Arsenic is a major environmental pollutant that exists in soil and minerals; it readily enters the groundwater system, contaminating drinking water. The concentration of arsenic in groundwater varies significantly in different geographic areas. Arsenic concentrations are highest in East Asia, including Bangladesh; West Bengal, India; and China (Kumagai and Sumi 2007; Smith et al. 2000; Tchounwou et al. 2003). Many efforts have been made to reduce arsenic damage as exemplified by the guideline for arsenic in drinking water set by the World Health Organization (Smith and Smith 2004) and by local governments. Nevertheless, a large number of populations are still at risk of arsenic-induced adverse effects, such as hypertension, arteriosclerosis, diabetes, hyperkeratosis, neuropathy, and skin, liver, bladder, and lung cancer (Kumagai and Sumi 2007; Smith et al. 2006; Steinmaus et al. 2000; Tseng 2002). Clearly, the best way to protect humans from arsenic-induced damage is to reduce arsenic intake. However, it is not always practical because many people have no choice but to consume drinking water and rice heavily contaminated with arsenic, as these are their only sources of food and water. Therefore, an alternative choice, of equal importance, is to subvert the detrimental effects of arsenic by modulating the body’s defense system.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a critical transcription factor that regulates a cytoprotective response. Many of its downstream target genes are important in maintaining the cellular antioxidant response and xenobiotic metabolism. For example, γ-glutamylcysteine synthetase (GCS) and the xCT cysteine antiporter are the key enzymes for synthesis of glutathione and maintenance of cellular redox homeostasis (Chan and Kwong 2000; Sasaki et al. 2002; Wild et al. 1999). Conjugating enzymes, such as glutathione S-transferases (GSTs) and UDP-glucuronosyl transferase, facilitate the removal of toxic and carcinogenic chemicals by increasing their solubility and excretion (Kobayashi and Yamamoto 2006; Zhang 2006). Many transporters such as multidrug resistance proteins and p-glycoprotein are important in uptake and removal of xenobiotics (Hayashi et al. 2003; Maher et al. 2005; Vernhet et al. 2001; Xu et al. 2005). Activation of the Nrf2 signaling pathway is tightly regulated by Kelch-like ECH-associated protein 1 (Keap1) according to changes in the intracellular redox state when cells are exposed to exogenous stimuli. Under normal conditions, cells maintain low constitutive levels of Nrf2-target genes through constant ubiquitination and degradation of Nrf2, which is accomplished by the Keap1-dependent E3 ubiquitin ligase complex. Upon induction, Nrf2 is stabilized because of impaired Keap1-E3 ubiquitin ligase activity, which results in activation of the Nrf2 signaling pathway (Cullinan et al. 2004; Furukawa and Xiong 2005; Kobayashi et al. 2004; Sun et al. 2007; Zhang et al. 2004b). Chemopreventive compounds are able to activate the Nrf2-dependent adaptive response and thus confer protection against subsequent toxic or carcinogenic damage (Jeong et al. 2006; Yates and Kensler 2007).

In addition to the beneficial antioxidants and many chemopreventive compounds, the Nrf2 signaling pathway can also be induced by many harmful chemicals such as arsenic, hydrogen peroxide, and even anticancer drugs including cisplatin (Aono et al. 2003; He et al. 2006; Massrieh et al. 2006; Pi et al. 2003; Purdom-Dickinson et al. 2007; Wang et al. 2006). This paradox may be explained by the balance between the induction of the Nrf2 defensive response and the toxic outcome elicited by a particular compound. The most attractive chemopreventive compounds are those that potentially induce the Nrf2-dependent defensive response without eliciting toxic effects, that is, those that tip the balance toward the Nrf2-dependent beneficial response. In accordance with this notion, many chemopreventive compounds extracted from dietary sources or plants activate the Nrf2-dependent response at low doses and do not elicit detectable toxic effects. Nrf2 activators identified so far can be classified into categories that include phenolic antioxidants (caffeoic acid, epigallocatechin-3-gallate, butylated hydroxyanisole), dithiolethiones (oltipraz, 3H-1,2-dithiole-3-thione), isothiocyanates (sulforaphane), and triterpenoids [1-(2-cyano-3,12-dioxooleane-1,9[11]-dien-28-oyl)imidazole] (Yates and Kensler 2007;
Bcl-2, which promotes release of cytochrome c-dependent apoptotic pathway through oridonin (et al. 2003). Oridonin activated the caspase 3–molecular basis by which oridonin inhibits efficacy of the cancer drug cisplatin in mouse (Li et al. 2007). In addition, oridonin enhances the HPB-ALL cells (Liu et al. 2006; Zhou et al. 2003b). The active ingredients in Rubus rubesecens A are rubesecensin A (oridonin) and rubesecensin B. Currently the major research focus on oridonin is in its antiproliferation and antitumor activities. The anticancer activity of oridonin is thought to rely on its ability to inhibit cell growth, reduce angiogenesis, and enhance apoptosis (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Meade-Tollin et al. 2004; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a). Oridonin inhibits cell growth and induces apoptotic cell death in many cancer cell lines, including leukemia (NB4, HL-60, HPB-ALL, Kasumi-1), glioblastoma (U118, U138), melanoma (A375-S2), cervical carcinoma (A431), glioblastoma (U118, U138), breast carcinoma (MCF-7, MDA-MB231), non–small-cell lung carcinoma (NCI-H520, NCI-H460, NCI-H1299) (Chen et al. 2005; Ikezoe et al. 2003; Liu et al. 2004, 2006; Zhang et al. 2004a).

Here, we report that oridonin belongs to a novel class of Nrf2 activators. Similiar to tert-butyldihydroquinone (tBHQ), it inhibits ubiquitination and degradation of Nrf2, resulting in stabilization of Nrf2 and activation of the Nrf2 signaling pathway. Furthermore, the chemopreventive activity of oridonin was demonstrated using a previously established arsenic-UROtsa cell model. Pretreatment of UROtsa cells with 1.4 μM oridonin significantly enhanced the cellular redox capacity, reduced formation of reactive oxygen species (ROS), and improved survival of UROtsa cells after arsenic exposure.

**Materials and Methods**

**Chemicals.** Most chemicals, including sodium arsenite, tBHQ, and Hoechst 33258, were from Sigma Chemical Co. (St. Louis, MO, USA). Rubesecensin A (oridonin) was purchased from LKT Laboratories Inc. (St. Paul, MN, USA).

**Cell cultures.** We obtained human MDA-MB-231 breast carcinoma cells from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Eagle’s minimal essential medium (MEM) supplemented with 3 μg/mL puromycin. For the reporter gene assay, the ARE-luciferase plasmid was electroporated into MDA-MB-231 cells using BioTek Synergy 2 microplate reader (Winooski, VT, USA). We performed the reporter gene assay in triplicate and calculated the mean ± SD.

**Luciferase reporter gene assay.** For the dual luciferase reporter gene assay, MDA-MB-231 cells were transfected with the same ARE-luciferase plasmid along with the renilla luciferase expression plasmid, pGL4.74[hRLuc/TK], from Promega. At 24 hr posttransfection, the transfected cells were treated with compounds for 24 hr, and both firefly and renilla luciferase activities were measured with the dual luciferase reporter assay system from Promega. Firefly luciferase activity was normalized to renilla luciferase activity. The experiment was carried out in triplicate and expressed as the mean ± SD.

**mRNA extraction.** Total mRNA was extracted from cells using TRIZOL reagent (Invitrogen), and equal amounts of RNA were reverse-transcribed to cDNA using the Transcriptor First Strand cDNA synthesis Kit (Roche, Indianapolis, IN, USA). The PCR condition, as well as Taqman probes and primers for Nrf2, NQO1, heme oxygenase-1 (HO-1), and GAPDH were previously published (Wang et al. 2007). Briefly, we obtained the following Taqman probes from the universal probe library (Roche): hNrf2 (#70), hNQO1 (#87), hHO-1 (#25), and hGAPDH (#25). The following primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA): hNrf2: forward (acagctgctagactc) and reverse (tgcaatccatctgcttg); hNQO1: forward (attgtagcaagagacccct) and reverse (ctcttcagagcactagag); hHO-1: forward (aacttcagagcgacccct) and reverse (ctggctctctccgtg); and hGAPDH: forward (aagggctcctctgctgctgc) and reverse (tcctcagagcgagag).
Reported gene assays were run in triplicate and luciferase activities in cell lysates. All luciferase activity in MDA-MB-231 cells cotransfected with a herpes simplex virus thymidine kinase promoter and a plasmid encoding renilla luciferase driven by the ubiquitous conjugating sites) were either left untreated or treated with oridonin for 24 hr. Cells were lysed by boiling in a buffer containing 2% SDS, 150 mM NaCl, 10 mM Tris-HCl, and 1 mM DTT. These lysates were then diluted 5-fold in buffer lacking SDS and incubated with anti-Nrf2 or anti-Keap1 antibodies. Immunoprecipitated proteins were analyzed by immunoblot with antibodies directed against the HA epitope (Zhang and Hannink 2003).

Ubiquitination assay. To detect endogenous Nrf2 ubiquitination, the UROtsa cells were treated with 10 μM MG132 and lysed and diluted in the same way. Nrf2 was immunoprecipitated with an anti-Nrf2 antibody and subjected to immunoblot analysis with an antiubiquitin antibody (Sigma Chemical Co.).

Protein half-life measurement. To measure the half-life of Nrf2, cells were either left untreated or treated with oridonin for 4 hr. To block protein synthesis, we added 50 μM cycloheximide. Total cell lysates were collected at different time points and subjected to immunoblot analysis with an anti-Nrf2 antibody. The relative intensity of bands was quantified by the ChemiDoc CRS gel documentation system and Quantity One software from BioRad.

Transient transfection of siRNA and measurement of glutathione concentration. We transfected Nrf2-siRNA and the control siRNA from Qiagen (Valencia, CA, USA). Transient transfection of siRNA was performed using HiPerFect Transfection Reagent according to the manufacturer’s protocol (Qiagen). Intracellular glutathione concentration was measured using the QuantiChrom glutathione assay kit from BioAssay Systems (Hayward, CA, USA). All the procedures were carried out according to the manufacturer’s instructions.

Detection of cell viability. Cell viability was measured by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Wang et al. 2007) and by colony formation assays. We performed colony formation assays in 35-mm plates with 200 UROtsa cells. Attached cells were left untreated or treated with oridonin for 24 hr, followed by treatment with different doses of As(III) for another 48 hr. After exposure, the medium was replaced with fresh medium, and cells were incubated for 12–14 days. The cells were then fixed and stained with crystal violet (0.5% in ethanol).

**Figure 1.** (A) Structure of the diterpenoid oridonin. (B, C) Luciferase reporter gene assays in MDA-MB-231 cells expressing ARE-luciferase. (B) Luciferase activity reporting oridonin as an Nrf2 activator using a high-throughput screening system. The stable MDA-MB-231 cells expressing ARE-luciferase were seeded in 96-well plates; cells were grown to 90% confluence and treated with oridonin for 24 hr before analysis of luciferase activity. (C) Luciferase activity in MDA-MB-231 cells cotransfected with a plasmid containing an ARE-luciferase reporter gene and a plasmid encoding renilla luciferase driven by the herpes simplex virus thymidine kinase promoter. The transfected cells were treated with oridonin for 24 hr prior to measurement of firefly and renilla luciferase activities in cell lysates. All luciferase reporter gene assays were run in triplicate and expressed as mean ± SD.

**Figure 2.** Effects of oridonin on MDA-MB-231 cells. (A) An aliquot of cell lysates from the dual luciferase reporter gene assay was subjected to immunoblot analysis with anti-Nrf2, anti-Keap1, and anti-β-actin. (B) Total cell lysates from MDA-MB-231 cells treated with oridonin for 24 hr were subjected to immunoblot analysis with anti-Nrf2, anti-Keap1, and anti-β-actin antibodies. (C) mRNA from similarly treated cells was extracted and reverse transcribed into cDNA prior to real-time PCR analysis for detection of mRNA for Nrf2 (top), NQO1 (center), and HO-1 (bottom).
Identification of oridonin as a novel Nrf2 activator.

Using the stable ARE luciferase reporter cell line derived from MDA-MB-231 cells combined with a high-throughput screening system established in our laboratory, we identified a novel Nrf2 activator that belongs to the class of diterpenoids (Figure 1A). The MDA-MB-231 cell line was used to show Nrf2 activation for two reasons: first, MDA-MB-231 cells can be easily transfected; and second, the Nrf2 pathway is most sensitive in this cell line in response to Nrf2 inducers. Oridonin induced transcription of the ARE-dependent luciferase gene in a dose-dependent manner in this stable cell line (Figure 1B). To confirm oridonin activation of Nrf2 using the high-throughput screening method, we also performed a dual luciferase reporter gene assay in which we included a renilla luciferase gene as an internal control for transfection efficiency and for toxicity induced during oridonin exposure. Consistent with the data obtained from the high-throughput screening, oridonin induced the ARE-dependent luciferase activity in a dose-dependent manner (Figure 1C). Slight induction (1.5-fold) was observed at as low as 1.4 μM and reached maximum induction (11.3-fold) at 14 μM. There was no obvious toxicity at 14 μM, as judged by cell morphology and renilla luciferase activity.

**Oridonin activated the ARE-dependent response primarily through up-regulation of the Nrf2 protein level.** Previous studies have demonstrated that ARE-dependent reporter gene activity correlated very well with the protein level of Nrf2. Therefore, we used the same cell lysates from the dual luciferase reporter gene assay for immunoblot analysis for detection of Nrf2, Keap1, and β-actin. Although the Keap1 levels remained constant, oridonin enhanced the levels of Nrf2 protein in a dose-dependent manner, with the highest induction at 14 μM (Figure 2A). During the reporter gene assay, any doses > 14 μM caused marked toxicity, as indicated by an increased number of rounded and floating cells. A large body of literature indicates that the antitumor activity of oridonin relies on its ability to inhibit cell growth and to induce cell death (Zhou et al. 2007a). Because Nrf2 regulates a cellular survival response, we envisioned that treatment with high doses of oridonin could inhibit Nrf2, allowing cells to undergo cell death. Therefore, we tested Nrf2 protein levels in response to high doses of oridonin. After treatment of MDA-MB-231 cells with different doses of oridonin for 24 hr, we collected all cells, including floating cells. Equal amounts of proteins were subjected to immunoblot analysis with Nrf2, Keap1, and β-actin antibodies. Interestingly, at doses > 28 μM, Nrf2 protein levels decreased in a dose-dependent manner, whereas the expression of Keap1 and β-actin had no significant change (Figure 2B, lanes 7–9). Previously, it has been demonstrated that Nrf2 activators, including tBHQ, induce the Nrf2 signaling pathway primarily through stabilization of the Nrf2 protein, rather than up-regulation of its mRNA (Nguyen et al. 2003).

Next, we measured mRNA expression of Nrf2 and its target genes, NQO1 and HO-1, in response to oridonin using real-time RT-PCR. Nrf2 mRNA increased slightly in a dose-dependent manner in response to oridonin, whereas tBHQ had no effect (Figure 2C, top panel). As expected, mRNA of NQO1 and HO-1 were induced significantly by oridonin in a dose-dependent manner (Figure 2C, center and bottom panels). These results demonstrate that oridonin is able to induce the Nrf2 signaling pathway mainly through up-regulation of Nrf2 at the protein level.

**Oridonin blocked Nrf2 ubiquitination and enhanced Keap1 ubiquitination.** tBHQ enhances the Nrf2 protein level by interfering with the Keap1-dependent ubiquitin conjugation process. Therefore, we tested the ability of oridonin in modulating Nrf2 ubiquitination. For this assay, we used Gal4-Neh2, a model fusion protein previously used for the Nrf2 ubiquitination test (Zhang and Hannink 2003). In a manner similar to tBHQ, oridonin suppressed Nrf2 ubiquitination (Figure 3A, left panel).
Furthermore, Zhang et al. (2005) showed that tBHQ caused a shift of ubiquitination from the substrate Nrf2 to the substrate adaptor Keap1. As with tBHQ, oridonin treatment was also effective in enhancing ubiquitination of Keap1 (Figure 3A, center panel). These results demonstrate that oridonin can induce a shift of ubiquitination from Nrf2 to Keap1. One of the major roles for ubiquitin conjugation onto a protein is to target the protein for 26S proteasome-mediated degradation. Next, we measured the half-life of Nrf2 in the absence or presence of oridonin. Half-life of the endogenous Nrf2 protein in MDA-MB-231 cells was 19 min, whereas treatment with oridonin increased the half-life to 51 min (Figure 3B, left panel). Taken together, these results indicate that oridonin activates the Nrf2 pathway by inhibiting ubiquitination and degradation of Nrf2, leading to an increase in Nrf2 protein level and activation of the Nrf2-dependent response.

**Efficacy of oridonin in protecting against As(III)-induced toxicity.** To test the feasibility of using oridonin as a chemopreventive compound to elicit the Nrf2-mediated protective response to defend against environmental...
were left untreated or were pretreated with oridonin. These data in a significant reduction of ROS levels, especially with 5.6 μM oridonin. To confirm that protection against As(III)-induced cell death was attributed to the activation of Nrf2 by oridonin, the MTT assay was performed in UROtsa cells that were treated with Nrf2-siRNA for 48 hr. Immunoblot analysis confirmed the effectiveness of Nrf2-siRNA in reducing Nrf2 expression (Figure 4D, center panel). Inhibition of Nrf2 expression in UROtsa cells reverted the MTT curve [i.e., oridonin lost its protection against As(III) toxicity] and aggravated As(III)-induced cell death (Figure 4D, center panel). This result demonstrates that oridonin-mediated protection requires activation of the Nrf2 pathway.

Apoptotic cell death was quantified using Annexin V-FITC/flow cytometry. Treatment with 30 μM As(III) for 48 hr increased the percentage of apoptotic cells, whereas pretreatment followed by cotreatment with 1.4 μM oridonin reduced apoptotic cell death to a level comparable to the untreated cells (Figure 4E, top and center panels). Apoptotic cell death was not increased by 1.4 μM oridonin alone (data not shown). We used Hoechst staining to detect condensed chromosomes in the apoptotic cells. The number of positive-stained cells increased after treatment with 30 μM As(III), whereas pre-treatment followed by cotreatment with oridonin markedly reduced the number of apoptotic cells (Figure 4E, bottom panel). Together, these results demonstrate that a low dose of oridonin is able to protect cells from As(III)-induced damage, as illustrated by reduced ROS and increased survival in response to As(III).

**Discussion**

The pivotal role of Nrf2 in chemoprevention has clearly been shown in Nrf2 null mice. These mice express lower basal levels of the Nrf2 target genes such as NQO1, GST, GCS, UDP-glucuronosyltransferase, glutathione peroxidase-2, and HO-1 (Chang and Kwong 2000; Cho et al. 2002; Hayes et al. 2000; Kwak et al. 2001; McMahon et al. 2001). As a consequence, these mice are more susceptible to toxic and carcinogenic challenges such as butylated hydroxytoluene, benzo(a)pyrene, diesel exhaust, aflatoxin B_1, N-nitrosobutyl (4-hydroxybutyl) amine, pentachlorophenol, acetaldehyde, ovulaminon, butylaminon, cigarette smoke, and 4- vinyl cyclohexene diepoxyde (Aoki et al. 2001; Chan et al. 2001; Chan and Kan 1999; Enomoto et al. 2001; Hu et al. 2006; Iida et al. 2004; Iizuka et al. 2005; Rangasamy et al. 2005; Umemura et al. 2006). These results provide the basis for chemopreventive intervention targeting the Nrf2 signaling pathway. Many previously indentified, naturally occurring compounds, including sulforaphane, epigallocatechin-3-gallate, caffeic acid phenethyl ester, and curcumin, have proved to be Nrf2 activators, which further implies the importance of Nrf2 in chemoprevention (Jeong et al. 2006; Nishinaka et al. 2007; Zhang 2006). Identification, validation, and optimization of new Nrf2 activators are essential for the development of effective dietary supplements or therapeutic drugs that can be used to boost the Nrf2-dependent adaptive system to protect humans from various environmental insults.

Oridonin represents a novel class of Nrf2 activators that has not been demonstrated previously. Mechanistic studies presented here indicate that oridonin induced the Nrf2-dependent response primarily by enhancing the Nrf2 protein level. The increase in the Nrf2 protein level in response to oridonin is attributed mainly to the stabilization of Nrf2, with minor contribution from increased mRNA. Similar to BHQ, oridonin is able to block ubiquitination and degradation of Nrf2, resulting in the prolonged half-life of Nrf2. Furthermore, we demonstrated the effectiveness of a low dose of oridonin (1.4 μM) in eliciting the Nrf2-dependent cytoprotective response in an As(III)-toxicity model. Low doses of oridonin are able to enhance the cellular reducing capacity by significantly elevating the reduced glutathione level, thus inhibiting the formation of ROS, resulting in increased survival in response to As(III) exposure. Furthermore, glutathione is able to conjugate arsenic to facilitate arsenic excretion, thereby reducing As(III) toxicity (Shinkai et al. 2006). In addition to GCS, which modulates intracellular glutathione levels, other Nrf2 downstream genes, including GST, UDP-glucuronosyl transferase, and multidrug resistance proteins, also contribute to the Nrf2-mediated protection against arsenic toxicity (Hayashi et al. 2003; Kobayashi and Yamamoto 2006; Maher et al. 2005; Vernhet et al. 2001; Xu et al. 2005; Zhang 2006). Although the present study shows only the protection of oridonin against acute As(III) toxicity, oridonin certainly can be applied to other toxic and carcinogenic chemicals, because oridonin induces the well-characterized Nrf2-dependent defensive response.

This cell-based study provides evidence that low-dose oridonin can be used as a chemopreventive compound that specifically targets Nrf2. Further studies on the chemopreventive activity of oridonin in animal models are needed. If oridonin is shown to...
have great chemopreventive potential, then it has a great economic advantage because it can easily be extracted from Rabdosia rubescens “the Chinese grass.” In addition, identification of diterpenoids as a new class of Nrf2 activators will broaden the choice for new chemopreventive compounds. Moreover, the diterpenoid structure can serve as a scaffold for the development of chemopreventive drugs. Identification of naturally occurring diterpenoids or synthetic optimization of the diterpenoid oridonin, which potently and specifically induce the Nrf2 signaling pathway, will greatly improve the efficacy of chemopreventive drugs and decrease side effects, which will have a profound impact on human health.

High doses of oridonin promote anti-cancer activity by causing cell cycle arrest, inhibiting proliferation, and inducing apoptotic cell death. The dose range needed for oridonin to exhibit anticancer activities in these studies, conducted by different laboratories with a variety of cancer cell lines, is very broad, with 100-fold differences. Although this may be due partially to the purity of oridonin used among groups, it largely indicates a difference in sensitivity of cancer cells to the oridonin-induced apoptotic response. In the present study, the effect of oridonin in inducing the Nrf2 protein level was assessed in two different cell lines, breast carcinoma MDA-MB-231 cells and immortalized but non-transformed bladder urothelium UROtsa cells. UROtsa cells showed a narrower window of Nrf2 induction in response to different doses of oridonin. It is interesting to note that oridonin induced Nrf2 protein and reporter gene activity in a dose-dependent manner to a certain point at which the Nrf2 protein level and the reporter gene activity dropped suddenly. We observed an initial decrease in Nrf2 protein in MDA-MB-231 cells and UROtsa cells at 56 μM and 28 μM oridonin, respectively. This decrease in Nrf2 protein level in response to high doses of oridonin is not due solely to cell toxicity because Keap1 or β-actin levels decreased only slightly. Based on the important role of Nrf2 in cell survival, it is conceivable that Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death. In support of this notion, Nrf2 has to be repressed before the execution of cell death.
Identification of oridonin as a novel Nrf2 activator

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