Inorganic sodium arsenite (iAs), a ubiquitous element, is one of the most toxic metals present in the natural environment (Bagla and Kaiser 1996). Arsenicals are found as naturally occurring constituents of soil, food, and drinking water (Wu et al. 1989; Yoshida et al. 2004), and exposure to iAs has been associated with a variety of disease outcomes, including disorders of the skin, urinary bladder, liver, and lung (Tchounwou et al. 2004). In particular, skin hyperkeratosis is a characteristic dermatologic lesion associated with ingestion of arsenic from contaminated groundwater (McLellan 2002; Yoshida et al. 2004). There is also a significant association between hyperkeratosis, nonmelanoma skin cancer (e.g., basal cell carcinoma and squamous cell carcinoma), and Bowen disease (Col et al. 1999; Rossman et al. 2004). Furthermore, the pathologic features associated with arsenic-induced hyperkeratosis present as typical acanthotic types of keratosis present as typical acanthotic types of keratosis, with cornified layer and hyperkeratosis (McMahon et al. 2003). Upon exposure of the skin to arsenicals and arsenic compounds, the skin, esophagus, and forestomach of Keap1-deficient mice show cornified layer and hyperkeratosis phenotypes. In addition, previous studies have also shown that the expression of NRF2 and ARE-controlled genes is induced by iAs in some cell types (Pi et al. 2003; Sakurai et al. 2005). Furthermore, histochemical analyses have indicated that the expression of K16 is increased in Bowen disease, basal cell carcinoma, and squamous cell carcinoma induced by arsenicals (Yu et al. 1993). However, it remains to be determined whether NRF2 can regulate the transcriptional activation of K16 upon iAs exposure in human keratinocytes. Hence, these findings prompted us to investigate the molecular mechanisms underlying the regulation of the K16 gene by iAs-induced NRF2 mediation.

**Materials and Methods**

**Chemicals and reagents.** A purified preparation of inorganic sodium arsenite (iAs; NaAsO₂; Merck, Darmstadt, Germany) was dissolved in phosphate-buffered saline (PBS) and added directly to the culture medium. A fresh iAs solution was prepared for each new experiment. Cycloheximide (CHX), dimethylsulfoxide (DMSO), and a protease inhibitor cocktail were purchased from Sigma (St. Louis, MO, USA). CHX was dissolved in DMSO and stored –20°C until use.

**Cells and culture conditions.** The human keratinocyte HaCaT cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA). CHX was dissolved in DMSO and added directly to the cell culture medium. The cells were maintained in growth medium (Gibco) supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin, 50 units/mL and 50 µg/mL, respectively). The cells were passaged weekly to maintain a confluence of 70-80%.
products were separated on a 1.8% agarose gel and stained with ethidium bromide.

**Western blot analysis.** We performed Western blot analysis as described previously (Sugioka et al. 2004). Briefly, nuclei and cytoplasmic proteins were extracted using the NE-PER nuclear and cytoplasmic extraction kit (Pierce, Rockford, IL, USA) according to the manufacturer’s protocol. For protein extraction, the cells were lysed in a buffer containing complete protease inhibitor cocktail. We measured protein concentrations using the DC Protein Assay Kit (Bio-Rad, Richmond, CA, USA). Equal amounts of protein were then resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane (Amersham Biosciences, Bucks, UK). Immunoblotting was carried out with specific antibodies in Tris-buffered saline with 0.05% Tween 20. The primary antibodies were as follows: K16 (Neomarkers, Fremont, CA, USA), NRF2 and KEAP1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), c-Jun (Cell Signaling, Beverly, MA, USA), and β-actin (Sigma). After washing, the membranes were probed with horseradish peroxidase-conjugated secondary antibodies and developed by chemiluminescence using the ECL Plus Detection Kit (Amersham Biosciences).

**Plasmids, transfections, and luciferase assays.** Human K16 promoter regions of varying lengths (pXK-1, 3, 4, 5, 1, and 5-2) were provided by Y-N Wang (National Cheng Kung University, Taiwan). These DNA fragments were prepared from HaCaT cells and were ligated into the pXP-1 luciferase vector (Wang and Chang 2003). The p3xARE/Luc vector, harboring three tandem repeats of ARE, was donated by X.L. Chen (Discovery Research, AtheroGenics Inc., Alpharetta, GA, USA) (Chen et al. 2003). The wild-type NRF2 expression vector (WT-NRF2) was a gift from H.S. So (Wonkwang University School of Medicine, Korea) (So et al. 2006). NRF2 cDNA was subcloned into a pcDNA3.1(+) vector (Invitrogen, San Diego, CA, USA). For the transfection of reporter plasmids, we seeded HaCaT cells into six-well plates at a density of 80% the previous day. Cells were then transfected with a total of each luciferase reporter construct (2.5 µg) using LipofectAMINE plus (Invitrogen). To control for the efficiency of transfection, Renilla luciferase gene expression was monitored using either the pRL-CMV or pRL-TK vectors (Promega, Madison, WI, USA). For overexpression of WT-NRF2, we normalized the total plasmid concentration using the pcDNA3.1(+) empty vector. Thirty-six hours after transfection, the medium was replaced with fresh medium containing either vehicle (PBS) or iAs for 6 hr. After iAs exposure, we harvested cells and analyzed them for luciferase activity using a Dual-Luciferase Reporter Assay System (Promega).

For the investigation of the role of NRF2 in regulating K16 gene expression, transfection of an NRF2 expression plasmid into HaCaT cells was carried out using LipofectAMINE 2000 (Invitrogen). Cells were cultured in 100-mm plates 24 hr before transfection. The expression plasmid WT-NRF2 (15 µg) was then transfected into the cells for 48–60 hr. As a negative control, we used 15 µg of the pcDNA3.1(+) empty vector.

**Electrophoretic mobility-shift assays (EMSA).** We extracted and measured nuclear proteins as described above. Nuclear protein/DNA complexes were subjected to electrophoresis in nondenaturing 5% polyacrylamide gels containing 2% glycerol in 0.25% Tris-borate/EDTA buffer and transferred to Hybond-N+ nylon transfer membranes (Amersham Biosciences) for detection using the Light-Shift EMSA kit (Pierce) according to the manufacturer’s protocol, with minor modifications. We incubated 10-µg aliquots of nuclear extract with the DNA probe in a binding reaction buffer containing 10 mM Tris/HCl (pH 7.6), 50 mM KCl, 0.5 mM dithiothreitol, 0.25 mM EDTA, 5% glycerol, 2.5 mM MgCl2, 0.05% NP-40 detergent, and 2 µg of poly(dI-dC)/poly(dI-dC) for 30 min at room temperature. For supershift assays, 2 µg of either a polyclonal anti-NRF2 or an anti-c-Jun antibody (Santa Cruz Biotechnology) was added to the nuclear extract. For competition assays, we added 0.1 µM of an unlabeled 22-mer DNA oligonucleotide probe (Sugioka et al. 2004). For EMSA, we used a consensus ARE probe (Kanagawa, Japan) as the probe.
was purchased from Panomics, Inc. (Redwood City, CA, USA). For competition binding of the K16 ARE-complexes, we used an unlabeled AP-1 consensus oligonucleotide (5’-TATCGATAAAGCTATGAGTCATCCCCGGG-3’). The binding specificity was confirmed in each case by the addition of a 100-fold molar excess of unlabeled oligonucleotide.

**CHX chase experiment.** We investigated the posttranscriptional regulation of both the steady-state levels and half-life of the NRF2 protein by CHX chase analysis. Cells were incubated in serum-free medium in the absence or presence of iAs for 6 hr. The culture medium was then replaced with serum-free containing CHX (100 µg/mL). We prepared cell lysates at 0, 10, 30, 60, 120, and 240 min after iAs treatment. Whole-cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies against NRF2.

**Statistics.** All the data generated from at least three independent experiments and expressed as the mean ± SD were analyzed by the Student’s t-test. Statistical comparisons were made by logarithmic transformation of the normalized values. We considered p-values < 0.01 to be statistically significant.

**Results**

**K16 expression is induced by iAs in HaCaT cells.** We wanted to determine whether the K16 mRNA is transcriptionally regulated by iAs, and treated HaCaT cells with this compound for various time periods over a range of doses. After treatment of HaCaT cells with 1–20 µM iAs, the expression of K16 mRNA was increased compared with the control at 6 hr (Figure 1A) but had declined to basal levels at 24 hr. The increase in the K16 protein levels after 6 hr of iAs exposure was just detectable at 10–20 µM, but a dose-dependent increase was more evident at 10 hr (Figure 1B). This enhancement of K16 expression had declined to basal levels at 24 hr.

**Identification of the iAs responsive region in the K16 gene promoter.** To investigate the mechanisms underlying the enhancement of the K16 gene by iAs, we first examined the response of the K16 regulatory region to this compound using a luciferase reporter gene assay. The dose-dependent activation of K16 transcription following iAs treatment was observed with a construct containing a 515-bp fragment of the K16 promoter (Figure 2A). To further elucidate the region containing the iAs responsive element, we examined a series of deletions of the 5'-flanking region of K16 gene. The ARE sequence in the pXK-5–1 vector contains an activator protein-1 (AP-1)-like element followed by a GC box. As shown in Figure 2B, an enhancement in the reporter activity levels was observed for the promoter constructs, pXK-1, 3, 4, and 5–1, in response to 20 µM iAs. A decline in reporter activity, however, depended on the number of AP-1-like sites, and the results for the pXK-5–1 construct show also that ARE is activated by iAs. In contrast, no significant activation was observed using a pXK-5–2 construct in response to 20 µM iAs.

**Expression of AP-1 transcription factors and c-Jun production following iAs treatment.** We examined AP-1 transcription factors c-Jun and c-Fos expression in iAs-treated HaCaT cells by semiquantitative RT-PCR. iAs-induced c-Jun expression was observed during the first 3 hr after treatment (data not shown). An appreciable induction of c-Jun was also confirmed after 6 hr, but this was down-regulated by 24 hr after iAs treatment (Figure 3A). In contrast, the expression of c-Fos was only transiently detectable at 3 hr (data not shown) but was not observed during the 6–24 hr

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**Figure 2.** Response of the K16 regulatory region to iAs using a luciferase reporter gene assay, with relative luciferase activities normalized for pRL-CMV activity and calculated as a percentage of the promoter activity in HaCaT cells without iAs exposure (control). Abbreviations: +, presence; –, absence; Con, control. (A) Transcriptional activation of K16 gene by iAs (left) and the luciferase reporter gene construct harboring the –515-bp K16 gene promoter region (pXK–1; right). (B) Luciferase activity of the iAs responsive region in the K16 gene promoter in cells cotransfected with individual reporter constructs harboring various deletions of the 5’-flanking region of the K16 gene promoter (left) and normalized for pRL-CMV, and the luciferase reporter gene constructs for individual promoter regions (right). See “Materials and Methods” for details of experiments. The values shown indicate the ratio of the luciferase activities in untreated cells and in cells treated with 20 µM iAs for 6 hr; luciferase activity with no treatment was defined as 100%. Values represent the mean ± SD of five to eight independent experiments, each run in triplicate. Each p-value was obtained by t-test following logarithmic transformation of normalized values.

* p < 0.01, and ** p < 0.001 compared with the untreated group.
period of this experiment. As shown in Figure 3B, iAs-enhanced c-Jun production can be observed in a dose-dependent manner at 6 hr, but it declines from 10 to 24 hr.

iAs potently induces the translocation of NRF2 and activates the ARE of the K16 promoter. The results of our reporter assays suggested that iAs stimulates not only the AP-1–like sites but also the ARE site within the K16 gene promoter in HaCaT cells (Figure 3). In addition, several oxidative stress agents and toxic chemicals, including iAs, have been reported to induce the expression of ARE–dependent genes in several cell types (Pi et al. 2003; Sakurai et al. 2005). On the basis of our observations and some recent reports, we thus hypothesized that iAs would have the ability to activate the ARE of the K16 gene promoter directly, resulting in the induction of K16 expression in HaCaT cells. To confirm that the K16 ARE indeed functions as an iAs-responsive transcriptional control element, we performed transient transfections of HaCaT cells with a p3xARE/Luc construct and then subjected these cells to iAs for 6 hr. As shown in Figure 4A, treatment of HaCaT cells with iAs results in a dramatic increase in ARE-driven promoter activity. Likewise, EMSA using a consensus ARE probe show that iAs-induced ARE-binding complexes increase markedly, in a dose-dependent fashion (Figure 4B). These results indicate that iAs has the ability to activate the ARE-driven genes. We performed further EMSA experiments using an ARE probe specific to the K16 proximal promoter region (WT-K16ARE) and found that K16ARE–nuclear protein complexes formation is augmented by iAs in a dose-dependent manner (Figure 4C). Moreover, the formation of these complexes is specifically inhibited by the addition of excess unlabeled oligonucleotide competitor (Figure 4B,C), whereas an excess of an unlabeled AP-1 probe competes only marginally for K16ARE binding (Figure 4C).

The NRF2 transcription factor has been shown to bind to AREs upon translocation into the nucleus, resulting in the induction of ARE–mediated genes (Wakabayashi et al. 2004). To examine whether iAs induces and translocates NRF2 into the nucleus in HaCaT cells, we treated these cells with iAs for either 3 or 6 hr. As shown in Figure 4D, a dose-dependent accumulation of NRF2 protein was observed in the nucleus upon treatment with iAs for 6 hr. This was not observed in the parallel experiment performed over the 3-hr time course.

Supershift EMSA analysis using an NRF2 antibody showed that the iAs-induced and iAs-translocated NRF2 protein binds to the WT-K16ARE probe containing the ARE sequence of the K16 proximal promoter region (5’-GGAGTCAAG-3’) that comprises an AP-1–like site and a GC box, whereas the supershift of c-Jun was not observed (Figure 4E). To identify whether the GC box is dispensable for the iAs-stimulated binding activity of NRF2, we next performed EMSA analyses with either WT- or a Mut-K16ARE probe containing an intact AP-1–like element but a mutated GC box. As shown in Figure 4F, the K16ARE–nuclear protein complexes and supershifted bands that were enhanced by iAs treatment were largely abolished by the addition of the Mut-K16ARE probe.

iAs stabilizes the NRF2 protein. We examined the effects of iAs treatment on the function of KEAP1 in HaCaT cells. Treatment with iAs did not alter the expression levels of KEAP1 mRNA or protein over either a 3 or 6 hr time course (Figure 5A). Next, we examined the effects of iAs on the
expression of NRF2 mRNA in HaCaT cells. Exposure to iAs did not significantly alter the steady-state levels of NRF2 mRNA (data not shown). Production of NRF2 protein, however, was observed to increase in both a dose- and time-dependent manner (Figure 4D). To further examine the stabilization of NRF2 protein by iAs, we monitored the decay of basal and iAs-induced NRF2 proteins after inhibition of protein synthesis by CHX (Figure 5B). The results of this analysis revealed that the NRF2 protein levels decrease by approximately 50% within 30 min of treatment with CHX in cells that had not been exposed to iAs. Only trace amounts of NRF2 are then detectable after 60 min of exposure to CHX in these cells. The HaCaT cells were then pretreated with iAs for 6 hr before their exposure to CHX in a similar timecourse experiment. The levels of NRF2 in these iAs-treated cells were again found to decrease by about 50%, but only after 120 min of CHX exposure.

**NRF2 plays a crucial role in the regulation of K16 gene expression in HaCaT cells.** To confirm the functional role of NRF2 in the induction of K16 gene expression by iAs, we investigated whether the expression of K16 mRNA is induced by the overexpression of NRF2 (WT-NRF2) in HaCaT cells. We also investigated the expression of the detoxification gene TXN, which is highly induced by a variety of oxidative stimuli through NRF2-mediated ARE transactivation (Kim et al. 2001). The expression of TXN gene in untransfected cells after treatment with iAs was stronger than that of the control cells (Figure 6A). When the cells were transfected with WT-NRF2 and then treated with or without iAs, the expression of TXN mRNA was augmented markedly compared with the empty-vector control. Similarly, the expression of K16 mRNA was also induced in cells transfected with WT-NRF2 in the absence or presence of iAs. We next performed a transient transfection of HaCaT cells with the pXX-5–1 luciferase vector together with the WT-NRF2 vector. The overexpression of NRF2 in increasing concentrations resulted in significant enhancement of the ARE-mediated K16 promoter activation (Figure 6B).

**Discussion**

In the present study, we showed for the first time that iAs induces the transcriptional activation of K16 in the human keratinocyte cell line, HaCaT, through the ARE present in its gene promoter. It has been reported previously that treatment with iAs enhances the production and translocation of NRF2 into the nucleus in several cell types. However, until now it has remained uncertain whether the induction of NRF2 by iAs mediates the transcriptional activation of the K16 gene in keratinocytes. In our current experiments, we demonstrated that iAs elongates the half-life of the NRF2 protein, which results in its increased expression levels. Furthermore, this iAs-induced NRF2 protein was shown to bind to the ARE sequences in the promoter region of the K16 gene. Finally, by overexpressing NRF2, we have clarified that its induction is involved in not only the gene expression of the detoxification gene TXN, but also in the upregulation of K16 expression in HaCaT cells through the ARE in the K16 gene promoter. These experiments indicate an important and novel function for NRF2 in the regulation of K16 in keratinocytes and also help to further explain the molecular mechanisms underlying arsenic-mediated epidermal hyperkeratosis.

In our present experiments, the expression levels of K16 mRNA and protein were indeed found to be enhanced by iAs in a dose-dependent manner (Figure 1). In addition, luciferase assays of the K16 promoter revealed that iAs enhances its activity in a dose-dependent fashion, which is stimulated by AP-1–like sites and an ARE (Figure 2). The promoter of the human K16 gene was recently cloned and sequenced, and several AP-1–like sites were found within the –515–bp region of the gene (Wang and Chang 2003). AP-1 transcription factor can be formed by the dimerization of either Jun or Jun/Fos family members (Eferl and Wagner 2003). In the present study, the increased expression of c-Jun, but not c-Fos was evident in the nuclei of HaCaT cells after iAs treatment (Figure 3). Our findings thus suggest that the activation of c-Jun/AP-1 is one of the essential steps in the regulation of K16 gene expression by iAs exposure in HaCaT cells.

It has been well documented that the ARE core sequence includes an AP-1–like binding site (TGAC/GTCA), followed by a GC box (Rushmore et al. 1991; Xie et al.
1995). We have found in our current analyses that the AP-1–like site within the K16 promoter region from -148 to -140 bp (5′-GGAGTCAGC-3′) resembles a consensus ARE sequence. Recent studies have shown that AREs can be specifically bound by complexes of several basic-leucine zipper transcription factors, including NRF2 (Ishii et al. 2000; Moi et al. 1994). NRF2 heterodimerizes with either AP-1 or small MAF (MAFG, MAFK, and MAFF) proteins (Maejima et al. 1997; Wild et al. 2003). Laminae flow induction of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 278:703–711.

Cill C, Col C, Saron A, Sayil BS, Ozlik S. 1999. Arsenic-related disease, palmar keratosis, and skin cancer. Environ Health Perspect 107:687–698.

Dinkova-Kostova AT, Holczewski WD, Cole RN, Itoh K, Wakabayashi N, Kato Y, et al. 2002. Direct evidence that sulphydryl 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.

Eferl R, Wagner EF. 2003. AP-1: a double-edged sword in carcinogenesis. Nat Rev Cancer 3:773–782.

Endo et al. 2007. Distribution of protein kinase C in response to antioxidants leads to the release of NRF2 from IκB, but is not required for NRF2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H:quinoine oxidoreductase-1 gene expression. J Biol Chem 278:4465–44662.

Chen XL, Yanar SE, Rao AS, Grey JY, Thomas S, Cook CK, et al. 2003. Production of Nrf2 in a mouse skin model. J Invest Dermatol 121:1067–1073.

Kim YG, Psaroudi N, Leseur B, Crouse D, Green D, Kripke M. 2003. NRF2/Keap1 interaction blocks the nuclear translocation of NRF2 from the cytoplasm to the nucleus. J Biol Chem 278:43293–43302.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.

Lee CH, Chen JS, Sun YL, Liao WT, Zheng YW, Chai CZ, et al. 2006. Defective beta1-integrins expression in arsenical keratosis and arsenic-treated cultured human keratinocytes. J Cutan Pathol 33:129–138.

McCullen F. 2002. Arsenic contamination affects millions in Bangladesh. Lancet 359:1127.

McManus M, Itoh K, Yamamoto M, Chana S, Henderson CJ, McLellan LI, et al. 2001. The Cap’n’Collar basic leucine zipper transcription factor Nrf2 (NFE2) is required for the transcriptional activation of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 276:18399–18406.
Arsenic induces Nrf2-mediated keratin 16 gene expression

Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. J Biol Chem 278:4536–4541.

Pi J, Du W, Reece JM, Kumagai Y, Waalkes MP. 2003. Transcription factor Nrf2 activation by inorganic arsenic in cultured keratinocytes: involvement of hydrogen peroxide. Exp Cell Res 290:234–245.

Rosman TG, Uddin AN, Burns FJ. 2004. Evidence that arsenite acts as a cocarcinogen in skin cancer. Toxicol Appl Pharmacol 198:394–404.

Rushmore TH, Morton MR, Pickett CB. 1991. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. J Biol Chem 266:11632–11639.

Sakurai A, Nishimoto M, Himeno S, Imura N, Tsujimoto M, Kunimoto M, et al. 2005. Transcriptional regulation of thioredoxin reductase 1 expression by cadmium in vascular endothelial cells: role of NF-E2-related factor-2. J Cell Physiol 203:529–537.

So HS, Kim HJ, Lee JM, Park SY, Park C, Kim YH, et al. 2006. Flunarizine induces Nrf2-mediated transcriptional activation of heme oxygenase-1 in protection of auditory cells from cisplatin. Cell Death Differ 13:1763–1775.

Stewart D, Kileen E, Naquin R, Alam S, Alam J. 2003. Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium. J Biol Chem 278:2396–2402.

Sugio Y, Watanabe T, Inagaki Y, Kushida M, Nioka M, Endo H, et al. 2004. c-Jun Nterminal kinase pathway is involved in constitutive matrix metalloproteinase-1 expression in a hepatocellular carcinoma-derived cell line. Int J Cancer 109:867–874.

Takahashi K, Fölmer J, Coulombe PA. 1994. Increased expression of keratin 16 causes anomalies in cytoarchitecture and keratinization in transgenic mouse skin. J Cell Biol 127:505–520.

Tchounwou PB, Centeno JA, Patiolla AK. 2004. Arsenic toxicity, mutagenesis, and carcinogenesis—a health risk assessment and management approach. Mol Cell Biochem 255:47–55.

Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, et al. 2004. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. Proc Natl Acad Sci USA 101:2040–2045.

Wakabayashi N, Itoh K, Wakabayashi J, Motohashi H, Noda S, Takahashi S, et al. 2003. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. Nat Genet 35:238–245.

Wang YN, Chang WC. 2003. Induction of disease-associated keratin 16 gene expression by epidermal growth factor is regulated through cooperation of transcription factors Sp1 and c-Jun. J Biol Chem 278:45848–45857.

Wasserman WW, Fahl WE. 1997. Functional antioxidant responsive elements. Proc Natl Acad Sci USA 94:5361–5366.

Wild AC, Gipp JJ, Mulcahy T. 1998. Overlapping antioxidant response element and PMA response element sequences mediate basal and beta-naphthoflavone-induced expression of the human gamma-glutamylcysteine synthetase catalytic subunit gene. Biochem J 332(Pt 2):373–381.

Wu MM, Kuo TL, Hwang YH, Chen CJ. 1988. Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. Am J Epidemiol 130:1123–1132.

Xie T, Belinsky M, Xu Y, Jainwall AK. 1995. ARE- and TRE-mediated regulation of gene expression. Response to xenobiotics and antioxidants. J Biol Chem 270:6894–6900.

Yoshida T, Yamauchi H, Fan Sun G. 2004. Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review. Toxicol Appl Pharmacol 198:243–252.

Yu HS, Chiu KS, Chen DS, Yang RC, Chang SF. 1993. Progressive alterations of cytokeratin expressions in the process of chronic arsenicism. J Dermatol 20:741–745.