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Transactivation of RARE and GRE in the cellular response to arsenic

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Abstract

Arsenic compounds are a somewhat unique class of metals, which have been considered as both carcinogens and chemotherapeutic agents for cancers. Tumor promotion effects of arsenic are believed to be associated with its transactivational activities on transcription factors, such as AP-1 and NFκB, while the induction of cell apoptosis and differentiation by arsenic is considered to be a mechanism for the chemotherapeutic effects of arsenic. Here, we found that exposure of cells to arsenite and arsenate leads to transactivation of retinoic acid response elements (RARE) and glucocorticoid response elements (GRE) in mouse epidermal JB6 cells. These inductions occur in a time-dependent manner. Furthermore, induction of RARE activity by arsenic was synergistically enhanced by co-treatment of cells with retinoic acid, while GRE activation by arsenic was not affected by combined treatment of cells with fluocinolone acetonide (FA). In consideration of the important role of RARE and GRE in induction of cell differentiation, we speculate that transactivation of RARE and GRE by arsenic may be involved in its induction of cell differentiation and anti-cancer activities in addition to its induction of apoptosis. (Mol Cell Biochem **222**: 119–125, 2001)

Key words: arsenic, RARE, GRE, signal transduction

Abbreviations: RARE – retinoic acid response elements; RAR – retinoic acid receptor; RA – trans-retinoic acid; GRE – glucocorticoid response elements; FA – fluocinolone acetonide; NFκB – nuclear factor-κB; AP-1 – activator protein-1; Erks – extracellular signal-regulated protein kinases; MAPK – mitogen-activated protein kinase; JNKs – c-Jun N-terminal kinases; FBS – fetal bovine serum; MEM – minimal essential medium.

Introduction

A growing amount of evidence demonstrates that arsenic is a human carcinogen [1–5]. The International Agency for Research on Cancer (IARC) concluded in 1980 and again in 1987 that arsenic is a human carcinogen according to the available epidemiological findings and experimental data [6–8]. One of the most sensitive targets of arsenic toxicity is the skin. Both inorganic and organic forms are absorbed by human and animal skin [9, 10]. Animal experiments show that following chronic exposure, arsenic accumulates in the skin and hair [9, 10]. Although arsenic can be ingested and absorbed from the diet, the skin is a major target organ. Previous studies suggested that the accumulation of arsenic in the skin increases dermal sensitivity to ultraviolet (UV) light which may lead to enhanced carcinogenic effects [11, 12]. Many cases of skin cancer have been reported in people exposed to arsenic through medicinal use [9, 10]. Epidemiological evidence has also shown an excess of skin cancers following arsenic exposure. Interestingly, arsenic-containing

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compounds have been used for treatment of cancer and skin disease for hundreds of years in both western and traditional Chinese medicine [13–18]. Arsenite was routinely used to control elevated leukocyte counts in chronic myelogenous leukemia in the early 1990s [14, 15]. For more than 1000 years, the Chinese medications pi shuang and xiong huang (Realgar), which contain As_2O_3 and As_2S_2, respectively, have been used to treat cancers and other skin diseases [19]. Recently, As_2O_3 was reported to induce complete remission in a high proportion of patients with refractory acute promyelocytic leukemia [17]. Therefore, arsenic compounds are somewhat unique metalloids, which have been considered as both carcinogenic and cancer chemotherapeutic agents.

The carcinogenic effects of arsenic are believed to act at the level of tumor promotion by activating signal transduction pathways leading to activation of transcription factors, which in turn modulate gene expression [20–25]. It has been reported that arsenite is a potent stimulator for induction of c-fos and c-jun gene expression and AP-1 transactivational activity and JNK activity in Hela cells [22]. Ludwig *et al.* [23] reported that arsenite induces Erks activation through MAPK kinase 6/p38-dependent pathways. Because the skin is one of the major targets of arsenic, we recently addressed effects of arsenic on signal transduction pathways leading to activation of transcription factors and kinase in the mouse epidermal cell line, JB6 cells, and mouse skin [21, 26]. We showed that low concentrations of arsenite induced Erks activation and that Erks activation was required for arsenite-induced cell transformation [21]. However, high concentration of arsenite caused activation of JNKs, which mediated cell apoptosis in mouse epidermal JB6 cells [27]. It has been proposed that apoptosis and differentiation are two major mechanisms for the therapeutic effects of arsenic [17, 18, 27, 29, 30]. The arsenic-induced apoptosis was mediated by a JNK-dependent pathway [27], targeting tubulins [28] and down-regulation of Bcl-2 [18]. However, the molecular mechanism for induction of cell differentiation has not been investigated yet. In this report, we found that treatment of cells with arsenic resulted in significant activation of retinoic acid response elements (RARE) and glucocorticoid response elements (GRE).

Materials and methods

Cell culture and reagents

Mouse epidermal JB6, Cl41 cells, and their RARE-luciferase reporter as well as GRE-luciferase stably transfectants were cultured in monolayers in Eagle's minimal essential medium containing 5% fetal bovine serum (FBS), 2 mM L-glutamine, and 25 μg of gentamicin/ml [31]. Fetal bovine serum was from Life Technologies, Inc. (Rockville, MD, USA); Eagle's minimal essential medium (MEM) was from Calbiochem (San Diego, CA, USA); luciferase assay substrate was from Promega (Madison, WI, USA); both arsenite and arsenate were from Sigma (St. Louis, MO, USA).

Generation of stable transfectants with RARE-luciferase reporter

Cl41 cells were cultured in a 6-well plate until they reached 85–90% confluence. One μg of CMV-neo vector with 12 μg of RARE-luciferase reporter plasmid DNA and 15 μl of LipofectAMINE reagent, were used to transfect each well in the absence of serum. After 10–12 h, the medium was replaced with 5% FBS MEM. Approximately 30–36 h after the beginning of the transfection, the cells were freed from the plate with 0.033% trypsin, and cell suspensions were plated onto 75 ml culture flasks and cultured for 24–28 days with G418 selection (800 μg/ml). The stable transfectants were identified by measuring the basal level of luciferase activity. The stable transfectant, Cl41 RARE mass, was established and cultured in G418-free MEM for at least two passages before each experiment.

Generation of stable transfectants with GRE-luciferase reporter

Cl41 cells were cultured in a 6-well plate until they reached 85–90% confluence. One μg of CMV-neo vector with 12 μg of GRE-luciferase reporter plasmid DNA and 15 μl of LipofectAMINE reagent, were used to transfect each well in the absence of serum. After 10–12 h, the medium was replaced with 5% FBS MEM. Approximately 30–36 h after the beginning of the transfection, the cells were freed from the plate with 0.033% trypsin, and cell suspensions were plated onto 75 ml culture flasks and cultured for 24–28 days with G418 selection (800 μg/ml). The stable transfectants were identified by measuring the basal level of luciferase activity. The stable transfectant, Cl41 GRE mass, was established and cultured in G418-free MEM for at least two passages before each experiment.

Assay for activation of RARE by arsenic in JB6 Cl41 cells

Confluent monolayers of JB6 Cl41 RARE mass cells were trypsinized, and 8×10^3 viable cells suspended in 100 μl of 5% FBS MEM were added to each well of a 96-well plate. Plates were incubated at 37°C in a humidified atmosphere of 5% CO_2. Twelve to 24 h later, the cells were exposed to arsenic (diluted in 0.1% of FBS MEM at the concentration indicated; storage concentration of arsenic is 50 mM dissolved

in PBS) for RARE induction and maintained in culture. After different time periods, cultures were extracted with lysis buffer, and luciferase activity was measured using a luminometer (Monolight 2010). The results are expressed as relative RARE activity [32].

Determination of GRE activation in JB6 Cl41 cells by arsenic

Confluent monolayers of JB6 Cl41 GRE mass cells were trypsinized, and 8×10^3 viable cells suspended in 100 µl of 5% FBS MEM were added to each well of a 96-well plate. Plates were incubated at 37°C in a humidified atmosphere of 5% CO_2. Twelve to 24 h later, the cells were exposed to arsenic (diluted in 0.1% of FBS MEM at the concentration indicated; storage concentration of arsenic is 50 mM dissolved in PBS) for GRE induction and maintained in culture. After different culture periods, the cells were extracted with lysis buffer, and luciferase activity was measured using a luminometer (Monolight 2010). The results are expressed as relative GRE activity [32].

Results

Establishment of stable transfectants with RARE-luciferase and GRE-luciferase reporter in mouse epidermal JB6 cells

In order to study the activation of RARE and GRE in JB6 cell culture models, we used a LipofectAMINE kit to transfect CMV-neo vector and RARE-luciferase reporter plasmid DNA, and CMV-neo vector and GRE-luciferase reporter plasmid into JB6 cells, respectively. After G418 selection for 24–28 days, the stable transfectants were identified by measuring basal levels of luciferase activity. Stable transfectants, Cl41 RARE mass1 and Cl41 GRE mass1, were established. Both cells showed good responses to RA and FA, respectively (Fig. 1).

Induction of RARE in mouse epidermal JB6 cells by arsenic

Both retinoic acid and arsenic were reported to induce cell differentiation [33]. It is well accepted that retinoic acid (RA) induces leukemia cell differentiation through retinoic acid receptors resulting in RA-mediated RARE activation [34–36]. To investigate the molecular mechanism of arsenic-induced cell differentiation, we tested the effects of arsenic on RARE activation in JB6 RARE-luciferase stable transfectants, Cl41 RARE mass. We incubated Cl41 RARE mass1

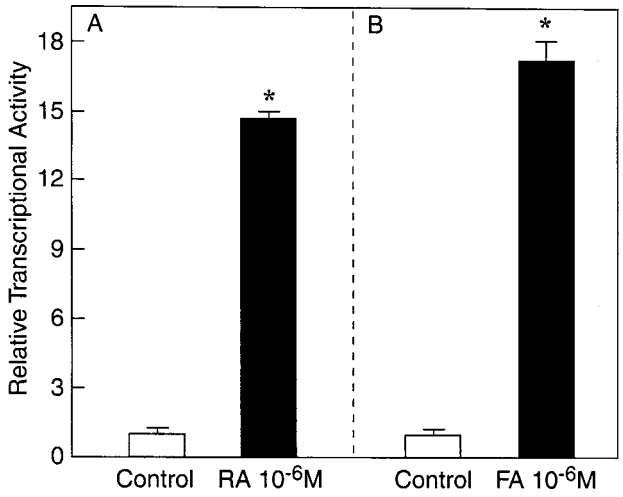

Fig. 1. Identification of RARE- and GRE-luciferase reporter stable transfectants. 8×10^3 Cl41 RARE mass1 (A) or Cl41 RARE mass1 (B) cells were seeded into each well of 96-well plates. After being cultured at 37°C overnight, the cells were starved for 12 h by replacing medium with 0.1% FBS MEM. Then the cells were treated with either (A) RA (1 µM) or (B) FA (1 µM). After being cultured for 24 h, the luciferase activity was measured. The results are presented as relative RARE or GRE activity. Each bar indicates the mean and S.D. of 4 identically treated assay wells. *Indicates a significant increase from control ($p < 0.05$).

cells, with either arsenite or arsenate. The results showed that treatment of Cl41 cells with either arsenite or arsenate, led to significant RARE induction (Fig. 2). Interestingly, the induction of RARE by arsenate (55 fold) was much higher than that induced by RA (22 fold) or arsenite (8 fold). A time course study suggested that treatment of cells with arsenic resulted in the maximum induction of RARE activity at 12–24 h post-arsenic treatment of cells (Fig. 3). After which the RARE activity returned to near basal levels (Fig. 3). In contrast, RA-induced RARE activity reached the peak between 12 and 48 h after RA treatment, then returned toward the basal level (Fig. 3). These results demonstrate that both arsenite and arsenate induce very strong activation of RARE activity in mouse epidermal cells.

Activation of GRE in the cellular response to arsenic

Previous studies indicated that arsenic compounds have effects on the function of the glucocorticoid receptor (GR) [37, 38]. However, the results from different groups were not consistent [37, 38]. Therefore, we investigated the GRE activation after JB6 cells were exposed to arsenic. As shown in Fig. 4, treatment of cells with arsenic results in marked GRE transactivation in mouse JB6 cells, with arsenate being more effective than arsenite. This activation appears to be time-dependent, with a maximal response at 24 h of exposure (Fig. 5). These data are not only consistent with previous findings

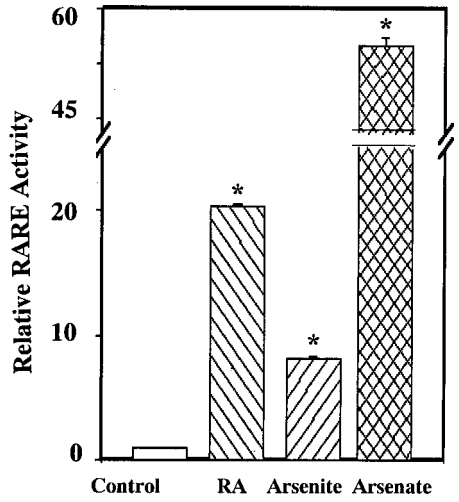

Fig. 2. Induction of RARE activity by arsenic in mouse epidermal JB6 cells. 8×10^3 Cl41 RARE mass1 cells were seeded into each well of 96-well plates. After being cultured at 37°C overnight, the cells were starved for 12 h by replacing medium with 0.1% FBS MEM. Then the cells were treated with RA (1 μM), arsenite (100 μM) or arsenate (100 μM). After being cultured for 24 h, the luciferase activity was measured. The results are presented as relative RARE activity. Each value indicates the mean and S.D. of 4 identically treated assay wells. *Indicates a significant increase from control ($p < 0.05$).

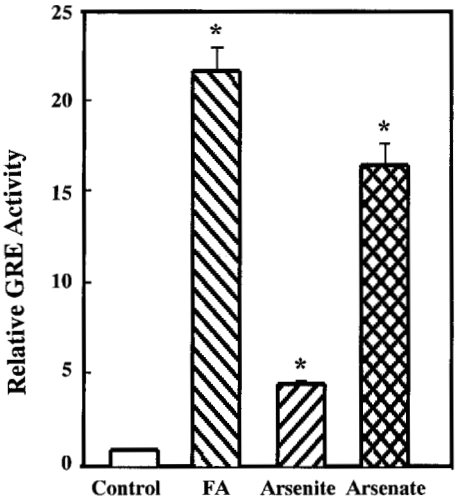

Fig. 4. Induction of GRE activity by arsenic in mouse epidermal JB6 cells. 8×10^3 Cl41 GRE mass1 cells were seeded into each well of 96-well plates. After being cultured at 37°C overnight, the cells were starved for 12 h by replacing medium with 0.1% FBS MEM. Then the cells were treated with FA (1 μM), arsenite (100 μM) or arsenate (100 μM). After being cultured for 24 h, the luciferase activity was measured. The results are presented as relative GRE activity. Each bar indicates the mean and S.D. of 4 identically treated assay wells. *Indicates a significant increase from control ($p < 0.05$).

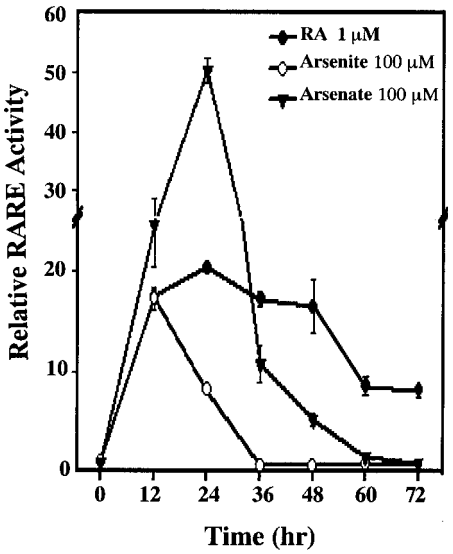

Fig. 3. Time course-study of RARE activation by arsenic. 8×10^3 Cl41 RARE mass1 cells were seeded into each well of 96-well plates. After being cultured at 37°C overnight, the cells were starved for 12 h by replacing medium with 0.1% FBS MEM. Then, the cells were treated with RA (1 μM), arsenite (100 μM) or arsenate (100 μM) for various times as indicated. The luciferase activity was measured as described in Materials and methods. The results are presented as relative RARE activity. Each value indicates the mean and S.D. of 4 identically treated assay wells.

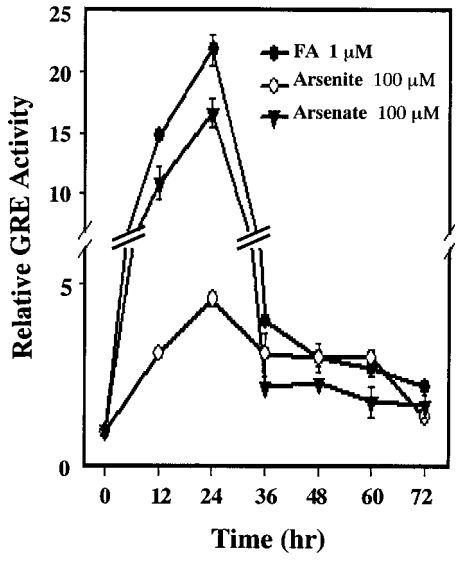

Fig. 5. Time course-study of GRE activation by arsenic. 8×10^3 Cl41 GRE mass1 cells were seeded into each well of 96-well plates. After being cultured at 37°C overnight, the cells were starved for 12 h by replacing medium with 0.1% FBS MEM. Then the cells were treated with FA (1 μM), arsenite (100 μM) or arsenate (100 μM) for various times as indicated and the luciferase activity was measured. The results are presented as relative GRE activity. Each value indicates the mean and S.D. of 4 identically treated assay wells.

that treatment of cells with arsenic caused a translocation of GR to the nucleus in an hormone independent manner [37, 38], but also demonstrate that arsenic induced the nuclear function of GR as a transcription factor.

Arsenic has synergistic effects on RA-induced RARE activation, but not on FA-induced GRE activation

It has been demonstrated that RA and arsenic have a synergistic effect on induction of NB4 cell differentiation [33]. To determine whether arsenic has synergistic effects on RA-induced RARE activation and FA-induced GRE activation, we exposed the cells to a combination of arsenic and RA or arsenic and FA. Results show that arsenic had a significant synergistic effect on RA-induced RARE activation (Fig. 6), while it did not show any effects on FA-induced GRE activation (data not shown). This result provides insight into the molecular mechanism involved in combined RA and arsenic therapy.

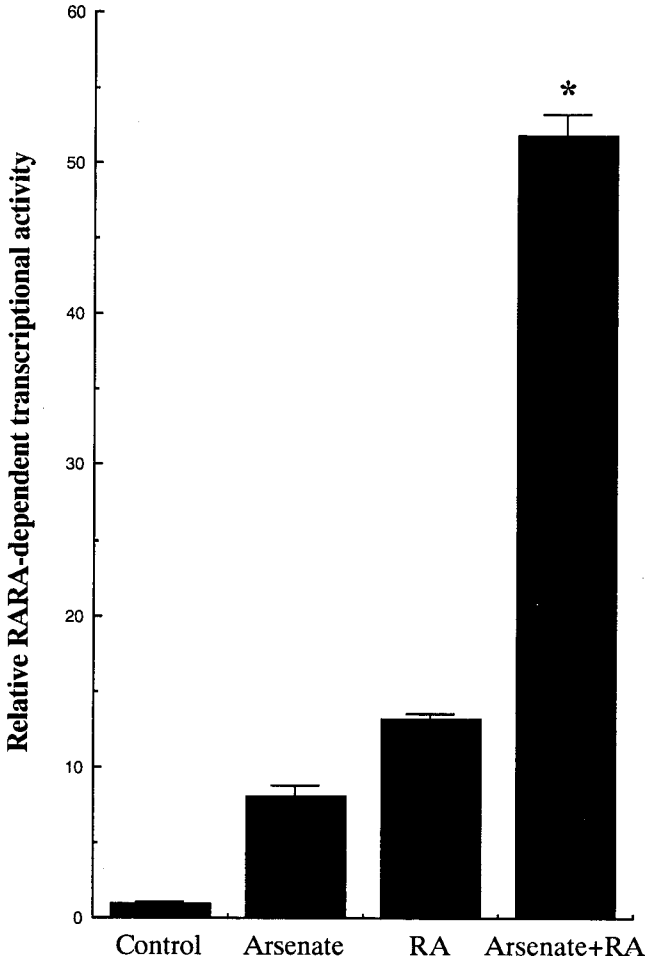

Fig. 6. Synergistic effects of arsenate on RA-induced RARE activation. 8 × 10^3 Cl41 RARE mass1 cells were seeded into each well of 96-well plates. After being cultured at 37°C overnight, the cells were starved for 12 h by replacing medium with 0.1% FBS MEM. Then the cells were treated with RA (1 μM) and arsenate (50 μM). After being cultured for 24 h, the luciferase activity was measured. The results are presented as relative RARE activity. Each bar indicates the mean and S.D. of 4 identically treated assay wells. *Indicates a significant increase from control ($p < 0.05$).

Discussion

In the present study, we investigated the effects of arsenic on RARE transactivation and GRE activation in mouse epidermal JB6 cell lines. The results show that treatment of mouse epidermal Cl41 cells with either arsenite or arsenate leads to significant transactivation of RARE and GRE. This activation appears to be time-dependent. The induction levels of activation of RARE and GRE by arsenate appears to be much higher than those by arsenite. The induction of RARE by arsenic could be synergistically enhanced with co-treatment of cells with RA, while arsenic did not show any effect on FA-induced GRE activation. These results demonstrate that both arsenite and arsenate could induce transactivation of RARE and GRE in mouse epidermal cells, and this effect may be responsible for induction of cell differentiation by arsenic.

Retinoids are a class of chemical compounds structurally related to vitamin A, which includes retinoic acid and its natural and synthetic analogs [34–36, 40]. Vitamin A has long been recognized as an indispensable nutritional factor necessary for the promotion of general growth, maintenance of visual function, regulation of differentiation of epithelial tissues, and embryonic development [34–36, 40]. The effect of vitamin A on epithelial tissue has attracted much attention for decades because vitamin A deficiency in animals and humans leads to hyperkeratosis of the skin and to hyperplastic and metaplastic changes in the epithelia of mucous membranes, which are observed in many skin diseases [34–37]. Previous studies have demonstrated that retinoids have a wide array of biological functions, such as inhibition of cell proliferation, induction of cell differentiation and apoptosis, immunomodulation, inhibition of angiogenesis, and influence on the expression of oncogenes and tumor suppressor genes [34–36, 40]. Retinoids can inhibit tumor cell growth and induce the differentiation and reversal of certain malignant cells to normal phenotype [34–36, 40]. Retinoic acid has been shown to be an effective treatment in inhibiting papilloma formation in the mouse model and tumor promoter-induced cell transformation in mouse epidermal JB6 cells [40, 41]. Thus, retinoids have been used for the treatment of many skin diseases, and as promising drugs for treatment of several cancers [34–41]. All of these biological activities of RA are believed to be mediated by transcriptional activation of RARE and inhibition of AP-1 activity, acting through distinct nuclear receptors, namely the retinoic acid receptors (RARs)

and the retinoid X receptors (RXRs) [36, 40, 41]. The binding of RA to RARs and/or RXRs leads to formation and activation of a RAR-RXR heterodimer. These activated RAR-RXR heterodimer complexes bind to RARE and regulate its targeted gene expression in a RA-dependent manner [34, 36, 42]. In the present study, we found that exposure of cells to arsenic led to marked transactivation of RARE in a time-dependent manner. This result indicates that arsenic compounds are stimulators for RARE activation. Considering the role of RARE activation in cell differentiation and treatment of many skin diseases by RA, we speculate that the arsenic-induced RARE activation may play some role in arsenic-induced cell differentiation and the efficiency of arsenic in treatment of skin disease and cancers. Our study also demonstrates that arsenic had a significant synergistic effect on RA-induced RARE activation. This may explain the synergism of cell differentiation induced by combined arsenic and RA treatment, and may provide a mechanistic basis for combined RA and arsenic therapies.

The glucocorticoid hormones are known to serve a variety of important functions in cells and tissues [43, 44]. They can promote cell differentiation and inhibit cell proliferation [43, 44]. Previous studies have also shown that glucocorticoid hormones prevent TPA-induced inflammation, skin hyperplasia and skin tumor formation [40, 43, 44]. The cellular response to glucocorticoid hormones is mediated through highly specific cytoplasmic glucocorticoid receptors (GR). Upon activation by the binding of glucocorticoid hormones, GR form a homodimer and migrates to the nucleus of the cell, where the GR homodimer binds to a glucocorticoid response element (GRE) and regulates gene expression [44, 45]. During this study, we found that exposure of mouse epidermal cells to arsenic resulted in marked activation of GRE. Unlike RARE, arsenic-induced GRE activation was not enhanced by the synthetic glucocorticoid fluocinolone acetonide (FA). Because GRE response is involved in FA-mediated anti-inflammatory and anti-cancer effects, we suggest that arsenic-induced GRE activation may be associated with the molecular mechanism for arsenic treatment of skin diseases and prevention of cancers, in addition to induction of apoptosis and RARE.

Arsenic, used as a drug in traditional Chinese medicine, has attracted a great deal of attention in recent years because of its ability to induce complete remission of disease in patients with acute promyelocytic leukemia [17, 18, 33]. It is well known that arsenic is a widely used traditional Chinese medicine for treatment of various cancers and skin diseases [13–18]. It is proposed that the anti-carcinogenic activity of arsenic is mainly through the induction of cell apoptosis and differentiation [17, 18, 27, 29, 30]. Previous studies have reported that several molecular mechanisms may be responsible for the apoptosis induction by arsenic, such as a JNK-dependent pathway, targeting tubulins and down-regulation of Bcl-2 [18, 27, 28]. However, the molecular mechanisms for induction of cell differentiation by arsenic remain unclear. In this study, we demonstrate that treatment of mouse epidermal JB6 cells with arsenic caused marked activation of RARE and GRE, which are considered to play important roles in induction of cell differentiation by RA and FA, respectively [34–36, 44, 45]. Therefore, we suggest that arsenic-induced activation of RARE and GRE may play an important role in its induction of cell differentiation and may be involved in the therapeutic effects of arsenic on cancers and skin diseases. We will test this hypothesis in our future studies.

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