophoresis; LC- MS, Liquid chromatography- Mass Spectrometry; GST-pi, glutathione S transferase-pi.

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Introduction.

Inorganic arsenic is a known human carcinoge[1]. Millions of people are exposed to it from naturally contaminated drinking water[1-4]. Chronic exposure to inorganic arsenic can cause cancer of the skin, lungs, urinary bladder, kidneys, and liver[1, 3, 5-8]. The toxic effects of inorganic arsenics are does depended and high exposure produce cytotoxicity[9] and carcinogenicity[9-11].Many mechanisms of arsenic toxicity and carcinogenicity have been suggested [12, 13] including chromosome abnormalities [14], oxidative stress [15, 16], altered growth factors [17], cell proliferation [18], altered DNA repair [19], altered DNA methylation patterns [20], inhibition of several key enzymes [21], gene amplification [22] etc. Some of these mechanisms result in alterations in protein expression. The mechanism of arsenic species having a +3 oxidation state biotransformants, methylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) has been claimed to be their reaction with thiols of proteins and conformational alteration of protein structure[13]. It is ultimately the change in protein expression that alters the cellular response. Evaluation of the proteome may lead to a more specific measure and mechanism of arsenic toxicity. Mice exposed to 0.01 % sodium arsenite in drinking water develop hyperplasia of the bladder urothelium within 4 weeks of exposure, as well as increased in DNA binding of the activating protein *(*AP)-1 transcription factor [17]. Although there has been no generally accepted animal model for inorganic arsenic carcinogenesis, the hamster is considered to be an excellent model for how the human process inorganic arsenic

because of its urinary arsenic profile and the relatively low affinity of its red blood cells for arsenic metabolites [12, 23-25].

Methods for analyzing multiple proteins have advanced greatly in the last several years. In particularly, mass spectrometry (MS) and tandem MS (MS/MS) are used to analyze peptides following protein isolation using DIGE and proteolytic digestion [26]. The use of Differential in Gel Electrophoresis (DIGE), an exquisitely sensitive and accurate proteomic technology to study arsenic toxicology now has the potential of obtaining more specific knowledge as to the exact role of specific proteins in arsenic intoxication and detoxification.

In the present study, DIGE coupled with Mass Spectrometry (MS) has been used to study some of the proteomic changes in the urinary bladder of hamsters exposed to sodium arsenite in their drinking water. Our results indicated that transgelin a protein important in tumor progression and a biomarker for cancer development [27-30] was down-regulated and GST-pi was up-regulated in the bladder tissues.

Materials and Methods

Chemicals.

Tris, urea, IPG strips, IPG buffer, CHAPS, Dry Strip Cover Fluid, Bind Silane, lodoacetamide, Cy3 and Cy5 were from GE Healthcare (formally known as Amersham Biosciences, Uppsala, Sweden). Thiourea, glycerol, SDS, DTT and APS were from Sigma-Aldrich (St. Louis, MO, USA). Glycine was from USB (Cleveland, OH, USA). Acrylamide Bis 40% was from Bio-Rad (Hercules, CA, USA). All other chemicals and biochemicals used *w*ere of analytical grade. All solutions were made with Milli-Q water.

Animals.

Male hamsters (Golden Syrian), 4 weeks of age, were purchased from Harlan Sprague Dawley, USA. Upon arrival, hamsters were acclimated in the University of Arizona animal care facility for at least 1 week and maintained in an environmentally controlled animal facility operating on a 12-h dark/12-h light cycle and at 22-24°C. They were provided with Teklad (Indianapolis, IN) 4% Mouse/Rat Diet # 7001 and water, ad libitum, throughout the acclimation and experimentation periods.

Sample preparation and labeling.

Hamsters were exposed to sodium arsenite (173 mg, 57 mg, or 1 mg As/L) in drinking water for 6 days, 21 days, or 10 days, respectively, and the control hamsters were given tap water. On the 6th, 21st, or 10th day hamsters were decapitated rapidly by guillotine. The urinary bladder samples were collected from the first and second experiments in which hamsters were exposed to sodium arsenite (173 mg, 57 mg, or 1 mg A*s*/L*)* in drinking water for 6 days, 21 days, or 10 days, respectively. The urinary bladder samples were also collected from the control hamsters. Urinary bladder tissues were removed, blotted on tissue papers (Kimtech Science, Precision Wipes), and weighed. Hamster urinary bladder tissues were homogenized in lysis buffer (30mm Tris, 2M thiourea, 7M urea, and 4% w/vCHAPS adjusted to pH 8.5 with dilute HCl), at 4°C using a glass homogenizer and a Teflon coated steel pestle; transferred to a 5 ml acid-washed polypropylene tube, placed on ice and sonicated 3 times for 15 seconds. The sonicate was centrifuged at 12,000 rpm for 10 minutes at 4°C. Small aliquots of the supernatants were stored at -80°C until use (generally within one week). Protein concentration was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin as a standard. Fifty micrograms of lysate protein was labeled with 400 pmol of Cy3 Dye (for control homogenate sample) and Cy5 Dye (for arsenic-treated urinary bladder homogenate sample). The samples containing proteins and dyes were incubated for 30 min on ice in the dark. To stop the labeling reaction, 1µL of 10 mM lysine was added followed by incubation for 10 min on ice in the dark. To each of the appropriate dye-labeled protein samples, an additional 200 µg of urinary bladder unlabeled protein from control hamster sample or arsenic treated hamster sample was added to the appropriate sample. Differentially labeled samples were combined into a single Microfuge tube (total protein 500 µg); protein was mixed with an equal volume of 2x sample buffer [2M thiourea, 7M urea, pH 3-10 pharmalyte for isoelectric focusing 2% (v/v*)*, DTT 2% (w/v*),* CHAPS 4% *(*w/v)); and was incubated on ice in the dark for 10 min. The combined samples containing 500 µg of total protein were mixed with rehydration buffer [CHAPS 4% (w/v)*,* 8M urea, 13mM DTT, IPG buffer (3-10) 1% (v/v*)* and trace amount of bromophenol blue]. The 450 µl sample containing rehydration buffer was slowly pipetted into the slot of the Immobiline DryStrip Reswelling Tray and any large bubbles were removed. The IPG strip (linear pH 3-10, 24 cm) was placed (gel side down) into the slot, covered with Drystrip cover fluid (Fig. 1), and the lid of the Reswelling Tray was closed. The Immobilline DryStrip was allowed to rehydrate at room temperature for 24 hours.

Two-dimensional gel electrophoresis.

First dimension Isoelectric focusing (IEF).

The labeled sample was loaded using the cup loading method on universal strip holder. IEF was then carried out on EttanIPGphor II using multistep protocol (6 hr @ 500 V, 6 hr @ 1000 V, 8 hr @ 8000 V). The focused IPG strip was equilibrated in two steps (reduction and alkylation) by equilibrating the strip for 10 min first in 10 ml of 50mM Tris (pH 8.8), 6M urea, 30% (v/v) glycerol, 2% (w/v) SDS, and 0.5% (w/v*)* DTT, followed by another 10 min in 10 ml of 50mM Tris (pH 8.8), 6M urea, 30% (v/v) glycerol, 2% *(*w/v) SDS, and 4.5% *(*w/v*)* iodoacetamide to prepare it for the second dimension electrophoresis.

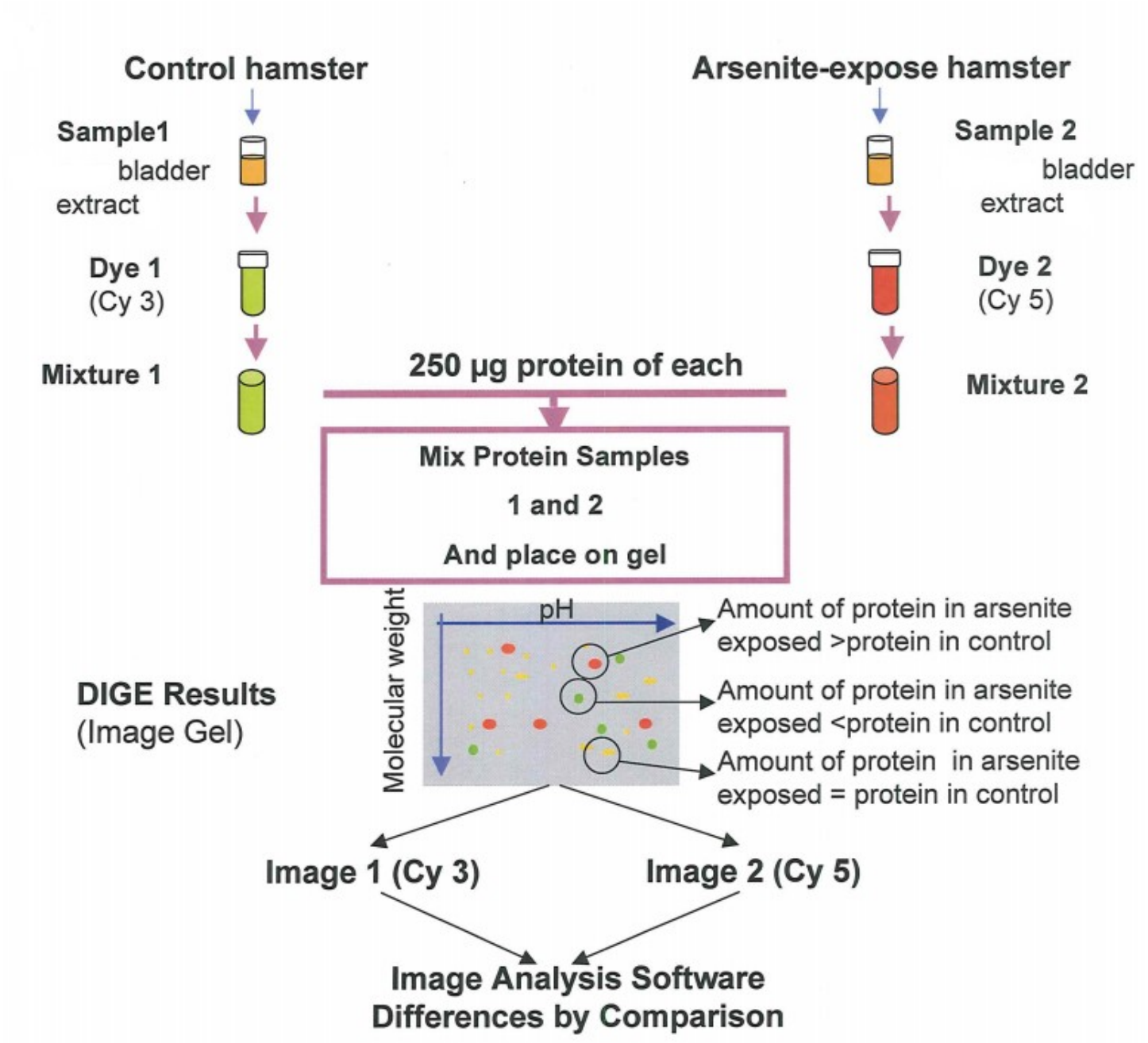

Figure 1. Flow sheet for Differential In-Gel Electrophoresis (DIGE) Experiments [31].

Second dimension SDS-PAGE.

The equilibrated IPG strip was used for protein separation by 2D-gel electrophoresis (DIGE). The strip was sealed at the top of the acrylamide gel for the second dimension (vertical) (12.5% polyacrylamide gel, 20x25 cm x 1.5 mm) with 0.5% (w/*v)* agarose in SDS running buffer [25 mM Tris, 192 mM Glycine, and 0.1% *(*w/v) SDS]. Electrophoresis was performed in an Ettan DALT six-electrophoresis unit (Amersham Biosciences) at 1.5 watts per gel, until the tracking dye reached the anodic end of the gel.

Image analysis and post-staining.

The gel then was imaged directly between glass plates on the Typhoon 9410 variable mode imager (Sunnyvale, CA, USA) using optimal excitation/emission wavelength for each DIGE fluor: Cy3 (532/580 nm) and Cy5 (633/670 nm). To see the proteins expression (green, red, and yellow spots), the DIGE images were previewed and checked with Image Quant software (GE Healthcare) where the two separate gel images could be viewed as a single gel image *(*overlapped). A green, red, or yellow spot indicated that the amount of protein was less, more,

or equal, respectively in the tissue sample of the arsenic-exposed hamster as compared to the control sample DeCyde v.5.02 was used to analyze the DIGE images as described in the Ettan DIGE User Manual (GE Healthcare). The appropriate up-/down-regulated spots were filtered based on an average volume ratio of ± over 1.2 fold. After image acquisition, the gel was fixed overnight in a solution containing 40% ethanol and 10% acetic acid. The fixed gel was stained with SyproRuby (BioRad) according to the manufacturer protocol (Bio-Rad Labs., 2000 Alfred Nobel Drive, Hercules, CA 94547).

Identification of proteins by MS.

Protein spot picking and digestion.

Sypro Ruby stained gels were imaged using an Investigator ProPic and HT Analyzer software, both from Genomic Solutions (Ann Arbor, MI). Protein spots of interest that matched those imaged using the DIGE Cy3/Cy5 labels were picked robotically, digested using trypsin as described previously [26] and saved for mass spectrometry identification.

Liquid chromatography (LC)- MS/MS analysis.

LC-MS/MS analyses were carried out using a 3D quadrupole ion trap mass spectrometer (Thermo Finnigan LCQ DECA XP PLUS; Thermo Finnigan, San Jose, CA) equipped with a Michrom Paradigm MS4 HPLC (Michrom Biosources, Auburn, CA) and a nanospray source, or with a linear quadrupole ion trap mass spectrometer (Thermo Finnigan LTQ), also equipped with a Michrom MS4 HPLC and a nanospray source. Peptides were eluted from a 15 cm pulled tip capillary column (100 µm I.D. x 360 µm O.D.; 3-5 µm tip opening) packed with 7 cm Vydac C18 (Vydac, Hesperia, CA) material (5 µm, 300 Å pore size), using a gradient of 0-65% solvent B (98% methanol/2% water/0.5% formic acid/0.01% triflouroacetic acid) over a 60 min period at a flow rate of 350 nL/min. The ESI positive mode spray voltage was set at 1.6 kV, and the capillary temperature was set at 200°C. Dependent data scanning was performed by the Xcalibur v 1.3 software on the LCQ DECA XP+ or v 1.4 on the LTQ [32], with a default charge of 2, an isolation width of 1.5 amu, an activation amplitude of 35%, activation time of 50 msec, and a minimal signal of 10,000 ion counts (100 ion counts on the LTQ). Global dependent data settings were as follows: reject mass width of 1.5 amu, dynamic exclusion enabled, exclusion mass width of 1.5 amu, repeat count of 1, repeat duration of a min, and exclusion duration of 5 min. Scan event series were included one full scan with mass range of 350-2000 Da, followed by 3 dependent MS/MS scans of the most intense ion.

Database searching.

Tandem MS spectra of peptides were analyzed with Turbo SEQUEST, version 3.1 (Thermo Finnigan), a program that allows the correlation of experimental tandem MS data with theoretical spectra generated from known protein sequences. All spectra were searched against the latest version of the non-redundant protein database from the National Center for Biotechnology Information (NCBI 2006; at that time, the database contained 3,783,042 entries). Sequest DTA/OUT files were cross-validated by Scaffold 2.0 (Proteome Software, Inc.). Positively identified proteins were selected using the following parameters: minimum protein confidence 95%, minimum number of peptides 2, and minimum peptide confidence 95%.

Limitations.

Wepreferred to study the most intense spots seen in DIGE gels. Other spots were visible but not as pronounced. Because of limited funds, we did not identify these other protein spots.

Statistical analysis.

The means and standard error were calculated. The Student's t-test was used to analyze the significance of the difference between the control and arsenite exposed hamsters. P values less than 0.05 were considered significant. The reproducibility was confirmed in separate experiments.

Results

Dose of arsenic in drinking water and physical changes of hamsters

Hamsters were exposed to 0.01 % sodium arsenite (57 mg As/L) in drinking water, observed that the movement of hamsters became slow on day 21. On that day, hamsters were decapitated rapidly. For a new group of hamsters the arsenic concentration was increased three fold, i.e., 0.03 % sodium arsenite (173 mg As/L) in drinking water. The movement of the hamsters became very slow (sometimes jumping occurred and sometimes they kept their head down) on the day 6. The hamsters were decapitated immediately. Hamsters also were exposed to sodium arsenite (1 mg As/L) in drinking water for 10 days to determine the protein expression levels a lower dose of arsenite and for a shorter period of time. The movement of hamsters was normal on the day 10.

Analysis of proteins expression

After DIGE, the gel was scanned by a Typhoon Scanner and the relative amount of protein in the sample of arsenic treated hamster as compared to the sample of control hamster was determined (Fig. 2). A green spot indicated that the amount of protein from sodium arsenite-treated hamster sample was less than that of the control sample (i.e., under-expressed). A red spot indicated that the amount of protein from the sodium arsenite-treated hamster sample was greater than that of the control sample (i.e., over-expressed). A yellow spot indicated that each had the same amount of that protein (i.e., equally expressed).

Several protein spots were up-regulated (red) or down-regulated (green) in the urinary bladder samples of hamsters exposed to sodium arsenite (173 mg As/L) for 6 days as compared with the urinary bladder of controls (Fig. 3A). Most of the protein spots were unchanged (yellow) in the urinary bladder samples of hamsters exposed to sodium arsenite (57 mg As/L or 1 mg As/L) for 21 days or 10 days, respectively, as compared with the urinary bladder control samples (Figs. 3B and C).

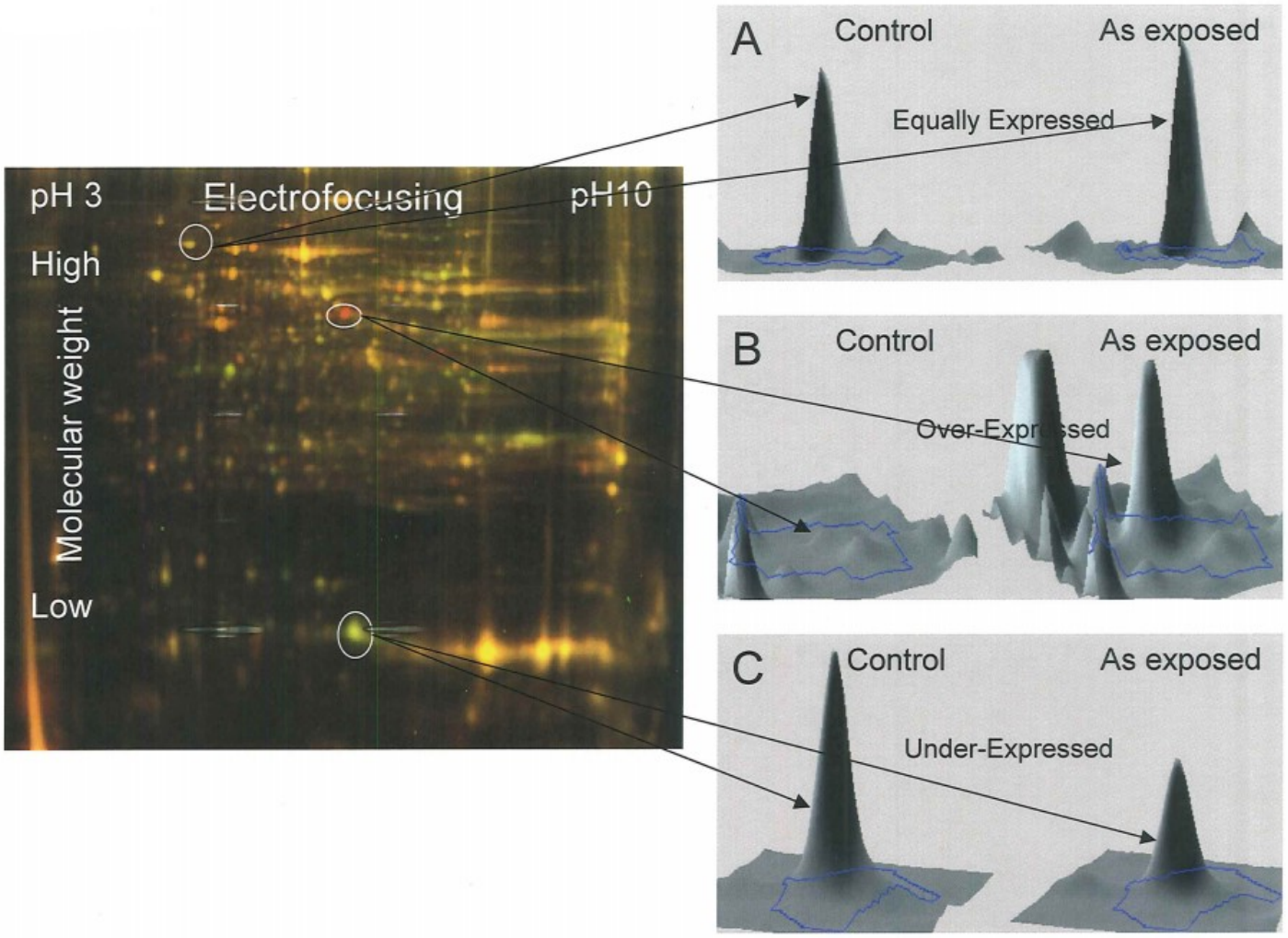

Figure 2. Three-dimensional stimulation of under-, over-, and equally expressed protein spots in the tissue ofhamster exposed to sodium arsenite as compared to the sample of control hamster using DeCyder software [31].

The urinary bladder samples were collected from the first and second experiments in which hamsters were exposed to sodium arsenite (173 mg A*s*/L) in drinking water for 6 days and the controls were given tap water. The urinary bladder samples from the 1st and 2nd experiments were run 5 times in DIGE gels on different days. The volume ratio change of the protein spots in the urinary bladder of hamsters exposed to arsenite were significantly different than that of the control hamsters. The protein expression is shown in Figure 3A and Table 1.

In other experiments hamsters were exposed to sodium arsenite (57 mg A*s*/L or 1 mg As/L) in drinking water for 21 days and 10 days, respectively. The urinary bladder samples were run in DIGE gels. After DIGE analysis most of the protein spots were unchanged (yellow) in the urinary bladder sample of hamster exposed to arsenite, as compared to control sample. The proteins expression are shown in Figures 3B and C & Table 1.

Protein spots identified by LC-MS/MS

The spots of interest were removed from the gel, digested, and their identities determined by LC-MS/MS (Figs. 3A-C & Table 1). Spots 1, 2, & 3 from the gel were analyzed and the procedure repeated for the confirmation of the results (experiments; 173 mg As/L). The proteins for the spots 1, 2, and 3 were identified as transgelin, transgelin, and glutathione S-transferase Pi, respectively (Fig. 3A). Mass numbers of peptides detected by MS, their amino acid sequence as well as their position in the primary sequence for each protein identified, areshown in Tables 2 and 3.

The proteins were identified in spot # 3 as GST-pi (Fig. 3B and Table 1; experiment; 57 mg A*s*/L) and as transgelin for spots 1 & 2 (Fig. 3C and Table 1; experiment; 1 mg As/L). The same protein was identified in the same spot found on different gels after running the samples on DIGE gels from different

experiments (Figs. 3A-C).

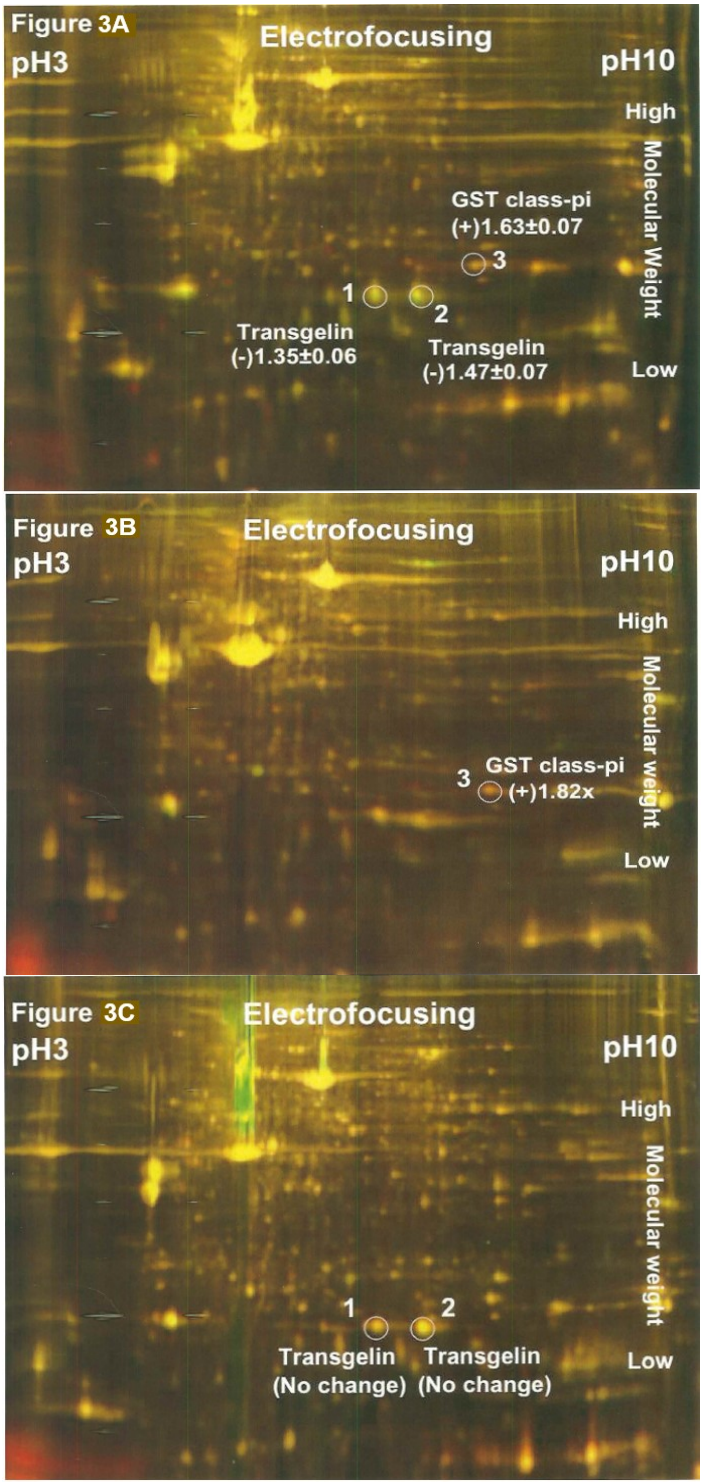

Figure 3. A Typhoon image of a DIGE gel of urinary bladder tissue samples labeledwith CyDye DIGE Fluors. Before adding to the gel, the bladder sample of controlhamster was labeled with Cy3 minimal dye, and the bladder sample of sodium arsenite-treated hamster was labeled with Cy5 minimal dye. The control hamsters were given tap water. [Figs. (3A) 173 mg; (3B) 57 mg; and (3C) 1 mg As/L in the drinking water for 6 days, 21 days and 10 days, respectively]

Table 1. Urinary bladder proteins that are down-regulated, up-regulated, or showed no change in hamsters exposed to sodium arsenite in drinking water. Proteins in the DIGE gels were identified by using LC-MS/MS. Values (fold change) are the mean ± SE; (n=5).

Spot No.	Accession no.[a]	Identification Protein	Mol. Wgt. (kDa)	No.of Peptides	Percent Coverage	Fold Change (n=5)	p value (n=5)
(A) 173 mg As/L in drinking water for 6 days							
1	CAA929	Transgelin	22.58	6	27.9%	(-)1.35±0.06	p<0.001
2	CAA929	Transgelin	22.58	7	28.4%	(-)1.47±0.07	p<0.001
3	Q60550	GST-class pi	23.43	13	57.6%	(+)1.63±0.07	p<0.001
(B) 57 mg As/L in drinking water for 21 days							
3	Q60550	GST-class pi	23.43	13	57.6%	(+)1.82[b]	
(C) 1 mg As/L in drinking water for 10 days							
1	CAA929	Transgelin	22.58	6	27.4%	No change[c]	
2	CAA929	Transgelin	22.58	6	27.4%	No change[c]	

[a]Accession number from NCBI (2006); [b & c] Dose response (n=1)

Table 2. Mass numbers of peptides detected by mass spectrometry in the protein of urinary bladder of hamster exposed to sodium arsenite in drinking water (173 mg As/L).

Spot No.	Identification protein	Peptides detected	Observed Mass (m/z)	Charge	Position in the protein
1	Transgelin	KYDEELEER	605.84	2	21 - 29
		LVEWIVVQCGPDVGRPDR	699.20	3	30 - 47
		LGFQVWLK	495.84	2	50 - 57
		NGVILSK	365.83	2	58 - 64
		LVNSLYPEGSK	603.84	2	65 - 75
		LVNSLYPEGSKPVK	765.91	2	65 - 78
2.	Transgelin	KYDEELEER	605.71	2	21 - 29
		YDEELEER	541.65	2	22 - 29
		LVEWIVMQCGPDVGRPDR	710.36	3	30 - 47
		NGVILSK	366.10	2	58 - 64
		LVNSLYPEGSK	603.65	2	65 - 75
		LVNSLYPEGSKPVK	765.80	2	65 - 78
		AAEDYGVTK	953.26	1	100 - 108
3.	GST class-pi	LLLADQGQSWK	1260.77	1	20 - 30
		LLLADQGQSWKEEVVTGDSWVK	830.35	3	20 - 41
		EEVVTGDSWVK	1248.70	1	31 - 41
		STCLYGQLPK	583.84	2	46 - 55
		FEDGDLILYQSNAILR	934.01	2	56 - 71
		EAALVDMANDGVEDLR	859.42	2	86 - 101
		YVTLIYTK	500.76	2	104 - 111
		YEEGKDDYVK	623.50	2	112 - 121
		ALPGHLKPFETLLSQNQGGK	1068.14	2	122 - 141
		PFETLLSQNQGGK	709.90	2	129 - 141
		AFLSSPDHVNR	621.85	2	192 - 202
		AFLSSPDHVNRPINGNGK	962.01	2	192 - 209
		AFLSSPDHVNRPINGNGKQ	1026.03	2	192 - 210

Table 3. Amino acid sequence (bold) in the protein corresponds to the detected peptide in the urinary bladder tissues of hamsters exposed to sodium arsenite in drinking water (173 mg As/L).

Spot No.	Identification protein	Amino acid sequence in the protein of the detected peptides
Urinary bladder		
1	Transgelin	MANKGPSYGM SREVQSKIEK **KYDEELEERL VEWIVVQCGP DVGRPDRGRL GFQVWLKNGV ILSKLVNSLY PEGSKPVK**VP ENPPSMVFKQ MEQVAQFLKA AEDYGVIKTD MFQTVDLYEG KDMAAVQRTL MALGSLAVTK NDGNYRGDPN WFMKKAQEHK RDFTDSQLQE GKHVIGLQMG SNRGASQAGM TGYGRPRQII S
2	Transgelin	MANKGPSYGM SREVQSKIEK **KYDEELEERL VEWIVMQCGP DVGRPDRGRL** GFQVWLKNGV **ILSKLVNSLY PEGSKPVK**VP ENPPSMVFKQ MEQVAQFLKA **AEDYGVTK**TD MFQTVDLFEG KDMAAVQRTV MALGSLAVTK NDGHYRGDPN WFMKKAQEHK REFTDSQLQE GKHVIGLQMG SNRGASQAGM TGYGRPRQII S
3	GST class-pi	MPPYTIVYFP VRGRCEAMRL **LLADQGQSWK EEVVTGDSWV KGSLKSTCLY GQLPKFEDGD LILVQSNAIL RHLGRSLGLY GKDQKEAALV DMANDGVEDL RCKYVTLIYT KYEEGKDDYV KALPGHLKPF ETLLSQNQGG KAFIVGDQIS FADYNLLDLL LIHQVLAPGC LDNFPLLSAY** VARLSARPKI **KAFLSSPDHV NRPINGNGKQ**

Discussion

The identification and functional assignment of proteins is useful for understanding the molecular events involved in disease. We exposed hamsters to sodium arsenite at different levels in drinking water and tissue was harvested at different time periods.The exquisitely sensitive DIGE procedure coupled with LC-MS/MS was then used to study the proteomic change in arsenite-exposed hamsters. After electrophoresis DeCyder software indicated that several protein spots were down-regulated (green), several were up-regulated (red), and several were unchanged (yellow) (Figs 3A-C).

In the case of the urinary bladder tissue of hamsters exposed to sodium arsenite (173 mg A*s*/L) in drinking water for 6 days, transgelin was down regulated and GST-pi was up-regulated (Fig 3A). These are the first evidence that transgelin is down-regulated and GST-pi is up-regulated in the bladders of animals exposed to sodium arsenite a human carcinogen. The proteins were identified in spot # 3 as GST-pi (Fig. 3B and Table 1) and as transgelin for spots 1 & 2 (Fig. 3C and Table 1) where hamsters were exposed to sodium arsenite (57 mg A*s*/L or 1 mg As/L) in drinking water for 21 days and 10 days, respectively. The same protein was identified in the same spot found on different gels after running the samples on DIGE gels from different experiments (Figs. 3A-C).

Transgelin, which is identical to SM22 or WS3-10, is an actin cross linking/gelling protein found in fibroblasts and smooth muscle [33, 34]. It has been suggested that the loss of transgelin expression may be an important early event in tumor progression and a diagnostic marker for cancer development [27-30]. It may function as a tumor suppressor via inhibition of ARA54 (co-regulator of androgen receptor)-enhanced AR (androgen receptor) function. It has been suggested that loss of transgelin and its suppressor function in prostate cancer might contribute to the progression of prostate cancer [29]. Down-regulation of transgelin has been noted in the urinary bladders of rats having bladder outlet obstruction [27]. Ras-dependent and Ras-independent mechanisms can cause the down regulation of transgelin in human breast and colon carcinoma cell lines and patient-derived tumor samples [28].

We observed that when hamsters were exposed to sodium arsenite (173 mg A*s*/L) their movements were very slow (sometimes they jumped excessively and sometimes they kept their head down) on the 6th day. Zeiden et al. [35] suggested that transgelin (SM22∞) might play a role in contractility, possibly by affecting actin filament organization. Decreased actin expression might be a factor behind the decreased contractility [35, 36]. A change in the aggregation pattern of actin filaments in intestinal and vascular smooth muscle has been found in SM22∞ $^{-/-}$ mice. A possible explanation for the decreased contractility is altered structure/function of the thin filament system [35, 36]. In our experiments, arsenic-exposed caused loss of transgelin expression. This may be associated with or preliminary to bladder cancer. Arsenic is a human carcinogen [1]. LC-MS/*M*S analysis showed that two spots (1 and 2) represent transgelin (Figs. 3A &C and Table 1). In human colonic neoplasms there is a loss of transgelin expression and the appearance of transgelin isoforms [30].

GST-pi protein was up-regulated in the bladders of the hamsters exposed to sodium arsenite. GSTs are a large family of multifunctional enzymes involved in the phase II detoxification of foreign compounds [37]. The most abundant GSTs are the alpha, mu, and pi classes [38]. They participate in protection against oxidative stress [39]. GST omega has arsenic reductase activity [40]. Over-expression of GST-pi has been found in colon cancer tissues [41]. Strong expression of GST-pi also has been found in gastric cancer [42], malignant melanoma [43], lung cancer [44], breast cancer [45] and a range of other human tumors [46]. GST-pi has been up-regulated in transitional cell carcinoma of human urinary bladder [47]. Up regulation of glutathione - related genes and enzyme activities have been found in cultured human cells exposed to sub-lethal concentrations of inorganic arsenic [48]. There is evidence that arsenic induces DNA damage via the production of reactive oxygen species (ROS) [49]. GST-pi may be over expressed in the urinary bladder to protect cells against arsenic-induced oxidative stress.

In summary, transgeline was down-regulated and GST-pi was up-regulated in the urinary bladder tissue of hamsters exposed to Na-As(III) (173 mg/L) in drinking water for 6 days, the movement of the hamsters became very slow on day 6. In an another experiment, GST-pi protein was up-regulated in the urinary bladder tissue of hamsters exposed to Na-As(III) (57 mg/L) in drinking water for 21 days, the movement of the hamsters became slow on day 21. There was no volume ratio change of the protein spots (transgeline) in the urinary bladder of hamsters exposed to arsenite (1 mg/L) in drinking water for 10 days than that of the control hamsters, the movement of hamsters were normal on day 10. This is the evidence (in vivo) that cytotoxicities of inorg-As depend on does and duration of exposure time. These proteomic results may be of help to investigators studying arsenic carcinogenicity and toxicity.

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The Author wants to dedicate this paper to the memory of his former supervisor Dr. H. Vasken Aposhian who passed away in September 6, 2019. He was an emeritus professor of the Department of Molecular and Cellular Biology at the University of Arizona. This research work was done under his sole supervision and with his great contribution. Author also would like to thanks Dr. George Tsapraills, Center of Toxicology, The University of Arizona for identification of proteins by MS.

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