Application of cDNA Microarray to the Study of Arsenic-Induced Liver Diseases in the Population of Guizhou, China

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Arsenic is an environmental toxicant and a human carcinogen. Epidemiology studies link human arsenic exposure to various diseases and cancers, including liver diseases and hepatocellular carcinoma. However, the molecular mechanisms for arsenic toxicity and carcinogenicity are poorly understood. To better understand these mechanisms, we used the human cancer cDNA expression array to profile aberrant gene expression in arsenic-exposed populations in Guizhou, China. The selected patients had a history of exposure to environmental arsenic for at least 6–10 years, and had arsenic-induced skin lesions and hepatomegaly. Samples were obtained by liver needle biopsy. Histology showed degenerative liver lesions, such as chronic inflammation, vacuolation, and focal necrosis. The University of North Carolina Hospitals provided normal human liver tissues from surgical resection or rejected transplants. Microarray was performed with total RNA from liver samples, and signal intensities were analyzed with AtlasImage software and normalized with 9 housekeeping genes. Means and SEM were calculated for statistical analysis. Approximately 60 genes (10%) were differentially expressed in arsenic-exposed human livers compared to controls. The differentially expressed genes included those involved in cell-cycle regulation, apoptosis, DNA damage response, and intermediate filaments. The observed gene alterations appear to be reflective of hepatic degenerative lesions seen in the arsenic-exposed patients. This array analysis revealed important patterns of aberrant gene expression occurring with arsenic exposure in human livers. Aberrant expressions of several genes were consistent with the results of array analysis of chronic arsenic-exposed mouse livers and chronic arsenic-transformed rat liver cells. Clearly, a variety of gene expression changes may play an integral role in arsenic hepatotoxicity and possibly carcinogenesis.

Key Words: arsenic; chronic exposure; liver degeneration; liver biopsy; aberrant gene expression; cDNA microarray.

Arsenic is a metalloid that occurs naturally in soil, water, and air. Arsenicals are also by-products of production of various metals, as well as coal consumption (ATSDR, 1999; National Research Council, 1999). A major concern for environmental arsenic exposure and human health is its carcinogenic potential in multiple organs. Arsenic is a human carcinogen, causing cancers of the skin, lung, urinary bladder, and liver (Abernathy et al., 1999; National Research Council, 1999; Goering et al., 1999). However, the molecular mechanisms of arsenic toxicity and carcinogenesis are only poorly understood.

The liver is a major target organ of inorganic arsenic toxicity. In rats given oral arsenate (20–60 ppm) for up to 6 weeks, swollen mitochondria and altered liver function were evident (Fowler et al., 1977). Hepatic fatty infiltration and degenerative lesions (such as vacuolation) became evident after 12 months of oral exposure of mice to a mixture of arsenite and arsenate, which can progress to liver fibrosis by 15 months (Santra et al., 1997). In mice chronically exposed to arsenite or arsenate via drinking water for 48 weeks, liver parenchymal cell degeneration and fatty infiltration are widespread, with inflammation, focal necrosis and early proliferative lesions (Liu et al., 2000). Hepatocellular proliferative lesions (adenoma and foci of cellular alteration) were increased by repeated arsenate injections in female Swiss mice in a recent 2-year study (Waalkes et al., 2000b). Additionally, chronic exposure to arsenite in vitro can cause malignant transformation of rodent liver cells (Zhao et al., 1997). Thus, it is clear that chronic arsenic exposure produces liver toxicity and, possibly, carcinogenicity in rodents, which is consistent with its hepatic effects in humans (National Research Council, 1999).

Arsenic-induced liver toxicity in humans is, in fact, a common phenomenon. Liver injury induced by arsenic exposure, either from the long-term use of arsenic-containing Fowler’s solution in the treatment of psoriasis (Morris et al., 1974; Nevens et al., 1990), or from arsenic exposure through contaminated drinking water (Mazumder et al., 1998), food, or air (Liu et al., 1992; Zhou et al., 1993, 1994), typically manifests initially as degenerative lesions with jaundice, and often progresses to noncirrhotic portal hypertension, fibrosis, and cirrhosis. In addition, liver neoplasia, including hepatocellular carcinoma, occurs in humans (ATSDR, 1999; National Research Council, 1999).

An area of endemic arsenic toxicity due to environmental exposure has recently been reported in Guizhou, China (Liu et al., 1992; Finkelman et al., 1999; Zhang et al., 2000; Zhou et
et al., 1998). This region is located on a high, mountainous plateau in the southwestern part of Guizhou province, where the local coal has extraordinarily high concentrations of arsenic (100–9000 ppm). Normally, coal arsenic content is less than 10 ppm (Finkelman et al., 1999; Zheng et al., 1999; Zhou et al., 1993). Coal became the main source of energy for domestic cooking and heating since the 1960s when the local natural forest was depleted. The residents frequently bring foodstuffs indoors and place them above their coal-burning stoves to dry. Coal is burned daily inside the home for cooking and crop drying over non-vented ovens. As a result, the indoor air arsenic concentrations are 5–100 times higher than China’s Air Quality Permission Standard (3 μg As/m³ vs. 400 μg As/m³) (Zhou et al., 1993, 1998). Arsenic in the air coasts and permeates the food being dried and becomes highly concentrated. Although arsenic concentrations in fresh food are in the normal range, in corn dried in a coal-burning stove, arsenic is 5–15 times higher than normal, and in dried chili (a favorite local food flavoring ingredient) arsenic levels are 100–500 times higher than normal (An et al., 1996; Finkelman et al., 1999; Zhou et al., 1993). The geological localization of high-arsenic coal varies among different villages, and a clear dose-response relationship exists between local arsenic content in coal, arsenic content in major food items (corn and chili), and arsenic concentration in the urine (Zhou et al., 1993), which is a reasonable surrogate for exposure.

Liver toxicity and hepatocellular carcinoma are major health concerns in this area of endemic arsenic poisoning (Guizhou). The incidence of liver toxicity is very high in people from Guizhou compared to that in other areas of China where arsenic poisoning commonly occurs from arsenic-contaminated drinking water, as in Xinjiang, China (Wang et al., 1993), or in Inner Mongolia, China (Zhou et al., 1994), as well as in West Bengal, India (Mazumder et al., 1998). Hepatomegaly is the major sign of arsenic-induced liver injury. Serum enzymes (ALT, AST) may be increased or unchanged. Liver toxicity is accompanied by chronic indigestion and abdominal pain, and can progress to liver fibrosis. Cirrhosis, ascites, and hepatocellular carcinoma are the most serious outcome of chronic arsenic-induced hepatotoxicity, and they can often be the cause of death. According to recent reports, approximately 70–85% of the mortality from arsenic intoxication resulted from cirrhosis and ascites (An et al., 1996; Liu et al., 1992; Zhou et al., 1993, 1998). However, the aberrant gene expression pattern associated with arsenic-induced liver toxicity and carcinogenesis are not known.

We have recently shown that chronic in vitro exposure (≥ 18 weeks) of rat liver epithelial cells to very low concentrations of sodium arsenite (125 to 500 μM), concentrations relevant to environmental arsenic contamination levels, resulted in malignant cellular transformation (Zhao et al., 1997). In fact, these transformed cells could produce tumors capable of metastasis upon inoculation into Nude mice (Zhao et al., 1997). The gene array analysis of these arsenic-transformed cells revealed several important patterns of aberrant gene expression, including altered expression of oncogenes, cell-cycle regulators, signal transduction modulators, stress- and apoptosis-related genes, cytokines, growth factors, and hormone receptors (Chen et al., 2001). The microarray analysis of chronic arsenic-exposed mouse liver also revealed remarkable aberrant expression of a variety of genes associated with arsenic-induced hepatotoxicity and liver pre-neoplastic lesions (unpublished data). Thus, the present study was designed to examine whether a similar pattern of aberrant gene expression also occurs in arsenic-exposed human livers, by using liver biopsy samples obtained from arsenic-exposed population in Guizhou, China. This initial gene array study could be important in identifying the aberrant gene expression associated with arsenic toxicity and carcinogenicity, which, in turn, could be critical as the basis of future studies on the molecular mechanisms of arsenic toxicity and carcinogenicity.

**MATERIALS AND METHODS**

**Materials and chemicals.** Patients from an area of endemic arsenic exposure in Guizhou province were selected for this study based on their history of arsenic-exposure, skin lesions (keratosis, hyperpigmentation, etc.), and gastrointestinal symptoms indicative of arsenic poisoning. These patients did not have a history of hepatitis, and serology for HBV and HCV was negative. They were selected to receive a regimen of traditional Chinese-medicine therapy for arsenic-induced liver diseases under the Human Subject Study Protocol approved by the Chinese Ministry of Public Health. To evaluate the efficacy of the therapy, liver biopsy was performed both pre- and posttreatment with traditional Chinese medicine in local hospitals by certified physicians. Only pre-treatment biopsies were used in the present study; a total of 20 were performed, and a random selection of 6 samples without identifiers to the present authors were used for RNA extraction. Six normal human liver tissues from rejected transplant donors or surgical resections were obtained from the University of North Carolina Hospitals and used as controls. The average age of arsenic-exposed subjects was 40 years old, while for controls it was 52. Both males and females were included. The cDNA expression arrays and AtlasImage software were purchased from Clontech (Palo Alto, CA). [α-32P]dATP was obtained from Amersham (Piscataway, NJ).

**Histopathology.** A portion of each arsenic-exposed human liver biopsy sample was fixed in 10% neutral formalin, processed by standard histological techniques, and stained with hematoxylin and eosin (H&E) by the Pathology Department of Guiyang Medical College. The images were analyzed with a Zeiss microscope connected to a charge-coupled device camera (DAGE-MTI, Michigan City, IN) and analyzed with the MetaMorph Image System (Universal Image Co., West Chester, PA).

**Microarray analysis.** Total RNA was extracted with Trizol reagent in China (biopies) and at the laboratory of Dr. Judy Munford, U.S. EPA (controls), followed by RNase-free, DNase-I digestion (2 U/100 μg RNA). Microarray analysis was performed according to manufacturer’s instructions. Briefly, 2 to 5 μg of total RNA was converted to 32P-labelled cDNA probes using MMLV reverse transcriptase and [α-32P]dATP with the Clontech Atlas human cancer CDS primer mix (Clontech). The 32P-labelled cDNA probe was purified using chroma spin-200 columns, denatured in 1.0 M NaOH and 10 mM EDTA at 68°C for 20 min, followed by neutralization with an equal volume of 1.0 M NaH2PO4, for another 10 min. The membranes were prehybridized with Ultrahyb (Ambion, Austin, TX) for 30–60 min at 42°C, followed by hybridization overnight at 42°C. The arrays were washed 3 times in 2X SSC/0.1% SDS, 5–10 min each, and twice in 0.1X SSC/0.1% SDS for 15–30 min. The membranes were then sealed in plastic bags and subjected to exposure in phosphorimage screens or X-ray film. The arrays were analyzed densitometrically using AtlasImage software (version 1.5).
RESULTS AND DISCUSSION

The selected patients had a history of exposure to environmental arsenic for at least 6–10 years, and all had prominent skin lesions, such as hyperpigmentation and hyperkeratosis, indicative of arsenic poisoning according to established criteria (Wang et al., 1993, Zhou et al., 1998). Representative microphotographs from the liver biopsy samples are shown in Figure 1. Hepatocellular vacuolation and balloon-like degenerative lesions were observed in every liver biopsy from the arsenic-exposed patients. Several samples had foci of inflammation, protein cast filtration, foci of necrosis, and foci of collagen fibrosis in the periportal areas. These patients did not have a history of hepatitis, and serology for HBV and HCV was consistently negative. Thus, the liver lesions were in all likelihood caused by chronic arsenic exposure. Indeed, the observed widespread liver degenerative lesions were quite similar to those produced by chronic arsenic exposure in the mouse (Liu et al., 2000; Waalkes et al., 2000b), where liver cell vacuolation and fatty infiltration occur along with preneoplastic proliferative lesions and chronic inflammation.

Representative Atlas human cancer cDNA microarray images are shown in Figure 2. Each gene on the array is duplicated, and the second line from the bottom of the membrane contains nine housekeeping genes. The gene spots on the right side and on the bottom are “genomic DNA spots,” which serve as orientation marks. The top panel is an image from a normal liver, and the bottom panel is an image from an arsenic-exposed liver. In arsenic-exposed human livers, the increase in E2F5, and the down-regulation of the endothelial plasminogen activator inhibitor 1 (PAI-1) can be readily visualized.

The microarray was performed with 6 individual arsenic-exposed human liver biopsy samples and 6 individual normal human livers. The AtlasImage software was used to quantify the hybrid intensity of each gene. The hybrid intensity of each gene was then normalized with the sum of all the 9 housekeeping genes on the arrays (see Methods). Means and SEM from 6 arsenic-exposed livers and 6 normal livers were calculated for statistical analysis for the data presented in Figures 3–6.

The alterations in expression of genes encoding for cell-cycle regulators are shown in Figure 3. The expression of E2F3, E2F5, and E2F dimerization-partner 2 were increased in arsenic-exposed human livers. The E2F family of transcription factors plays an important role in cell-cycle regulation, oncogenesis, and differentiation (Black and Azizkhan-Clifford, 1999; Muller and Helin, 2000). Chronic exposure of cells to arsenite has been shown to increase the expression of E2F-1 and the c-myc oncogene, resulting in enhanced cell proliferation (Trouba et al., 2000). Similarly, in chronic arsenite-transformed rat liver TRL1215 cells, dramatic increases in c-myc, proliferating cellular antigen (PCNA), and cyclin D1 were accompanied by a marked enhancement of cell proliferation (Chen et al., 2001). In intact animals chronically treated with arsenite or arsenenate, proliferative, preneoplastic lesions in the liver were also observed (Liu et al., 2000; Waalkes et al., 2000b), and continuous cell cycle disruption may well have resulted in hyperproliferation. Likewise, in arsenic-induced preneoplastic uterine hyperplasias in mice, PCNA is highly over-expressed (Waalkes et al., 2000b). The transcription factor E2F plays an important role in the G(1) to S-phase transition of the cell cycle, and is dependent on its association with cyclin A/ckd2, as well as induction of cyclin E, cyclin G1, and p19ink4 (Arata et al., 2000; Kato, 1999). In the present study, cyclin A, cyclin E, cyclin G1, and p19-ink4 were all increased in arsenic-exposed human livers, consistent with the hypothesis of cell-cycle dysregulation. However, in contrast to marked induction of cyclin D1 (CDKN1A) in chronic arsenic-transformed cells (Chen et al., 2001), CDKN1A and cyclin-dependent kinase 4 (CDK4) were decreased in arsenic-exposed human livers. Perhaps the overexpression of cyclin D1 and CDK4 is mainly associated with overt carcinogenic events (Johnson and Walker, 1999), as would occur in arsenic-induced malignant cellular transformation (Chen et al., 2001; Zhao et al., 1997). Additionally, E2F-mediated S-phase transition is not dependent on CDKN1A and CDK4 (Arata et al., 2000). Overall, the present results in human livers are consistent with the concept that cell-cycle dysregulation may well be an important element of arsenic-induced preneoplasia and carcinogenesis (Chen et al., 2001; Trouba et al., 2000; Waalkes et al., 2000a,b).

The alterations in apoptosis-related genes in arsenic-exposed human livers are shown in Figure 4. In contrast to induction of pro-apoptotic genes and initiation of apoptosis following acute arsenic treatment of leukemia cells (Akao et al., 2000; Huang et al., 1999), the expression of apoptosis-related genes during chronic arsenic exposure was quite different: the expression of caspase 3 and caspase 8, which are dramatically increased during acute arsenic treatment (Akao et al., 2000), is basically unchanged in chronic arsenic-exposed human livers. Furthermore, caspase 4 and caspase 6 expression were suppressed while caspase 9 and caspase 10 were increased in human livers from arsenic-exposed populations. Interestingly, several tumor necrosis factor (TNF)-associated components, such as TNF-converting enzyme, and TNF-receptor 1 (TNFR1) were also suppressed. TNF-related Fas ligand (FasL) and TNFR1-associated death domain protein, TRADD, were reduced, but the TNF-related apoptosis-inducing ligand TRAIL, was not decreased. Overall, it appears that during chronic arsenic exposure cellular apoptosis machinery may be blunted. Indeed, in rodent cells transformed by chronic arsenic-exposure (Zhao et al., 2000). The alterations in expression of genes encoding for cell-cycle regulators are shown in Figure 3. The expression of E2F3, E2F5, and E2F dimerization-partner 2 were increased in arsenic-exposed human livers. The E2F family of transcription factors plays an important role in cell-cycle regulation, oncogenesis, and differentiation (Black and Azizkhan-Clifford, 1999; Muller and Helin, 2000). Chronic exposure of cells to arsenite has been shown to increase the expression of E2F-1 and the c-myc oncogene, resulting in enhanced cell proliferation (Trouba et al., 2000). Similarly, in chronic arsenite-transformed rat liver TRL1215 cells, dramatic increases in c-myc, proliferating cellular antigen (PCNA), and cyclin D1 were accompanied by a marked enhancement of cell proliferation (Chen et al., 2001). In intact animals chronically treated with arsenite or arsenenate, proliferative, preneoplastic lesions in the liver were also observed (Liu et al., 2000; Waalkes et al., 2000b), and continuous cell cycle disruption may well have resulted in hyperproliferation. Likewise, in arsenic-induced preneoplastic uterine hyperplasias in mice, PCNA is highly over-expressed (Waalkes et al., 2000b). The transcription factor E2F plays an important role in the G(1) to S-phase transition of the cell cycle, and is dependent on its association with cyclin A/ckd2, as well as induction of cyclin E, cyclin G1, and p19ink4 (Arata et al., 2000; Kato, 1999). In the present study, cyclin A, cyclin E, cyclin G1, and p19-ink4 were all increased in arsenic-exposed human livers, consistent with the hypothesis of cell-cycle dysregulation. However, in contrast to marked induction of cyclin D1 (CDKN1A) in chronic arsenic-transformed cells (Chen et al., 2001), CDKN1A and cyclin-dependent kinase 4 (CDK4) were decreased in arsenic-exposed human livers. Perhaps the overexpression of cyclin D1 and CDK4 is mainly associated with overt carcinogenic events (Johnson and Walker, 1999), as would occur in arsenic-induced malignant cellular transformation (Chen et al., 2001; Zhao et al., 1997). Additionally, E2F-mediated S-phase transition is not dependent on CDKN1A and CDK4 (Arata et al., 2000). Overall, the present results in human livers are consistent with the concept that cell-cycle dysregulation may well be an important element of arsenic-induced preneoplasia and carcinogenesis (Chen et al., 2001; Trouba et al., 2000; Waalkes et al., 2000a,b).

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al., 1997), the ability of arsenic to induce apoptosis is markedly perturbed (Qu et al., 2000). Immunocytochemical analysis (TUNEL assay) of these liver biopsy samples did not show an

FIG. 1. Representative photomicrographs of hepatic lesions in arsenic-exposed patients. (A) Patient #3: Big arrow indicates focal necrosis, small arrowhead indicates hepatocellular balloon-like degenerative lesions and small arrow indicates apoptosis. (H&E, magnification ×174). (B) Patient #6: Small arrow indicates hepatocellular degenerative lesions. Big arrow indicates inflammatory cell infiltration in the periportal area. (H&E, magnification ×175). (C) Patient #5: Big arrow shows the protein casts in the sinusoid and in the hepatocytes, and small arrow shows the cell vacuolation. (H&E, magnification ×87).

FIG. 2. Representative cDNA microarray phosphoimages. Top panel, normal human liver; bottom panel, arsenic-exposed human liver. Note that the upregulation of the E2F5 gene and the down-regulation of the plasminogen activator inhibitor (PAI)-1 gene in the arsenic-exposed human liver were substantial enough to visualize, as compared to the normal human liver tissues.

FIG. 3. Expression of several cell-cycle regulator genes in arsenic-exposed human biopsy samples and normal human liver tissues. Data are means ± SEM (n = 6). *Significantly different from normal human liver tissues, p < 0.05.
increase in apoptic cells (unpublished data). This apoptotic resistance may be important in both early (initiation) and later (promotion/progression) stages of carcinogenesis.

The most dramatic change in apoptosis-related gene expression was the down-regulation of the myeloid leukemia cell differentiation protein MCL-1 (Fig. 4). MCL-1 is a Bcl-2 family protein, and functions as an anti-apoptotic element (McDonnell et al., 1996). In primary biliary cirrhosis, the expression of MCL-1 was markedly reduced (Iwata et al., 2000), and this likely decreases the threshold for apoptosis in the damaged bile duct. In the present study, a 65% suppression of MCL-1 may have clinical implications in arsenic-exposed human livers, because the down-regulation of MCL-1 may be associated with liver/biliary cirrhosis, a major health concern in this arsenic-exposed population (Liu et al., 1992; Zhou et al., 1998). Taken together, the dysregulation of various apoptosis-related genes is complex, but it may well have an integral role in arsenic-induced liver diseases and carcinogenesis.

The alterations in expression of stress- and DNA damage-related genes are shown in Figure 5. Arsenic is known to produce DNA damage and related events, such as DNA-protein crosslinks, micronuclei (Schaumloffel and Gebel, 1998), DNA strand breaks (Liu and Jan, 2000; Lynn et al., 1998), or alterations in DNA repair enzymes (Hartwig, 1998). Superoxide scavengers such as Cu,Zn-SOD suppress arsenic-induced DNA damage (Hartwig, 1998; Liu and Jan, 2000; Lynn et al., 1998). In the present study, the expression of the Cu,Zn-SOD gene was increased by 50% in the livers of arsenic-exposed patients, consistent with the observation of strong immunocytochemical staining for Cu,Zn-SOD in skin samples from the same arsenic-exposed population of Guizhou (Zhang et al., 2000). Indeed, in chronic arsenic-transformed rat liver cells, Cu,Zn-SOD is also significantly increased (Chen et al., 2001). The nucleotide excision repair genes such as ERCC2, ERCC5, and RAD23A were increased, suggesting the activation of DNA-repair machinery in response to arsenic-induced DNA damage. Human topoisomerase II (TOP2A), an essential nuclear enzyme involved in DNA replication (Lang et al., 1998), was also upregulated in arsenic-exposed human livers in the present study. In contrast to acute arsenic exposure in mice, the growth arrest and DNA damage-inducible proteins GADD45 and GADD153 were not increased in chronic arsenic-exposed human livers. In arsenic-exposed human livers, RFC38 and RFC40 were increased. Replication factor C (RFC) is a complex of 5 subunits and an essential eukaryotic protein involved in both DNA replication and repair (Podust and Fanning, 1997). RFC is closely associated with PCNA functioning in DNA replication. The enhanced expression of RFcs and PCNA could induce hyperproliferation and DNA replication in an attempt to respond to arsenic-induced DNA damage.

The alterations in other cellular regulators and growth factors in arsenic-exposed human livers are shown in Figure 6. It has been reported that the expression of cytokeratins are altered in arsenite-treated human liver cells (Ramirez et al., 2000), arsenate-treated human keratinocytes (Kachinskas et al., 1997), and arsenic-induced skin lesions in arsenic-exposed population in Taiwan, China (Yu et al., 1993). Cytokeratins constitute a diverse group of intermediate filament proteins expressed as pairs in keratinized and nonkeratinizing epithelial cells. Recently, aberrant expression of cytokeratins in liver diseases and hepatocellular carcinomas has been reported (Omary and Ku, 1997). The pair of cytokeratin-8 and cytokeratin-18 is known to be liver-specific. In the present study, the expressions of cytokeratin-8 and cytokeratin-18 were suppressed, while the expression of cytokeratin-1 was increased.
The exact role for aberrant expression of these cytokeratins is not immediately clear, but could be related to liver degenerative lesions and fibrosis (Omary and Ku, 1997).

Endothelin-2 and endothelin-3 were overexpressed in arsenic-exposed human livers (Fig. 6). Endothelins are potent vasoconstrictors and play an important role in increasing intrahepatic vascular resistance, which in turn contributes to portal hypertension in cirrhosis (Petrowsky et al., 1999). Indeed, in Canadian and Chinese populations, higher expression of endothelin-1 and endothelin-3 was found to be associated with cirrhosis and liver fibrolamellar carcinomas (Cai et al., 1999). In addition, vascular endothelial growth factor was also increased. Overexpression of endothelins and vascular endothelial growth factor B (VEGF-B) was also seen in arsenic-transformed rat liver epithelial cells (Chen et al., 2001), and could have an integral role in arsenic-induced portal hypertension and cirrhosis, a major health concern in areas of endemic arsenic poisoning. The type-1 plasminogen-activator inhibitor (PAI-1) is a serine protease inhibitor, which inactivates urokinase-type plasminogen activator (uPA) and regulates degradation of the extracellular matrix. In arsenic-exposed human livers, expression of endothelial PAI-1 was dramatically suppressed (Fig. 6), a result similar to the observation seen in chronic arsenic-treated mouse livers (unpublished data). The exact role for PAI-1 in hepatocellular tumor progression and fibronolysis has been the subject of controversy. However, lower PAI-1 levels are found in human alcohol-cirrhotic livers (Aleman-Valls et al., 2000), and an inhibitory role for PAI-1 towards invasion and proliferation of hepatocellular carcinoma cells has been proposed (Morita et al., 1999). Thus, down-regulation of PAI-1 in arsenic-exposed patients could have implications in arsenic toxicity and carcinogenesis.

Consistent with possible increased cell proliferation in arsenic-exposed human livers, the hepatocyte growth factor (HGF), interleukin-6 receptor, and interleukin-1β were all increased (Fig. 6). These growth factors have been shown to be important in arsenic-induced enhancement of skin neoplasia through continuous chronic stimulation (Germolec et al., 1998). Thus, the upregulation of these positive hepatocellular proliferation regulators could be envisioned as cellular adaptive mechanism in response to arsenic-induced hepatocellular degenerative lesions, and may have an integral role with the upregulation of the E2F family (Fig. 3) in arsenic-induced proliferative lesions and overt hepatomegaly in this population.

In summary, the present study has utilized Atlas Human Cancer cDNA expression arrays to analyze the aberrant gene expression associated with arsenic-induced liver disease in humans. By using 6 arsenic-exposed human liver biopsy samples and 6 normal human liver tissues, we are able to calculate means and SEMs for statistical analysis. It should be kept in mind that the control population used in the present study does not precisely match the arsenic-exposed population. This is a frequent limitation of such studies, but the gene expression changes seen in this study are consistent among the group and generally substantial enough that minor differences in the age or genetic make-up of the subject populations probably cannot account for the changes. Most importantly, the alterations in expression of genes encoding for cell-cycle regulation and proliferation, the apoptosis-related genes, the DNA damage-related genes, and the genes encoding for cellular regulators and growth factors in arsenic-exposed human livers are largely in agreement with the results from a variety of nonhuman in vivo and in vitro systems (Liu et al., 2000; Parrish et al., 1999; Qu et al., 2000; Ramirez et al., 2000; Schaumloffel and Gebel, 1998; Trouba et al., 2000; Waalkes et al., 2000a,b; Zhang et al., 2000; Chen et al., 2001). This fortifies the concept that arsenic is a causative factor in the alterations in gene expression patterns seen in the present study. Finally, although this study is observational in nature, the initial gene array analysis has provided a relatively comprehensive picture of the aberrant gene expressions associated with arsenic-induced hepatotoxicity in a population living in Guizhou, China. This should then allow us to ask a multitude of hypothesis-based questions about the mechanism(s) of arsenic toxicity and carcinogenesis. This initial gene array work may well also help to formulate strategies for the treatment and the prevention of arsenic-induced liver injury, and, thereby, to protect human health.

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