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Transplacental exposure to inorganic arsenic at a hepatocarcinogenic dose induces fetal gene expression changes in mice indicative of aberrant estrogen signaling and disrupted steroid metabolism

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Abstract

Exposure to inorganic arsenic *in utero* in C3H mice produces hepatocellular carcinoma in male offspring when they reach adulthood. To help define the molecular events associated with the fetal onset of arsenic hepatocarcinogenesis, pregnant C3H mice were given drinking water containing 0 (control) or 85 ppm arsenic from day 8 to 18 of gestation. At the end of the arsenic exposure period, male fetal livers were removed and RNA isolated for microarray analysis using 22K oligo chips. Arsenic exposure *in utero* produced significant ($p < 0.001$) alterations in expression of 187 genes, with approximately 25% of aberrantly expressed genes related to either estrogen signaling or steroid metabolism. Real-time RT-PCR on selected genes confirmed these changes. Various genes controlled by estrogen, including X-inactive-specific transcript, anterior gradient-2, trefoil factor-1, CRP-ductin, ghrelin, and small proline-rich protein-2A, were dramatically over-expressed. Estrogen-regulated genes including cytokeratin 1–19 and Cyp2a4 were over-expressed, although Cyp3a25 was suppressed. Several genes involved with steroid metabolism also showed remarkable expression changes, including increased expression of 17β-hydroxysteroid dehydrogenase-7 (HSD17β7; involved in estradiol production) and decreased expression of HSD17β5 (involved in testosterone production). The expression of key genes important in methionine metabolism, such as methionine adenosyltransferase-1a, betaine-homocysteine methyltransferase and thioether S-methyltransferase, were suppressed. Thus, exposure of mouse fetus to inorganic arsenic during a critical period in development significantly alters the expression of various genes encoding estrogen signaling and steroid or methionine metabolism. These alterations could disrupt genetic programming at the very early life-stage, which could impact tumor formation much later in adulthood.

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Keywords

Arsenic; in utero exposure; fetal liver; gene expression; estrogen signaling; steroid metabolism

INTRODUCTION

Inorganic arsenic is a human carcinogen, associated with tumors of the skin, urinary bladder, lung, liver, prostate, kidney, and possibly other sites (NRC, 2001; Morales et al., 2000; Centeno et al., 2002; IARC, 2004). We have shown that short-term exposure in mice to inorganic arsenic *in utero* produces a variety of internal tumors in the offspring when they reach adulthood (Waalkes et al., 2003; 2004a; 2006a, 2006b). Gestation is a period of high sensitivity to chemical carcinogenesis in rodents and probably in humans (Anderson et al., 2000). Inorganic arsenic can readily cross the rodent and human placenta and enter the fetus (Concha et al., 1998; NRC, 2001). After *in utero* exposure to inorganic arsenic at carcinogenic doses, significant amounts of inorganic arsenic and its methylated metabolites (DMA and MMA) are detected in various mouse fetal tissues including the liver (Devesa et al., 2006). In arsenic-exposed human populations all life stages of exposure are involved (IARC, 2004). Thus, it is likely that significant *in utero* arsenic exposure occurs in human populations, and it is prudent to assume that the transplacental carcinogenic risks defined in rodents may predict similar effects in humans.

The liver is a major target organ of arsenic toxicity (Lu et al., 2001; Mazumder, 2005) and carcinogenesis in humans (Chen et al., 1997; Zhou et al., 2002; Centeno et al., 2002; Chen and Ahsan, 2004). In accord with human data, transplacental exposure to inorganic arsenic induced a marked, dose-related increase in hepatocellular tumors, including carcinoma, in adult male mice (Waalkes et al., 2003, 2004a, 2006b). Genomic analysis of liver samples taken at necropsy 1–2 years after gestational arsenic exposure alone or combined with postnatal exposure to 12-*O*-teradecanoyl phorbol-13-acetate (TPA), revealed a variety of hepatic genes to be aberrantly expressed in adulthood, including genes critical to the carcinogenic process (Liu et al., 2004, 2006a, 2006b). Although the expression changes in adult mouse liver are clearly associated with liver tumors, whether they are involved in cancer causation specifically by arsenic cannot be defined in fully developed tumors. Thus, genomic analysis of early molecular events following gestational arsenic exposure is clearly warranted.

The spectrum of tumors and/or proliferative lesions induced by *in utero* arsenic exposure, including tumors of liver, ovary, adrenal, uterus and oviduct, resembles the potential targets of carcinogenic estrogens (Waalkes et al., 2003; 2004a; 2006a; 2006b). This has led us to the hypothesis that arsenic could somehow produce estrogen-like effects, possibly through estrogen receptor-alpha (ER-α), as part of the mechanisms causing tumor formation (Waalkes et al., 2004b). Aberrant over-expression of ER-α is associated with a variety of human and rodent tumors (Fishman et al., 1995). Indeed, in livers and liver tumors from male mice exposed to arsenic *in utero*, the over-expression of the ER-α and estrogen-linked cyclin D1 is a prominent feature, and a feminized pattern of hepatic metabolic enzyme genes is evident (Waalkes et al., 2004b; Liu et al., 2004; 2006b). Samples of human livers from populations highly exposed to inorganic arsenic also show ER-α over-expression (Waalkes et al., 2004b).

Thus, this study investigated aberrant gene expression in the fetal male livers following *in utero* exposure to a hepatocarcinogenic dose of arsenic. Global genomic analysis was performed through the National Center for Toxicogenomics, using the Agilent 22K chip array. Expression of key genes was followed up by real-time RT-PCR analysis. This study clearly showed that *in utero* arsenic exposure produced dramatic alterations in gene expression in fetal liver, providing evidence for enhanced estrogen signaling and aberrant steroid metabolism in

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the developing fetus as a result of transplacental arsenic exposure. This arsenic-induced early life stage disruption of genetic programming could potentially lead to tumor formation much later in adulthood.

MATERIALS AND METHODS

Chemicals

Sodium arsenite ($NaAsO_2$) was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in the drinking water at 85 mg arsenic/L (85 ppm). The Agilent 22-K mouse oligo array was obtained from Agilent Technologies (Palo Alto, CA).

Animal Treatment and Sample Collection

Timed pregnant C3H mice were given drinking water containing 85 ppm arsenic or unaltered water *ad libitum* from day 8 to day 18 of gestation. At day 18 of gestation, mice were killed by CO_2 asphyxiation and fetuses removed. Only male fetal livers were used for the present study, as male offspring are most susceptible to arsenic hepatocarcinogenesis (Waalkes et al., 2003, 2004a, 2006b). Animal care was provided in accordance with the US Public Health Policy on the Care and Use of Animals, and the Institutional Animal Care and Use Committee approved this study proposal. Animals used in this study were treated humanely and with regard for the alleviation of suffering.

Microarray Analysis

Total RNA was isolated from liver samples with TRIzol reagent (Invitrogen, Carlsbad, CA), followed by purification and on-column DNase-I digestion with RNeasy mini kit (Qiagen, Valencia, CA). The high quality of RNA was confirmed by an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Total RNA was amplified using the Agilent Low RNA Input Fluorescent Linear Amplification Kit protocol. Starting with 500 ng of total RNA, Cy3 or Cy5 labeled cRNA was produced according to manufacturer's protocol. For each two-color comparison, 750 ng of each Cy3 and Cy5 labeled cRNAs were mixed and fragmented using the Agilent *In Situ* Hybridization Kit protocol. Hybridizations were performed on Agilent mouse 22K oligo assay for 17 hours in a rotating hybridization oven using the Agilent 60-mer oligo microarray processing protocol. Slides were washed as indicated in this protocol and then scanned with an Agilent Scanner. Data were obtained using the Agilent Feature Extraction software (v7.5), using defaults for all parameters. Two hybridizations with fluor reversals were performed for each RNA sample from each group.

Real-time RT-PCR Analysis

The levels of expression of the selected genes were quantified using real-time RT-PCR analysis. The forward and reverse primers for selected genes were designed using ABI Primer Express software (Applied Biosystems, Foster City, CA) and listed in Table 1. Total RNA was reverse transcribed with MuLV reverse transcriptase and oligo-dT primers, and subjected to real-time PCR analysis using SYBR green PCR master mix (Applied Biosystems, Cheshire, UK). The cycle time (Ct) values of genes of interest were first normalized with β-actin from the same sample, and then the relative differences between control and treatment groups were calculated and expressed as percentage of controls. Assuming that the Ct value is reflective of the initial starting copy and that there is 100% efficiency, a difference of one cycle is equivalent to a two-fold difference in starting copy.

Statistics

For microarray analysis, samples were cross-hybridized with Cy3 and Cy5. Images and GEML files, including error and p-values, were exported from the Agilent Feature Extraction software

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and deposited into the Rosetta Resolver system (version 4.0, build 4.0.1.0.7.RSPLIT) (Rosetta Biosoftware, Kirkland, WA). The resultant ratio profiles were combined. Intensity plots were generated for each ratio experiment and genes were considered "signature genes" if the p value was less than 0.001. For real-time RT-PCR analysis, means and SEM of individual samples (n = 6) were calculated. For the comparisons of gene expression between two groups, Students' *t* tests were performed.

RESULTS

Microarray analysis of aberrantly expressed genes

Total RNA from male fetal liver samples of control and arsenic-exposed mice were subjected to microarray analysis. Under the criteria of $p < 0.001$ by the Rosetta Resolver (v4.0) system, the transcript levels of 187 genes among 22,000 contained on the array were significantly altered by arsenic exposure compared to control. Clustering analysis of these altered genes is shown in Fig. 1. A cluster of increased genes (shown in red) included various genes related to estrogen signaling, and a cluster of decreased genes (shown in green) included various metabolic enzymes (Fig. 1). The 30 genes with increased expressions (cut-off at ratio of 1.9) are listed in Table 2, and 30 genes with decreased expressions (cut-off at ratio of 0.65) are listed in Table 3. The putative cDNA clones without fully defined corollary genes are excluded from this list.

Real-time RT-PCR analysis of aberrantly expressed genes

To help verify the microarray results, real-time RT-PCR analysis of selected genes was performed using individual samples from control and arsenic-exposed male fetal livers. Real-time RT-PCR generally confirmed microarray results, although RT-PCR appeared to be more sensitive and often showed more pronounced changes than microarray analysis. For consistency, quantitative description and discussion are henceforth based on the real-time RT-PCR analysis. For genes potentially regulated by estrogen (Fig. 2), there were marked increases in anterior gradient 2 (Agr2, 134-fold), small proline-rich protein 2a (Sprr2a, 125-fold), trefoil factor 1 (Tff1, 31-fold), CRP-ductin (14-fold), ghrelin (Ghrl, 10-fold), cytokeratin 1–19 complex (Krt1-19, 5-fold), X-inactive specific transcript (Xist, 4-fold), and corticosteroid-binding globulin (Cbg, 2-fold).

Figure 3 illustrates the altered expression of genes related to steroid metabolism. These included increased expression of hydroxysteroid 11-β dehydrogenase-7 (HSD17β7, 1.9-fold, involved in estradiol production) and decreased expression of HSD17β5 (0.52 ratio to control, involved in testestorone production), HSD11β1 (0.58 ratio to control) and aldo-keto reductase Akr1c18 (also known as 20α-hydroxysteroid dehydrogenase, 0.46 ratio to control). For sex-dependent cytochrome P450 enzyme genes, the expression of female dominant Cyp2a4 (steroid 15α-hydroxylase) was increased 2.3 fold, while the expression of male dominant Cyp3a25 (0.40 ratio to control) and Cyp2j5 (0.45 ratio to control) were decreased.

The expression of several genes involved in methyl metabolism in fetal male mouse liver were also decreased (Fig. 4). These include methionine adenosyltransferase 1a (Mta1a; 0.56 ratio to control), betaine-homocysteine methyltransferase (Bhmt; 0.50 ratio to control) and thioether S-methyltransferase (Temt; 0.25 ratio to control).

Discussion

The hypothesis that inorganic arsenic might somehow act through aberrant activation of estrogen signaling pathways (Waalkes et al., 2004b) comes from several lines of evidence. This includes the fact that the transplacental arsenic carcinogenesis shows consistent targets

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(i.e., liver, ovary, adrenal, uterus) which are also targets of broad range or tissue-selective carcinogenic estrogens (Birnbaum and Fenton, 2003; Newbold, 2004). In addition, estrogen-linked gene/protein overexpressions are evident in transplacental arsenic-induced tumors (Waalkes et al., 2004b, 2006a; Liu et al., 2004, 2006a, 2006b; Shen et al., 2007). Furthermore, transplacental arsenic enhances subsequent diethylstilbestrol (a synthetic estrogen) carcinogenesis (Waalkes et al., 2006a, 2006b) and enhances diethylstilbestrol-induced estrogen-related gene expression in neonatal tissues (Waalkes et al., 2006a). For instance, compared to the incidence of urogenital cancers in the control (0%), arsenic (9%), or diethystilbestrol (21%) alone, *in utero* arsenic plus postnatal diethylstilbestrol induces a synergistic 48% increase in urogenital malignancies (Waalkes et al., 2006a). Transplacental arsenic-induced lung tumorigenesis is associated with aberrant expression of pulmonary estrogen-related genes in the fetus (Shen et al., 2007). The current study demonstrates various estrogen-linked genes (such as Xist, Agr2, Tff1, CRP-ductin, Ghrl, Krt1-19, and Cyp2a4) were significantly over-expressed in fetal livers following *in utero* arsenic exposure, adding further evidence to support the hypothesis that aberrant estrogen signaling may play a role in transplacental arsenic carcinogenesis in mice.

Xist is a female specific gene essential for X-chromosome inactivation in female mammals (Goto and Monk, 1998; Plath et al., 2002). The expression of Xist was increased nearly 4-fold in arsenic-exposed male fetal livers. Epigenetic dynamics of imprinted X chromosome inactivation during early development is a critically important process for early genetic programming and development in mice and in humans (Goto and Monk, 1998; Okmoto et al., 2004). Xist is imprinted early in mammalian development (Okmoto et al., 2004). Aberrant regulation of Xist could lead to disruption of cell differentiation and could have implications in early life programming (Plath et al., 2002; Okmoto et al., 2004), and deserves further investigation.

Anterior gradient 2 (Agr2) expression was dramatically increased (140-fold) in arsenic-exposed fetal livers. Agr2 mRNA and protein are positively associated with ER-α positive, but not ER-α negative, breast carcinomas (Liu et al., 2005). In human breast cancer, Agr2 and Krt1-19, both estrogen-regulated genes, are over-expressed (Shen et al., 2005). Estrogen-linked trefoil factor 1 (Tff1) and CRP-ductin expression was increased 32- and 15-fold, respectively, in arsenic-exposed fetal livers. Estrogen receptor-positive breast cancer cells secrete high concentrations of Tff1, and dimeric Tff1 is a potent stimulator for migration of breast cancer cells (Prest et al., 2002). Tff1 is also required for the commitment programming of mouse oxyntic epithelial progenitors, and influences Tff2 and Tff3 expression during development (Kama et al., 2004). In mouse uterine adenocarcinoma and urinary bladder transitional cell carcinoma induced by *in utero* arsenic plus postnatal diethylstilbestrol treatment, ER-α and pS2 (Tff1) and were greatly over-expressed (Waalkes et al., 2006a). CRP-ductin fulfils some of the criteria for being a Tff receptor or a Tff binding protein (Thim and Mortz, 2000), and is an estrogen-responsive gene with a possible role in endometrial proliferation or differentiation. Ghrelin and small proline-rich protein 2a (Sprr2a) were increased 13- and 120-fold, respectively, in arsenic-exposed fetal livers. Ghrelin plays an important role in the control of some aspects of gonadal function in the testes and ovary (Tena-Sempere, 2005), and its expression is regulated by estrogen (Tena-Sempere, 2005). Ghrelin immunopositive cells also express ER-α (Matsubara et al., 2004). Similarly, expression of Sprr2 protein and the Sprr2 mRNA family (Sprr2a, 2b, 2c, 2d, 2e, 2f, and 2g) can be regulated by estrogen, and Sprr2a has been proposed to play a role in the estrous cycle, early pregnancy and implantation (Hong et al., 2004). Most of the above genes have cross-talk mechanisms with ER-α (Hewitt et al., 2005). The aberrant over-expression of these genes in fetal liver supports an early disruption by arsenic of estrogen signaling.

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Possibly as a contributing factor in aberrant estrogen signaling, the genes encoding steroid metabolism enzymes were also altered. For example, the expression of HSD17β7, a gene encoding for an enzyme involved with estradiol biosynthesis (Nokelainen et al., 1998), was increased ~2 fold, while the expression of HSD17β5, a gene encoding for an enzyme catalyzing the transformation of 4-androstenedione (4-dione) into testosterone (Luu-The et al., 2001), was decreased ~50%. Enhanced expression of female-dominant Cyp2a4 and the decreased expression of male-dominant Cyp2j5 and Cyp3a25 were also evident in arsenic-exposed fetal livers. Cyp2a4 (steroid 15α-hydroxylase) is an ER-α-regulated gene (Sueyoshi et al., 1999), Cyp3a25 encodes for a testosterone 6β-hydroxylase (Dai et al., 2001), and the expression of Cyp2j5 is up-regulated by androgen and down-regulated by estrogen (Ma et al., 2005). In addition, the expression of HSD11β1 and Akr1c18 were both decreased ~50% in arsenic-exposed fetal livers. HSD11β1 converts inactive cortisone to active cortisol (Tomlinson et al., 2004) and Akr1c18 encodes 20α-hydroxysteroid dehydrogenase (Vergnes et al., 2003). Thus, alterations in the expression of these genes are reflective of endocrine disruption effects of inorganic arsenic at a very early life stage. The fetal stage is a critical period of development which is susceptible to environmental estrogen insult (Birnbaum and Fenton, 2003). The impact of arsenic in fetal livers is quite similar to the liver feminization pattern seen in arsenic-induced hepatocellular carcinomas occurring much later in life (Waalkes et al., 2004b, Liu et al., 2004, 2006a, 2006b).

Another interesting category of aberrant gene expression after *in utero* arsenic is related to methionine metabolism. The expression of methionine adenosyltransferase 1(Mta1a), betaine-homocysteine methyltransferase (Bhmt) and thioether *S*-methyltransferase (Temt), were decreased in arsenic-exposed fetal livers. The aberrant expression of these enzyme genes would potentially contribute to abnormal S-adenosylmethionine production, a critical methyl donor for enzymatic DNA methylation. Altered methyl group metabolism could provide a potential mechanism for inducing epigenetic changes in the embryo (Steele et al., 2005), including DNA methylation changes, as DNA methylation is an important gene imprinting mechanism during development and can be altered in the adult during arsenic carcinogenesis (Chen et al., 2004; Waalkes et al., 2004b)

In summary, the present study clearly demonstrates that *in utero* arsenic exposure resulted in dramatic alterations in gene expression in the fetal liver involving a complex interplay between steroid metabolism and estrogen signaling pathways, which may well play an important role in early genetic reprogramming, leading to the formation of tumors later in life (Cook et al., 2005). As a corollary in humans, increased mortality occurs from lung cancers in young adults following *in utero* exposure to arsenic in the drinking water (Smith et al., 2006). Thus, the developing human and mouse fetus appear to be very sensitive to arsenic carcinogenesis. Reduction of arsenic intake in pregnant women may be a valid mechanism for reducing human cancer associated with environmental arsenic exposure.

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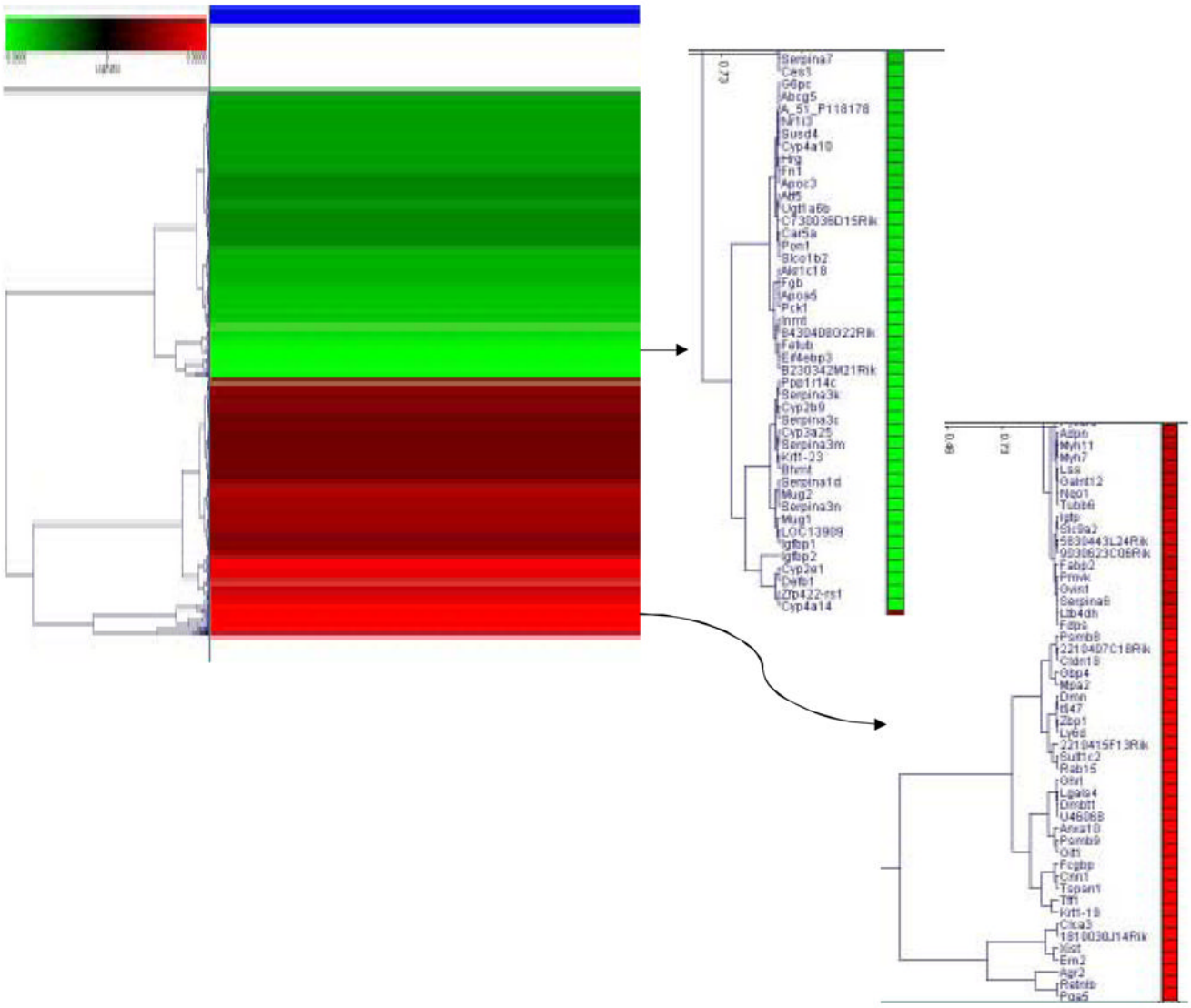

Figure 1.

Altered gene expression in fetal mouse liver exposed to arsenic *in utero.* The significantly altered genes under criteria of $p < 0.001$ were clustered for comparison. The increased genes are shown in red, and the decreased genes are shown in green.

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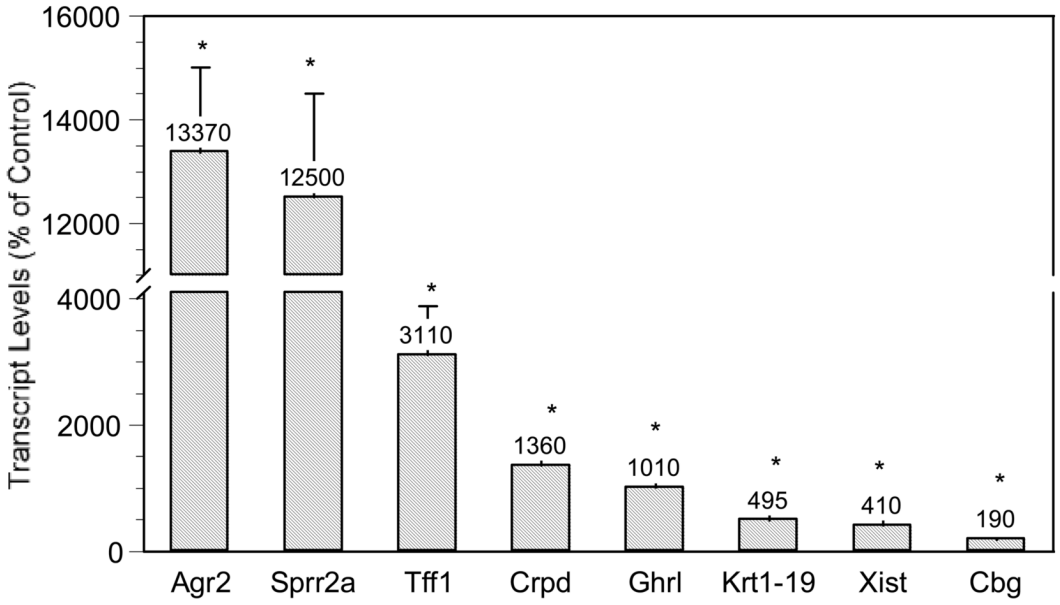

Figure 2.
Effect of *in utero* arsenic exposure on estrogen-signaling gene expression in the fetal liver. Data are mean + SEM of 6 mice. *Significantly different from controls, $p < 0.05$.

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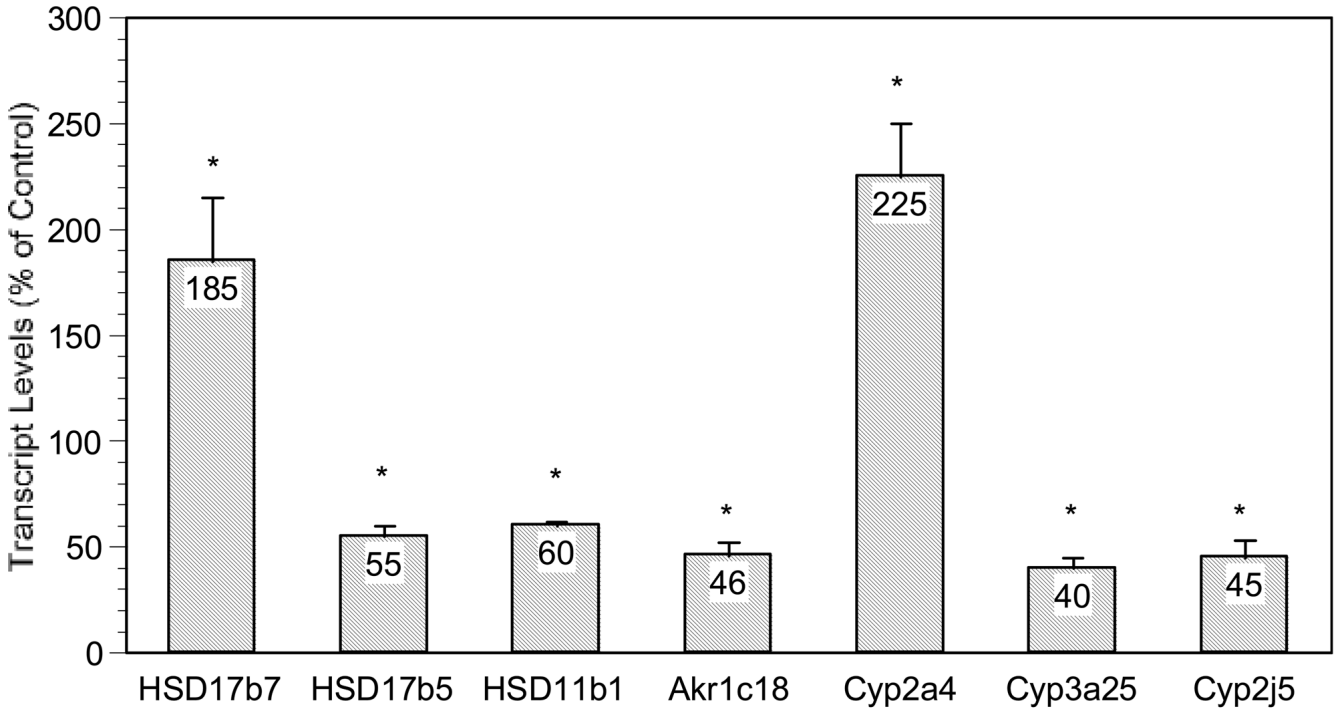

Figure 3.
Effect of *in utero* arsenic exposure on expression of genes related to steroid metabolism in the fetal liver. Data are mean + SEM of 6 mice. *Significantly different from controls, $p < 0.05$.

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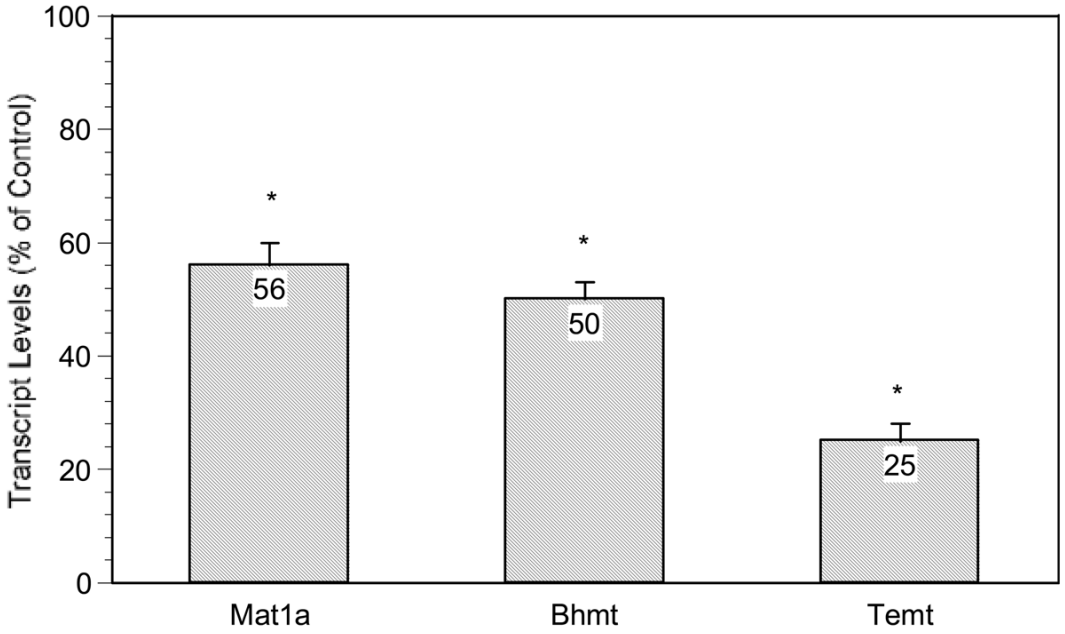

Figure 4.
Effect of *in utero* arsenic exposure on expression of genes related to methyl metabolism in the fetal liver. Data are mean + SEM of 6 mice. *Significantly different from controls, $p < 0.05$.

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Table 1

Primer sequences for real-time RT-PCR analysis

Gene name	Acceession #	Forward	Reverse
Agr2, anterior gradient 2	NM_011783	CCTTGCGGCTCACACAAAG	ATGGCCACAAGAAGCAGGAT
Akr1c18	NM_134066	GCAACCAGGTAGAATGCCATCT	GCACCATAGGCAACCAGAACA
β-actin	M12481	GTATGACTCCACTCACGGCAAA	GGTCTCGCTCCTGGAAGATG
Bhmt	AF033381	CACATCAGGGCGATTGCA	TCCCCAGCTGCCATGTTT
Cbg (corticocoid-binding protein)	NM_007618	CCATGCTGCAACTGGATGAA	GATTCGGAGGGCAGGTGTAC
CRP-ductin (Dmbt1)	NM_007769	GCCAACCTCCAAGTACTAAGTTGTG	GTCAGGCTCTCATGTCTTCCATCT
Cyp2a4	J03549	GGAAGACGAACGGTGCTTTC	CCCGAAGACGATTGAGCTAATG
Cyp2j5	NM_010007	CAGACATGGAAGGAGCAAAGG	GAATGCGCTCCTCCAAGCT
Cyp3a25	Y11995	TGGAGGCCTGAACTGCTAAAG	TAACCAGCAGCACCCAGGTT
HSD11β1	NM_008288	CTCTCTTCCATGACGACATCCA	CTGTGCTCATGACCACGTAGCT
HSD17β5 (Akr1c6)	NM_030611	GCCATGGAGAAATGCAAGGA	GTTGCACACAGGCTTGTACTTGAG
HSD17β7	NM_010476	AGCTGATGGAGGCGTTCCT	TGTGTATCACCCAGCGAGATG
Krt1-19	NM_008471	GAGGACTTGCGCGACAAGA	GGCGAGCATTGTCAATCTGTAG
Mat1a	NM_133653	CAGGTGTCCTATGCCATTGGT	TCTAGCAGCTCCCGCTCAGT
Sprr2a (samll proline-rich protein 2A)	NM_011468	GGGAAGCACAACAGGTATCTACTCT	GCAGTGACCATTGCCCTGAT
Temt (thioether S-methyltransferase)	NM_009349	CCTACGACTGGTCCTCCATAGTG	CTTCTGAGCTTGGCTTCCTTCT
Tff1	NM_009362	GGCCCAGGAAGAAACATGTATC	CCCCGGACACTGTCATCAA
XIST	L04961	GCTTCTGCGTGATACGGCTAT	AGCTAGCGCAGCGCAATT

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Table 2

Microarray analysis of increased gene expression in arsenic-exposed fetal mouse liver

Symbol	Accession	Gene Description	Control	Arsenic	As/Control
Agr2	NM_011783	anterior gradient 2 (Xenopus laevis)	133	3004	22.80
Pga5	NM_021453	Mus musculus pepsinogen 5, group I (Pga5), mRNA.	702	11166	15.77
Retnlb	NM_023881	resistin like beta	62	893	15.43
Clca3	NM_017474	chloride channel calcium activated 3	258	1903	7.47
Xist	L04961	inactive X specific transcripts	182	1195	6.68
Ern2	NM_012016	endoplasmic reticulum (ER) to nucleus signalling 2	163	927	6.04
Krt1-19	NM_008471	keratin complex 1, acidic, gene 19	368	1794	4.87
Tff1	NM_009362	trefoil factor 1	1191	5127	4.30
Tspan1	NM_133681	Mus musculus tetraspan 1 (Tspan1), mRNA	162	600	3.78
Cnn1	NM_009922	calponin 1	867	3191	3.71
Mtlrp(Ghrl)	NM_021488	motilin-related peptide	73	266	3.54
Crpd	NM_007769	crp-ductin	281	907	3.44
Lgals4	BC011236	Mus musculus galactose binding, soluble 4 (Galectin-4)	450	1540	3.41
U46068	U46068	Mus musculus von Ebner minor salivary gland protein mRNA	461	1558	3.39
Anxa10	AK020288	annexin A10	398	1260	3.17
Psmb9	NM_013585	proteosome (prosome, macropain) subunit, beta type 9	1724	5270	3.05
AK010206	AK010206	oncoprotein induced transcript 1, full insert sequence	365	1049	2.95
Cldn18	NM_019815	claudin 18	324	890	2.91
Psmb8	NM_010724	proteosome (prosome, macropain) subunit, beta type 8	1849	5301	2.86
Mpa2	NM_008620	macrophage activation 2	348	953	2.75
Gbp3	NM_018734	guanylate nucleotide binding protein 3	773	1996	2.58
Sult1c1	NM_026935	Mus musculus sulfotransferase family,1C, member 1 (Sult1c1)	361	778	2.19
Dlm1 (Zbp1)	NM_021394	tumor stroma and activated macrophage protein DLM-1	550	1173	2.13
Ly6d	NM_010742	lymphocyte antigen 6 complex, locus D	314	667	2.11
Scyb10	NM_021274	small inducible cytokine B subfamily (Cys-X-Cys), member 10	688	1426	2.10
Ifi47	NM_008330	interferon gamma inducible protein, 47 kDa	1689	3538	2.09
Iigp	NM_021792	interferon-inducible GTPase	14068	29009	2.05
Krt1-15	NM_008469	keratin complex 1, acidic, gene 15	494	999	2.04
Tacstd1	NM_008532	tumor-associated calcium signal transducer 1	1228	2452	2.01
Sprr2a	NM_011468	small proline-rich protein 2A	800	1528	1.93

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Symbol	Accession	Gene Description	Control	Arsenic	As/Control

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Table 3

Microarray analysis of decreased gene expression in arsenic-exposed fetal mouse liver

Symbol	Accession	Gene Description	Control	Arsenic	As/Control
Igfbp2	NM_008342	insulin-like growth factor binding protein 2	17969	5581	0.31
Cyp2e1	NM_021282	cytochrome P450, 2e1, ethanol inducible	13706	5259	0.38
Defb1	NM_007843	defensin beta 1	578	229	0.38
Cyp4a14	NM_007822	cytochrome P450, 4a14	16593	6851	0.41
Igfbp1	NM_008341	insulin-like growth factor binding protein 1	438719	186169	0.43
Est31	NM_144511	esterase 31, male-predominant carboxylesterase in mouse liver	8808	3835	0.44
Mug1	NM_008645	murinoglobulin 1	1313	595	0.45
Spi2-2	NM_009252	serine protease inhibitor 2-2	44864	20661	0.46
Mug-ps1	M65237	Mouse murinoglobulin (MUG3) mRNA, last 2 exons	508	246	0.46
Spi1-4	NM_009246	serine protease inhibitor 1–4	1102183	512408	0.46
Spi2	D00725	serine protease inhibitor 2	4930	2346	0.47
Klkbp	AI115771	kallikrein binding protein	23838	11520	0.48
Bhmt	NM_016668	betaine-homocysteine methyltransferase	12196	6020	0.49
Cyp2b9	NM_010000	cytochrome P450, 2b9, phenobarbitol inducible, type a	1120	546	0.49
Haik1	NM_033373	type I intermediate filament cytokeratin	1903	935	0.49
Cyp3a25	Y11995	cytochrome P450, 3a25	2707	1366	0.50
Spi2-rs1	NM_009253	serine protease inhibitor-2 related sequence 1	29480	14887	0.51
Akr1c18	NM_134066	Mus musculus aldo-keto reductase family 1, member C18 (Akr1c18)	769	390	0.51
Pck1	NM_011044	phosphoenolpyruvate carboxykinase 1, cytosolic	17597	9190	0.52
Temt	NM_009349	thioether S-methyltransferase	1262	694	0.55
Fetub	NM_021564	fetuin beta	30806	17606	0.56
Slc21a10	NM_020495	solute carrier family 21 (organic anion transporter), member 10	35255	20890	0.59
Pon1	NM_011134	paraoxonase 1	22003	13048	0.59
Car5a	NM_007608	carbonic anhydrase 5a, mitochondrial	2063	1226	0.59
Ugt1a1	S64760	UGTBr1=UDP-glucuronosyltransferase [mice, mRNA, 2215 nt].	48324	28752	0.60
Cyp4a10	AK002528	cytochrome P450, 4a10	1255	768	0.61
Fn1	X93167	M. musculus mRNA for fibronectin.	23405	14628	0.62
Cyp2f2	NM_007817	cytochrome P450, 2f2	11356	7185	0.63
Hsd11b1	NM_008288	hydroxysteroid 11-beta dehydrogenase 1	17620	11266	0.64
Cyp2j5	NM_010007	cytochrome P450, 2j5	2319	1511	0.65

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Symbol	Accession	Gene Description	Control	Arsenic	As/Control