ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 1 & 2, 2006

Induction of Endogenous Nrf2/Small Maf Heterodimers by Arsenic-Mediated Stress in Placental Choriocarcinoma Cells

WAEL MASSRIEH,[1,2*] ANNA DERJUGA,[1*] and VOLKER BLANK[1,2]

ABSTRACT

Exposure to inorganic arsenic has been associated with various forms of cancer, nervous system pathogenesis, and vascular diseases, as well as reproductive and developmental toxicity. Here, the effect of inorganic arsenic on placental JAR choriocarcinoma cells was assessed. The nuclear protein levels of the CNC transcription factor Nrf2 were strongly induced in the presence of arsenic. Dosage response experiments showed that 0.5 μ*M* of arsenic is sufficient to augment Nrf2 levels. The expression of the Nrf2 dimerization partners MafG and MafK appeared not to be modulated by arsenic, whereas MafF protein levels were slightly increased. Arsenic also induced the binding of endogenous Nrf2/small Maf DNA-binding complexes to a stress response element (StRE) recognition site. In addition, arsenic caused oxidative stress in the choriocarcinoma cell model as evidenced by an increase in intracellular H_2O_2 levels. Expression of the enzyme heme oxygenase-1 (HO-1), a known Nrf2 target gene, was upregulated by exposure of JAR cells to arsenic. These results suggest that Nrf2/small Maf heterodimers may play an important role in the response to arsenic-mediated stress in placental cells. *Antioxid. Redox Signal.* 8, 53–59.

INTRODUCTION

Arsenic is a ubiquitous contaminant, and humans are continually exposed to this semimetal. Trivalent arsenic is highly toxic and carcinogenic, but has been shown to induce apoptosis in certain tumor cells at low concentrations, and is thus currently used as a standard anticancer treatment (20, 28). Arsenic affects numerous intracellular signaling molecules including protein kinases, tyrosine phosphatases, NF-κB/IκB and caspases (20). Besides the induction of apoptosis, arsenic may also inhibit growth and angiogenesis, and promote differentiation. In addition, exposure to arsenic has been shown to cause oxidative stress by disturbing natural oxidation and reduction equilibria in the cell (20).

The CNC and Maf families of basic-leucine (bZIP) transcription factors play important roles in mammalian gene regulation, development, differentiation, oncogenesis, and cellular stress response (4, 23). Several studies have linked the CNC factor Nrf2 and small Maf proteins to arsenic induced stress. Induction of antioxidative stress proteins such as heme oxygenase-1 (HO-1) and peroxiredoxin I (Prx I) by arsenic is impaired in Nrf2 deficient macrophages (18). In keratinocytes, Nrf2 is activated by inorganic arsenic and it has been proposed that hydrogen peroxide (H_2O_2) is one of the mediators of this process (27). Treatment of osteoblasts with arsenite [As(III)] or arsenate [As(V)] also induces Nrf2 levels and leads to the transcriptional activation of Nrf2 target genes (3). In addition, it was shown that the transcript levels of the small Maf factor *mafG* gene, coding for an Nrf2 dimerization partner, are induced by high doses of sodium arsenite in HeLa cells (30). Hence, arsenic mediated stress is clearly linked to the activity of CNC and small Maf proteins. Recent evidence suggests that Nrf2 is the primary target of the broad complex–tramtrack–bric-a-brac (BTB) protein Keap1 (Kelch-like ECH associated protein 1) that retains Nrf2 in the cytoplasm (19, 24, 25). Keap1, functioning as a substrate adaptor protein, associates with cullin 3 and the Ring finger protein Rbx1/Roc1 to form a functional E3 ubiquitin ligase complex, that targets Nrf2 for degradation by the proteasome (13, 33). Thus, Nrf2 activity appears to be regulated by cytoplasmic sequestration and degradation via the ubiquitin pathway.

Arsenic has been shown to cross the placenta in both animals and humans, and experimental studies support a role for

[1]Lady Davis Institute for Medical Research and [2]Department of Medicine, McGill University, Montreal, Quebec, Canada.
[*]WM and AD contributed equally to this work.

arsenic as a developmental toxicant leading to spontaneous abortions, birth defects, and embryonic lethality (8, 16, 21). Placental levels of arsenic can be significantly increased in humans living in regions with high environmental contamination (31). Placental trophoblast cells are exposed to extensive oxidative stress during pregnancy, and there is evidence implicating oxidative stress in the pathogenesis of preeclampsia and miscarriage (6). Based on these previous data, we analyzed the effect of arsenic induced stress in placental cells. As a model we chose placental trophoblast derived JAR choriocarcinoma cells (26). We found that arsenic has a profound effect on Nrf2 expression and DNA-binding activity of endogenous Nrf2/small Maf complexes. There is evidence of significant oxidative stress in JAR cells in the presence of arsenic. Furthermore, the expression of HO-1, a major stress responsive protein, is also induced upon exposure to arsenic. Thus CNC/small Maf complexes are likely to be involved in the regulation of the cellular response to stress in placental cells.

MATERIALS AND METHODS

Cell culture and induction by arsenic

Human embryonic kidney 293T (HEK293T) cells were cultured in minimal essential medium alpha (MEM-α) and 10% bovine growth serum. The human choriocarcinoma JAR cell line (American Type Culture Collection, Manassas, VA) was maintained in RPMI-1640 medium plus 1 m*M* sodium pyruvate, 10 m*M* HEPES, and 10% fetal bovine serum. Penicillin and streptomycin was added to all media. For induction studies, JAR cells at 70–90% confluency were incubated with various concentrations of arsenic trioxide (As_2O_3) (Sigma, Oakville, ON) for the times indicated.

Plasmid constructs and transfections

The human *nrf2* cDNA was recovered by RT-PCR using the following oligonucleotides: hNrf2-F_{new} (5'-CCCAGCAG GACATGGATTTGAT-3') and hNrf2-R_{new} (5'-GGTCAAA TCCTCCTAAATCTAG-3'). The PCR fragments were then cloned into the pCR-BluntII-TOPO vector. As the *nrf2* cDNA comprises an internal *Eco*RI site, the insert was recovered from the pCR-BluntII-TOPO plasmid by a partial *Eco*RI digest, and subcloned into the *Eco*RI site of the pMT2 expression vector to yield pMT2Nrf2. The pMT2MafG, pMT2MafF and pMT2MafK expression constructs have been described elsewhere (5; Massrieh, Derjuga, Doualla-Bell, Ku, Sanborn, and Blank, in revision). Transient transfections of HEK293T cells were performed using the standard calcium-phosphate coprecipitation procedure. To this end, we cotransfected HEK293T cells at 30–50% confluency (100 mm dish) with 10 μg of both, the pMT2Nrf2 vector and either of the pMT2MafF, pMT2MafG or pMT2MafK expression plasmids.

Immunoblot analysis

To prepare cytoplasmic extracts, the cells were scraped using 1× PBS, centrifuged, and the pellets were then resuspended in NB buffer (250 m*M* sucrose, 10 m*M* Tris/HCl, 2 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 1% Triton X-100) and protease inhibitors. After incubation on ice for 10 min, samples were briefly centrifuged and the supernatant was collected. Nuclear extracts were prepared by resuspending the pellet from the cytoplasmic extract preparation in NB buffer plus 420 m*M* NaCl, centrifuging, and collecting the supernatant. The protein concentrations were determined using a protein assay kit (Bio Rad, Hercules, CA). 20–30 μg of the lysates were electrophoresed in 4–12% NuPage Novex Bis-Tris (Invitrogen, Carlsbad, CA) (Figs. 1 and 2) according to the instructions of the manufacturer or 12% SDS-polyacrylamide gels (Fig. 6). Resolved proteins were transferred electrophoretically to a PVDF membrane (Immobilon, Millipore, Bedford, MA). After transfer, the membrane was blocked for 1 hour at room temperature and was subsequently incubated overnight with primary antisera in 1× TBS plus 5% milk, 0.05% Tween-20, and 350 m*M* NaCl. After washing, the membrane was incubated for 1 hour at room temperature with secondary goat anti-rabbit or anti-mouse antibodies conjugated to horseradish peroxidase (Pierce, Rockford, IL). The proteins were detected using the Super-Signal West Pico chemiluminescent reagent (Pierce) following the manufacturer's instructions.

EMSA experiments

For electrophoretic mobility shift assay (EMSA) experiments, nuclear extracts were prepared as described previously (2) and then incubated at 4ºC for 30 min in the binding reaction containing 18 m*M* HEPES-KOH (pH 7.9), 80 m*M* KCl, 2 m*M* $MgCl_2$, 10 m*M* DTT, 10% glycerol, poly(dI-dC) 160 μg/ml, 0.2 mg/ml bovine serum albumin, and 7K-30K cpm γ-^{32}P–ATP labeled probe (15). Reaction mixtures were analyzed by native 5% polyacrylamide gel electrophoresis and autoradiography. The StRE (stress response element) DNA binding site oligonucleotide derived from the human HO-1 promoter (15), end-labeled with T4-polynucleotide kinase, was used as a probe. A 1200 molar excess of unlabeled oligonucleotide was used for competition. For supershift experiments 3 μl of either preimmune or immune serum was added to the reaction mixture as indicated.

Antisera

For immunoblot and/or EMSA analyses we used antisera specific for various human proteins. The Nrf1, Nrf2, MafK (Santa Cruz Biotech, Santa Cruz, CA), HO-1 (Stressgen, Victoria, BC), β-actin (Sigma) antisera were obtained commercially. The generation of the MafF (Massrieh, Derjuga, Doualla-Bell, Ku, Sanborn, and Blank, in revision), MafG (5) and Nrf3 (7) antisera has been described elsewhere.

Detection of intracellular H_2O_2 levels

Intracellular H_2O_2 is detected when membrane-permeable 2',7'-dichlorofluorescin diacetate (DCFH-DA) undergoes nonspecific cleavage by intracellular esterases, and the resulting DCFH is oxidized to the fluorescent compound, 2',7'-dichlorofluorescein (DCF) in the presence of H_2O_2 as previously described (11). JAR cells were seeded at 2×10^5 cells/ml in six-well plates and were treated the following day with 5 μ*M* As_2O_3 for 6 hours. Cells were then treated with 25 μg/ml DCFH-DA, and incubated at 37°C for 45 min. Positive control groups were treated with 1 m*M* H_2O_2, and incubated at 37°C for 15 min prior to time of collection (data not shown). The

fluorescent intensity of DCF was then measured with a Coulter flow cytometer.

Quantification and statistical analysis

Quantification was performed by densitometry using NIH image software (version 1.63). Values of $p < 0.01$ (**) were used as criteria for declaring significance. FACS analysis was performed using Cell Quest Pro software. All data are expressed as the mean ± SEM and were analyzed for significance using one way ANOVA or Student *t*-test.

RESULTS

Upregulation of Nrf2 protein levels by different doses of arsenic

We first performed dosage-response experiments exposing placental JAR choriocarcinoma cells for 6 hours to different concentrations of arsenic trioxide. Nuclear extracts of JAR cells were prepared and analyzed in immunoblot assays using an Nrf2 specific antiserum. We found that nuclear Nrf2 protein levels are upregulated by doses of 0.5 μ*M* arsenic (Fig. 1). Higher concentrations resulted in even stronger inductions of Nrf2 protein. At doses of 10 μ*M* and higher, cells looked unhealthy as evidenced by a rounded appearance resulting in increased numbers of cells that were lifting off the plate, especially at longer time points (data not shown). We thus chose to use 5 μ*M* of arsenic in further experiments as this dose resulted in a strong induction of Nrf2 protein levels with no visible toxic effects.

Time course of arsenic induction

We next analyzed the induction of Nrf2 after treatment of choriocarcinoma cells with 5 μ*M* arsenic by preparing nuclear extract at different time points. We found upregulation of Nrf2 at all the times analyzed, from 2 to 24 hours (Fig. 2A). The highest induction levels were observed at the 6 hour time point. Nrf2 was similarly induced by arsenic in the cytoplasm (data not shown). We also monitored the expression of the dimerization partners of Nrf2, the small Maf protein MafF,

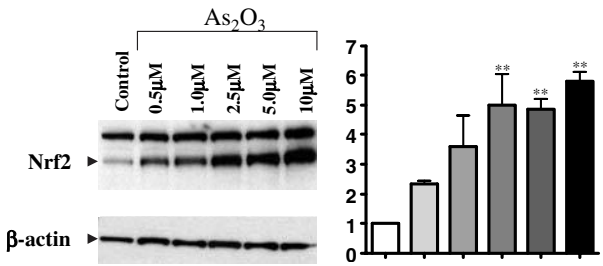

FIG. 1. Induction of Nrf2 by different doses of arsenic. Immunoblot of nuclear extracts prepared from JAR choriocarcinoma cells exposed to different concentrations of arsenic trioxide (0.5–10 μ*M*) or not (control) for 6 hours using antisera specific for Nrf2 and β-actin. 30 μg of protein was loaded per well. Quantification of 3 experiments is shown. Statistically significant differences are indicated by two asterisks ($p < 0.01$).

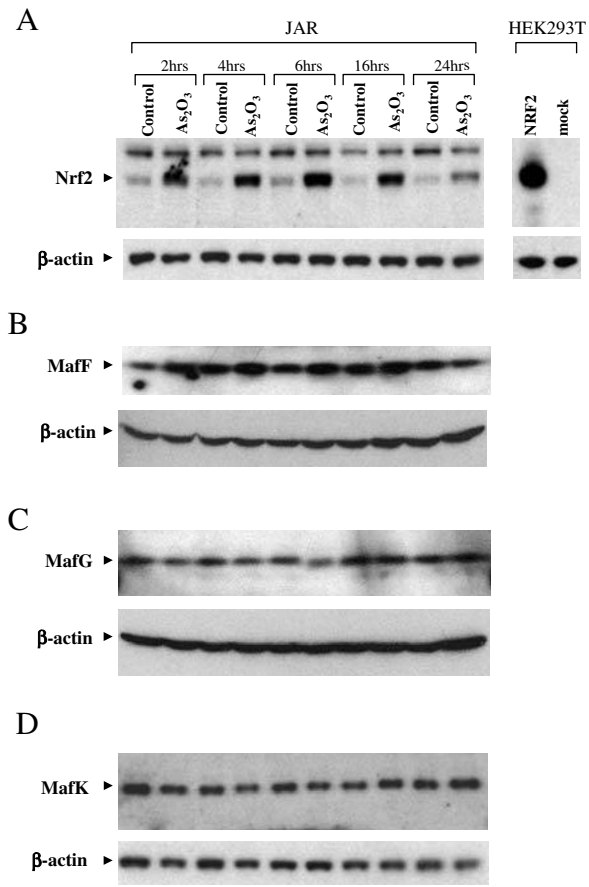

FIG. 2. Time course of arsenic treatment of nuclear Nrf2 and small Maf levels. Immunoblot of nuclear extracts prepared from JAR choriocarcinoma cells treated or not (control) with 5 μ*M* arsenic trioxide for 2 to 24 hours using antisera specific for (**A**) Nrf2, (**B**) MafF, (**C**) MafG, (**D**) MafK and β-actin. 30 μg of protein was loaded per well. Control extracts from HEK293T cells transfected with an expression vector coding for Nrf2 or not (*mock*) have been analyzed in parallel.

MafG, and MafK. We used a set of antisera shown earlier to be specific for each of the small Maf proteins in immunoblot experiments (Massrieh, Derjuga, Doualla-Bell, Ku, Sanborn, and Blank, in revision). We found no induction for the small Maf factors MafG and MafK, whereas expression of MafF was slightly upregulated, in particular at 2, 4, 6 and 16 hours following arsenic treatment (Fig. 2B–D).

Increase of Nrf2/small Maf DNA binding activity following exposure to arsenic

Since we detected a strong increase in nuclear Nrf2 levels, we performed electrophoretic mobility assays (EMSAs) to analyze the DNA binding activities in JAR choriocarcinoma cells. First, we assessed the specificities of antisera used in this study. To this end, we prepared extracts from human embryonic kidney 293T (HEK293T) cells that were transiently transfected with expression constructs coding for Nrf2 and either one of the small Mafs MafF, MafG, or MafK. Cotransfection

of Nrf2 and either of the small MAF constructs resulted in a complex binding to a stress responsive element (StRE) recognition site, derived from the human HO-1 promoter (15) (Fig. 3). DNA binding was specific as addition of an unlabeled oligonucleotide corresponding to the recognition site abolished the interaction. The Nrf2 antiserum disrupted the NRF2/small Maf heterodimer, whereas Nrf1 or Nrf3 specific antisera did not appear to recognize the complex. As expected, the MafG specific antiserum supershifted the complex containing MafG, but also crossreacted with DNA binding complexes comprising MafF or MafK. In contrast, both the MafF and MafK antisera seem to be highly specific and recognized only complexes that comprise either of these small Maf factors. Next, we examined the endogenous DNA binding complexes in JAR cells that have been exposed to 5 μ*M* arsenic for 6 hours. Uninduced cells showed only a minor band, although this basic DNA binding activity appeared to be specific, as competition with nonlabeled oligonucleotides abolished this interaction (Fig. 4, left panel). Exposure to arsenic resulted in the induction of a prominent DNA binding complex (Fig. 4, right panel). This DNA binding activity comprised Nrf2, as addition of the Nrf2 antiserum clearly disrupted the complex. In contrast, antisera specific for the highly homologous Nrf1 and Nrf3 proteins did not appear to have an effect. Addition of the MafG antiserum that crossreacts with all three small Mafs also completely abolished the DNA binding complex, whereas in the presence of the specific MafF and MafK antisera, the complex did not fully disappear. Our results suggest that the arsenic-induced complex in JAR choriocarcinoma cells consists of Nrf2/small Maf heterodimers (Fig. 4), in agreement with the fact that all three small Maf proteins are expressed in these cells (Fig. 2).

Arsenic treatment results in oxidative stress and increase of HO-1 expression

As it has been shown previously, arsenic treatment can lead to increased oxidative stress. We analyzed the production of hydrogen peroxide (H_2O_2) in JAR cells and showed that levels are significantly increased in JAR choriocarcinoma cells (Fig. 5) at 6 hours following exposure to arsenic. As further evidence for oxidative stress, we examined the expression of the enzyme HO-1, a major protein involved in the oxidative stress response, and a target of Nrf2/small Maf DNA binding complexes (1, 15). We found that HO-1 protein levels are strongly upregulated in the presence of arsenic (Fig. 6).

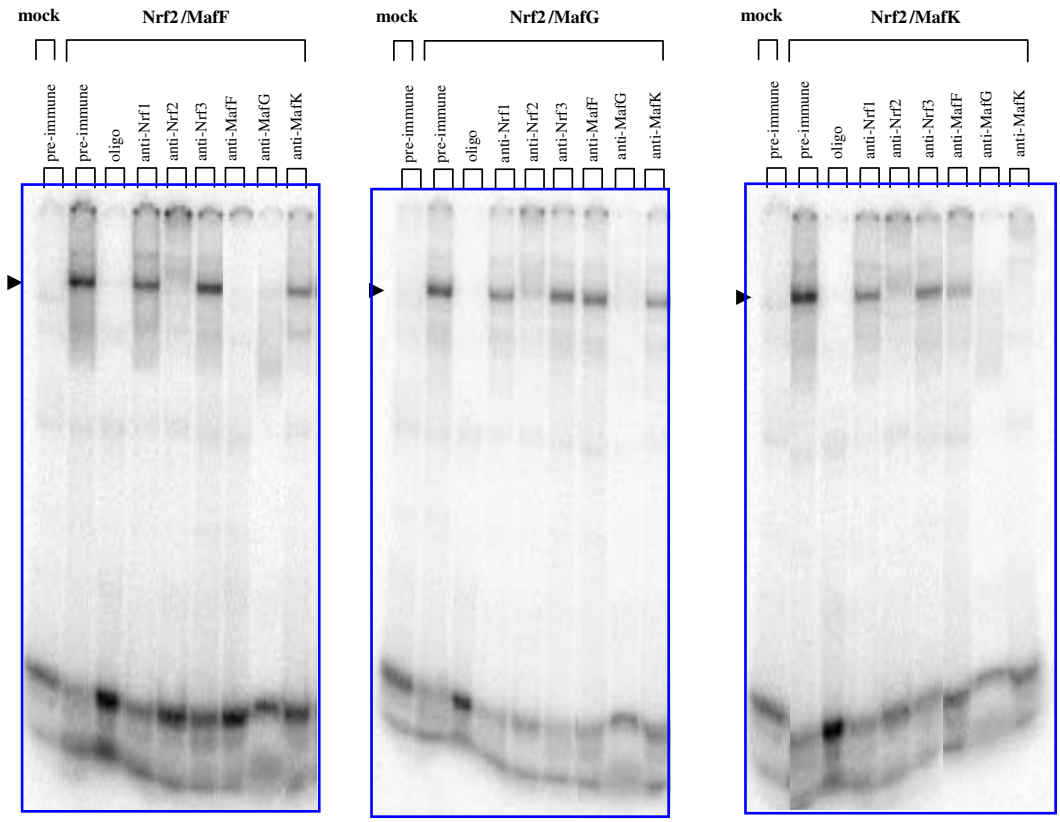

FIG. 3. Specificities of Nrf2 and small Maf antisera. EMSA analysis of nuclear extracts for HEK293T cells transfected or not (*mock*) with expression vectors coding for Nrf2 and either MafF (*left panel*), MafG (*center panel*), or MafK (*right panel*). A stress responsive element (StRE) recognition site derived from the HO-1 promoter has been used as a probe. Nonlabeled competitor StRE oligonucleotide (*oligo*), preimmune serum and Nrf1, Nrf2, Nrf3, MafF, MafG and MafK specific antisera were added to the reaction mix as indicated. *Arrows* indicate the position of the different Nrf2/small Maf heterodimers.

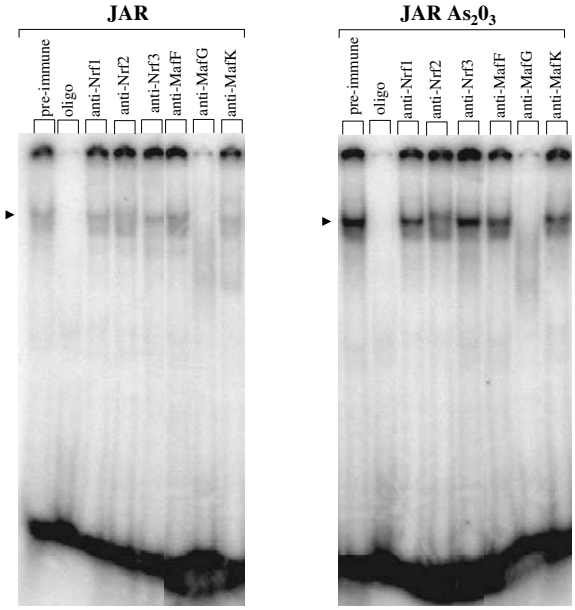

FIG. 4. Induction of Nrf2/small Maf heterodimers by arsenic in choriocarcinoma cells. EMSA analysis of nuclear extracts for JAR choriocarcinoma cells not treated (*left panel*) or treated (*right panel*) with 5 μ*M* arsenic trioxide for 6 hours. A stress responsive element (StRE) recognition site derived from the HO-1 promoter has been used as a probe. Nonlabeled competitor StRE oligonucleotide (*oligo*), preimmune serum and Nrf1, Nrf2, Nrf3, MafF, MafG, and MafK specific antisera were added to the reaction mix as indicated. The *arrow* indicates the position of the endogenous NRF2/small Maf heterodimers.

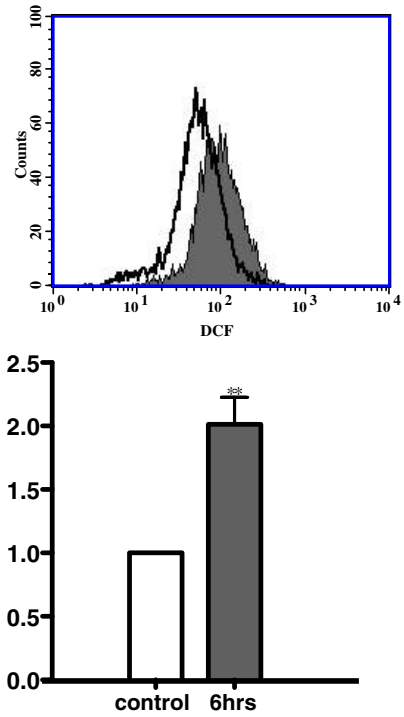

FIG. 5. Intracellular H_2O_2 levels in choriocarcinoma cells upon exposure to arsenic. JAR choriocarcinoma cells were treated with 5 μ*M* arsenic trioxide for 6 hours, and stained with DCFH-DA for detection of intracellular H_2O_2. Fluorescein-positive cells were detected through flow cytometry. The histogram shows the plot for the untreated group (*unshaded*) overlaying that for the treated group (*shaded*). The graph is representative of five experiments.

DISCUSSION

Arsenic is well known as a lethal toxicant, human carcinogen, and neurotoxicant (14). There is also evidence that arsenic is able to cross the placenta, and experimental studies support a role for this semimetal as a reproductive and developmental toxicant (8, 16, 21). In addition, arsenic levels in the placenta are increased in pregnant women living in areas with significant environmental contamination (31). Thus, we analyzed here the effect of arsenic trioxide-induced stress on placental cells, in particular with respect to the cellular levels and DNA binding activity of CNC and small Maf transcription factors. As a model, we used JAR choriocarcinoma cells that have been derived from trophoblastic tumors of the placenta. We clearly established a link between arsenic exposure and the presence of Nrf2 in this placental cell line. Arsenic rapidly induces nuclear Nrf2 levels (Figs. 1 and 2). In addition, we found a strong upregulation of endogenous DNA binding complexes comprising this CNC transcription factor (Fig. 4). Our results thus identify Nrf2 as an important stress regulator in placental cells and confirm earlier data showing that Nrf2 is involved in the response to arsenic insult in other cell types such as peritoneal macrophages, keratinocytes, and osteoblasts (3, 18, 27). From the work of many laboratories, Nrf2 has been established as a major regulator in the response to oxidative and xenobiotic insult in numerous cell types (24).

It has become clear that the BTB protein Keap1 acts as a cytoplasmic repressor of Nrf2 activity (19, 24, 25). Keap1, in association with cullin 3 and the ring finger protein Rbx1/Roc1, appears to form an E3 ubiquitin ligase complex that mediates the rapid degradation of Nrf2 via the proteasome (29, 33). It is known that arsenic perturbs the natural oxida-

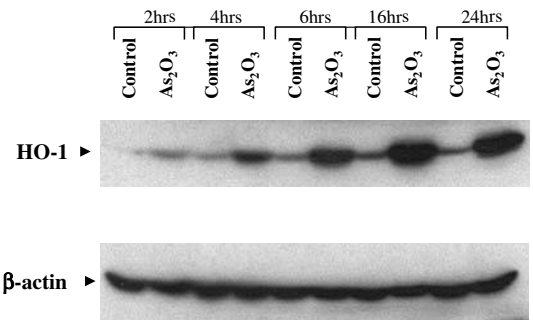

FIG. 6. Arsenic induced upregulation of HO-1 expression. Immunoblot analysis of cytoplasmic extracts prepared from JAR choriocarcinoma cells treated or not (*control*) with 5 μ*M* arsenic trioxide for various lengths of time using antisera specific for HO-1 and β-actin. 30 μg of protein was loaded per well.

tion and reduction balance through a variety of mechanisms that are involved in redox reactions with endogenous oxidants and cellular antioxidant systems (20). This is of interest, as the redox capacities of many proteins reside in the sulfhydryl groups on cysteines. As proposed earlier (12), we believe that the accumulation of nuclear Nrf2 is at least in part mediated by modification of the sulfhydryl groups on cysteines in Keap1 by arsenic. Recent results confirm the existence of redox-sensitive cysteine residues in Keap1 and mutation of a single cysteine in Keap1 has been shown to confer significant resistance to inhibition by either oxidative stress or sulforaphane, a cancer-preventive isothiocyanate (12, 33). It is not clear yet whether oxidative stress and xenobiotics regulate the association of Nrf2 with Keap1 or the association of Keap1 with the degradation machinery or both (24). Recently, it was proposed that it is the ability of Keap1 to assemble into a functional E3 ubiqutin ligase complex that is the critical determinant that controls the steady-state levels of Nrf2 (33). In addition, it has been suggested by several groups that activation of Nrf2 is also regulated by phosphorylation of this transcription factor (24). ERK mitogen activated protein kinase (MAPK) activation has been shown to be required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase gene expression in HepG2 cells (34). Intriguingly, we found that ERK MAPKs are activated in the presence of arsenic in our choriocarcinoma cell model (data not shown). Induction of ERK MAPKs by arsenic has been observed previously in the human HaCat keratinocyte and JP6 mouse epidermal cell lines (9, 17).

In addition to Nrf2, it appears that the small MafF factor expression is induced by arsenic, although the levels of the two other small Mafs MafG and MafK seem not to be modulated. This is of interest, as the small Maf proteins are highly homologous, and functional redundancy has made it difficult to assign specific roles *in vitro* and *in vivo* for each of these factors (4, 23). In our previous study, we found that *mafG* mRNA levels are induced by high concentrations (80 μ*M*) of sodium arsenite at the 2 hour time point in HeLa cells (30). In the present study we analyzed placenta-derived JAR cells and used a concentration of 5 μ*M* arsenic trioxide. Interestingly, in this earlier study we also noticed the induction of a *maf* homologous mRNA species by sodium arsenite, with a transcript size corresponding to that of the *mafF* gene (30). Our results in JAR cells hint at a possible role for MafF in the cellular response to arsenic. Previous studies showed induction of small Maf transcript levels in the response to oxidative stress and electrophiles (10, 22, 30, 32). Arsenic clearly causes oxidative stress in our JAR choriocarcinoma cell model, as detected by an increase in intracellular H_2O_2 levels (Fig. 5). In addition, the expression of the HO-1 protein, the rate-limiting enzyme for heme degradation and a major stress responsive protein, is significantly induced by arsenic in JAR cells (Fig. 6). Our results are in agreement with earlier reports showing upregulation of HO-1 in the presence of arsenic (3, 18).

In conclusion, we provide here the first evidence that CNC/small Maf complexes may also play a role in the cellular stress response in placental cells. This is important as arsenic appears to cause developmental toxicity leading to spontaneous abortions, birth defects, and embryonic lethality (8, 16, 21). In addition, oxidative stress in placental cells is associated with common pregnancy-related pathologies including miscarriage and preeclampsia (6). In future studies, it will be of interest to identify the placenta-specific target genes of Nrf2/small Maf heterodimers. Further investigation of the signaling mechanisms involved in the cellular response to toxic insults in placental cells should provide novel insights into gestational disorders as well as into other arsenic-associated pathologies such as cancer.

ABBREVIATIONS

BTB, broad complex–tramtrack–bric-a-brac; EMSA, electrophoretic mobility shift assay; HO-1, heme oxygenase-1; H_2O_2, hydrogen peroxide; Keap1, Kelch-like ECH associated protein 1; StRE, stress response element.

ACKNOWLEDGMENTS

The authors thank Myrian Colombo for help with FACS analysis, and Benoît Chénais for discussions. Special thanks go to Koren Mann and Zuanel Diaz for their generous advice and helpful discussions. VB acknowledges a *Chercheur Boursier* award of the *Fonds de la Recherche en Sante du Quebec*. This study was supported by a McGill Major studentship to WM and by a Cancer Research Society Inc. grant to VB.

REFERENCES

1. Alam J, Killeen E, Gong P, Naquin R, Hu B, Stewart D, Ingelfinger JR, and Nath KA. Heme activates the heme oxygenase-1 gene in renal epithelial cells by stabilizing Nrf2. *Am J Physiol Renal Physiol* 284: F743–F752, 2003.
2. Andrews NC, and Faller DV. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res* 19: 2499, 1991.
3. Aono J, Yanagawa T, Itoh K, Li B, Yoshida H, Kumagai Y, Yamamoto M, and Ishii T. Activation of Nrf2 and accumulation of ubiquitinated A170 by arsenic in osteoblasts. *Biochem Biophys Res Commun* 305: 271–277, 2003.
4. Blank V, and Andrews NC. The Maf transcription factors: regulators of differentiation. *Trends Biochem Sci* 22: 437–441, 1997.
5. Blank V, Kim MJ, and Andrews NC. Human MafG is a functional partner for p45 NF-E2 in activating globin gene expression. *Blood* 89: 3925–3935, 1997.
6. Burton GJ, and Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig* 11: 342–352, 2004.
7. Chenais B, Derjuga A, Massrieh W, Red-Horse K, Bellingard V, Fisher SJ, and Blank V. Functional and placental expression analysis of the human NRF3 transcription factor. *Mol Endocrinol* 19: 125–137, 2005.
8. Concha G, Vogler G, Lezcano D, Nermell B, and Vahter M. Exposure to inorganic arsenic metabolites during early human development. *Toxicol Sci* 44: 185–190, 1998.

9. Cooper KL, Myers TA, Rosenberg M, Chavez M, and Hudson LG. Roles of mitogen activated protein kinases and EGF receptor in arsenite-stimulated matrix metalloproteinase-9 production. *Toxicol Appl Pharmacol* 200: 177–185, 2004.
10. Crawford DR, Leahy KP, Wang Y, Schools, GP, Kochheiser JC, and Davies KJ. Oxidative stress induces the levels of a MafG homolog in hamster HA-1 cells. *Free Radic Biol Med* 21: 521–525, 1996.
11. Davison K, Cote S, Mader S, and Miller WH. Glutathione depletion overcomes resistance to arsenic trioxide in arsenic-resistant cell lines. *Leukemia* 17: 931–940, 2003.
12. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, and Talalay P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci USA* 99: 11908–11913, 2002.
13. Furukawa M, and Xiong Y. BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. *Mol Cell Biol* 25: 162–171, 2005.
14. Golub MS. Maternal toxicity and the identification of inorganic arsenic as a developmental toxicant. *Reprod Toxicol* 8: 283–295, 1994.
15. Gong P, Hu B, Stewart D, Ellerbe M, Figueroa YG, Blank V, Beckman BS, and Alam J. Cobalt induces heme oxygenase-1 expression by a hypoxia-inducible factor-independent mechanism in Chinese hamster ovary cells: regulation by Nrf2 and MafG transcription factors. *J Biol Chem* 276: 27018–27025, 2001.
16. Hopenhayn C, Ferreccio C, Browning SR, Huang B, Peralta C, Gibb H, and Hertz-Picciotto I. Arsenic exposure from drinking water and birth weight. *Epidemiology* 14: 593–602, 2003.
17. Huang C, Ma WY, Li J, Goranson A, and Dong Z. Requirement of Erk, but not JNK, for arsenite-induced cell transformation. *J Biol Chem* 274: 14595–14601, 1999.
18. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, Bannai S, and Yamamoto M. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J Biol Chem* 275: 16023–16029, 2000.
19. Jaiswal AK. Nrf2 signaling in coordinated activation of antioxidant gene expression. *Free Radic Biol Med* 36: 1199–1207, 2004.
20. Miller WH Jr, Schipper HM, Lee JS, Singer J, and Waxman S. Mechanisms of action of arsenic trioxide. *Cancer Res* 62: 3893–3903, 2002.
21. Milton AH, Smith W, Rahman B, Hasan Z, Kulsum U, Dear K, Rakibuddin M, and Ali A. Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. *Epidemiology* 16: 82–86, 2005.
22. Moran JA, Dahl EL, and Mulcahy RT. Differential induction of mafF, mafG and mafK expression by electrophile-response-element activators. *Biochem J* 361: 371–377, 2002.
23. Motohashi H, O'Connor T, Katsuoka F, Engel J, and Yamamoto M. Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors. *Gene* 294: 1–12, 2002.
24. Motohashi H, and Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. *Trends Mol Med* 10: 549–557, 2004.
25. Nguyen T, Yang CS, and Pickett CB. The pathways and molecular mechanisms regulating Nrf2 activation in response to chemical stress. *Free Radic Biol Med* 37: 433–441, 2004.
26. Pattillo RA, Ruckert AC, Hussa RO, Bernstein R, and Delfs E. The JAR cell line, continuous human multihormone production and controls. *In Vitro* 6: 398–399, 1971.
27. Pi J, Qu W, Reece JM, Kumagai Y, and Waalkes MP. Transcription factor Nrf2 activation by inorganic arsenic in cultured keratinocytes: involvement of hydrogen peroxide. *Exp Cell Res* 290: 234–245, 2003.
28. Pulido MD, and Parrish AR. Metal-induced apoptosis: mechanisms. *Mutat Res* 533: 227–241, 2003.
29. Stewart D, Killeen E, Naquin R, Alam S, and Alam J. Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium. *J Biol Chem* 278: 2396–2402, 2003.
30. Suzuki T, Blank V, Sesay JS, and Crawford DR. Maf genes are involved in multiple stress response in human. *Biochem Biophys Res Commun* 280: 4–8, 2001.
31. Tabacova S, Baird DD, Balabaeva L, Lolova D, and Petrov I. Placental arsenic and cadmium in relation to lipid peroxides and glutathione levels in maternal-infant pairs from a copper smelter area. *Placenta* 15: 873–881, 1994.
32. Wild AC, Moinova HR, and Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J Biol Chem* 274: 33627–33636, 1999.
33. Zhang DD, Lo SC, Cross JV, Templeton DJ, and Hannink M. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol Cell Biol* 24: 10941–10953, 2004.
34. Zipper LM, and Mulcahy RT. Erk activation is required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells. *Toxicol Sci* 73: 124–134, 2003.

Address reprint requests to:
Dr. Volker Blank
Lady Davis Institute for Medical Research
Department of Medicine
McGill University
3755 Cote Sainte-Catherine Road
Montreal, Quebec H3T 1E2
Canada

E-mail: volker.blank@mcgill.ca

Received for publication June 12, 2005; accepted July 1, 2005.

This article has been cited by:

1. Meenakshi B. Kannan, Vera Solovieva, Volker Blank. 2012. The small MAF transcription factors MAFF, MAFG and MAFK: Current knowledge and perspectives. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1823**:10, 1841-1846. [CrossRef]
2. Dona Sinha, Jaydip Biswas, Anupam Bishayee. 2012. Nrf2-mediated redox signaling in arsenic carcinogenesis: a review. *Archives of Toxicology* . [CrossRef]
3. Hongyi Qi, Baowei Chen, X. Chris Le, Jianhui Rong. 2012. Concomitant Induction of Heme Oxygenase-1 Attenuates the Cytotoxicity of Arsenic Species from Lumbricus Extract in Human Liver HepG2 Cells. *Chemistry & Biodiversity* **9**:4, 739-754. [CrossRef]
4. Xinyan Zhang, Ying Su, Min Zhang, Zheng Sun. 2011. Opposite effects of arsenic trioxide on the Nrf2 pathway in oral squamous cell carcinoma in vitro and in vivo. *Cancer Letters* . [CrossRef]
5. Roberta Venè , Patrizia Castellani , Laura Delfino , Maria Lucibello , Maria Rosa Ciriolo , Anna Rubartelli . 2011. The Cystine/Cysteine Cycle and GSH Are Independent and Crucial Antioxidant Systems in Malignant Melanoma Cells and Represent Druggable Targets. *Antioxidants & Redox Signaling* **15**:9, 2439-2453. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
6. Bing Li, Xin Li, Bo Zhu, Xinyu Zhang, Yi Wang, Yuanyuan Xu, Huihui Wang, Yongyong Hou, Quanmei Zheng, Guifan Sun. 2011. Sodium arsenite induced reactive oxygen species generation, nuclear factor (erythroid-2 related) factor 2 activation, heme oxygenase-1 expression, and glutathione elevation in Chang human hepatocytes. *Environmental Toxicology* n/a-n/a. [CrossRef]
7. Swaran J.S. Flora, Vidhu Pachauri, Geetu SaxenaArsenic, cadmium and lead 415-438. [CrossRef]
8. Marie Vahter. 2009. Effects of Arsenic on Maternal and Fetal Health. *Annual Review of Nutrition* **29**:1, 381-399. [CrossRef]
9. Yu Du, Nicole F. Villeneuve, Xiao-Jun Wang, Zheng Sun, Weimin Chen, Jixue Li, Hongxiang Lou, Pak Kin Wong, Donna D. Zhang. 2008. Oridonin Confers Protection against Arsenic-Induced Toxicity through Activation of the Nrf2-Mediated Defensive Response. *Environmental Health Perspectives* **116**:9, 1154-1161. [CrossRef]
10. Volker Blank. 2008. Small Maf Proteins in Mammalian Gene Control: Mere Dimerization Partners or Dynamic Transcriptional Regulators?. *Journal of Molecular Biology* **376**:4, 913-925. [CrossRef]
11. Marie Vahter. 2008. Health Effects of Early Life Exposure to Arsenic. *Basic & Clinical Pharmacology & Toxicology* **102**:2, 204-211. [CrossRef]
12. Z NOUHI, G CHEVILLARD, A DERJUGA, V BLANK. 2007. Endoplasmic reticulum association and N-linked glycosylation of the human Nrf3 transcription factor. *FEBS Letters* . [CrossRef]
13. Dr. Jawed Alam . 2006. The Mammalian Cap and Collar Family of Transcription Factors. *Antioxidants & Redox Signaling* **8**:1-2, 39-42. [Citation] [Full Text PDF] [Full Text PDF with Links]