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A cell-based system to identify and characterize the molecular mechanism of drug-metabolizing enzyme (DME) modulators

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Abstract

Many naturally occurred or synthetic compounds can modulate the body's drug-metabolizing enzymes to enhance carcinogen detoxification, and some have demonstrated remarkable cancer prevention effects. Understanding the molecular mechanism behind each candidate agent is critically important in designing rational cancer chemoprevention strategies. In this work, we have employed a set of molecular mechanism-based assays and characterized eight classes of known drug-metabolizing enzyme (DME) modulators in a cellular system. Examination of mRNA and protein levels of representative phase I and phase II enzymes validated the results obtained in our cell-based system. Our data confirmed that the antioxidant ethoxyquin (EQ) and the isothiolcyanate sulfurophane (SFP) exclusively activate the antioxidant response element (ARE), and thus represent monofunctional inducers. We were also able to reclassify some compounds, and to use the system to identify structure–activity relationships among structurally related but different compounds. Finally, this cell-based system permitted us to identify a potential novel mechanism for cross-talk between the ARE and the xenobiotic response element (XRE)-mediated pathways.

Keywords: Cancer; Chemoprevention; Drug-metabolizing enzyme; Antioxidant response element; Xenobiotic response element; Molecular classification

1. Introduction

Cancer development is closely related to environmental carcinogen exposure, therefore, enhancing the body's carcinogen-detoxifying capability provides a promising approach to cancer prevention [1,2]. The body's detoxifying system is comprised of two sets of enzymes, referred to as phase I and phase II drug-metabolizing enzymes (DMEs). The phase I enzymes consist of a gene superfamily of cytochrome P450s (CYPs), while the phase II enzymes contain multiple detoxifying and antioxidant enzymes, such as glutathione *S*-transferase (GST), NADP(H):quinone oxidoreductase (NQO) and UDP:glucuronosyl transferase (UGT) [3]. A large number of naturally derived or synthetic compounds have demonstrated significant cancer prevention effects through DME modulation in experimental models [4,5]. Of these active chemopreventive agents, some activate both phase I and phase II enzymes and are called bifunctional inducers; while others selectively activate phase II detoxifying enzymes, and are thus named monofunctional inducers [3,6]. In recent years, the underlying molecular mechanisms of gene activation in this context have been unveiled. The antioxidant responsive element (ARE), a *cis*-acting regulatory element, is present in many phase II genes and is implicated in transcriptional activation by monofunctional inducers; while the xenobiotic responsive element (XRE), a different *cis*-acting regulatory element present in both phase I and phase II genes, appears to be responsible for transcriptional activation by bifunctional inducers [3]. The XRE motif is ultimately the target of a protein complex composed of a ligand-activated aryl hydrocarbon receptor (AhR) translocating to the nucleus after binding to AhR nuclear

Abbreviations: DME, drug-metabolizing enzyme; ARE, antioxidant response element; XRE, xenobiotic response element; β-NF, β-naphthoflavone; D3T, 1,2-dithiole-thione; DAS, dially sulfide; DADS, dially disulfide; DATS, dially trisulfide; EGCG, epigallocatechin; KAW, kahweol; EQ, ethoxyquin; SFP, sulfurophane; BHQ, *tert*-butylhydroquinone; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AhR, aryl hydrocarbon receptor; CYP, cytochrome P450; GST, glutathione *S*-transferase; NQO, NAD(P)H:quinone oxidoreductase

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translocator (ARNT). The AhR–ARNT complex binds to the XRE motif and activates a battery of genes [3,7]. In the case of ARE pathway, electrophilic compounds can stimulate kelch-like ECH-associated protein1 (KEAP1), activating and releasing the transcription factor Nrf2 from KEAP1–Nrf2 complex. In the nucleus the activated Nrf2 forms a complex with Maf, and this protein complex binds to the ARE motif, activating the ARE gene battery [3,8,9]. The action of some phase I CYPs can be a double-edged sword. On the one hand they can transform some carcinogens into non-toxic-forms and help to detoxify them, and on the other they may metabolize some procarcinogens into carcinogenic metabolites and eventually increase the chances of cancer development. The phase II enzymes mainly function to detoxify carcinogens by various conjugation reactions [3,6]. Therefore, it is generally believed that an ideal cancer chemopreventive agent should preferably be a monofunctional inducer selectively activating phase II detoxifying enzymes. Obviously, understanding the molecular mechanism of each individual agent is of critical importance in predicting the optimal cancer chemopreventive effect and in designing a rational cancer preventive strategy.

Traditionally, a DME modulator is characterized by measuring its effect on enzyme activity, and protein or gene expression of multiple members of phase I and phase II enzymes. This procedure is time consuming and labor intensive and may also be dependent on other factors variably expressed in different cells. In the absence of a standard protocol, different research groups select distinct set of enzymes, making comparison very difficult. Although there are many candidate DME modulators reported in the literature, in many cases the molecular mechanism(s) of their action are not fully elucidated. Since there is in general a vigorous search for novel DME modulators, thousands of new compounds need to be characterized in an efficient way. There is a need to establish a quick and accurate characterization tool for large-scale screening purposes. In this work, we have used a set of molecular mechanism-based assays to characterize eight classes of known candidate chemopreventive agents.

2. Materials and methods

2.1. Chemicals and antibodies

β-Naphthoflavone (β-NF), *tert*-butylhydroquinone (BHQ), and ethoxyquin (EQ) were purchased from Sigma–Aldrich; diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS) and sulforaphane (SFP) were obtained from LKT laboratory; D3T was kindly provided by Dr. Thomas Kensler (John Hopkins University). CYP1A1/2 antibody was from Santa Cruz Biotechnology. The rat GSTA1/2 antibody was kindly provided by Dr. John Haynes (University of Dundee) (Fig. 1).

2.2. Selection of promoter sequences for our DME molecular activity assays

Since XRE is present in both phase I and phase II genes, we chose the DRE3 sequence, a prototypical XRE sequence found in the phase I system of human *CYP1A1* genes, and we also tested another XRE (A2-XRE) found in the phase II human *GSTA2* gene. As ARE is only present in phase II genes, one representative ARE (A2-ARE) from *GSTA2* was used in this work (the sequence of each enhancer is shown in Fig. 2A). The DRE3, A2-XRE and A2-ARE were cloned into the *Kpn*I and *Mlu*I sites of pGL3-promoter vector, which contains a heterologous SV40 promoter and a firefly luciferase reporter gene (Fig. 2B). Rat hepatoma cell line H4IIE was transfected with these constructs before being exposed to the test compounds.

2.3. Plasmid construction

Double stranded nucleotides containing ARE and XRE sequences were cloned into the *Kpn*I and *Mlu*I sites of the pGL3-promoter vector (Promega). The sequences of XRE and ARE are shown in Fig. 2A.

2.4. Cell culture, transfection and luciferase assay

Rat hepatoma H4IIE cell line, and murine hepatoma 1c1c7, tao and c37 were obtained from American Tissue Culture Collection (ATCC). Cells were maintained in DMEM supplemented with 10% FBS. For luciferase assay, cells were seeded at 1×10^5 per well using 24-well plate, grown overnight before being transfected with pGL3 constructs using LipofectAMINE (Invitrogen) for 5 h. The plasmid pRL, containing a renilla reniformis luciferase reporter gene, was cotransfected as internal control to correct transfection efficiency. After transfection, cells were treated with test compounds for 24 h prior to harvesting. The luciferase activities were analyzed in 20-μl cell extracts with the Dual luciferase assay™ kit (Promega) on a Lumat LB 9507 luminometer (Berthold Technologies). The related luciferase activities reported are expressed as a ratio of the pGL3 reporter activity to that of the control plasmid pRL. The fold induction is expressed as ratio of induction from treated cells versus untreated. Values represent measurements from three independent transfections and are presented as the mean ± S.E.M. All experiments were repeated at least three times, and average fold induction was shown in figures. For statistics, Student's *t*-tests were carried out with GraphPad Prism, and significance (*) was reached at $P < 0.05$.

2.5. Reverse transcriptase–polymerase chain reaction (RT–PCR)

Rat hepatoma H4IIE cells were treated with test compounds and harvested at 2, 6, 16 and 24 h. Total RNA was

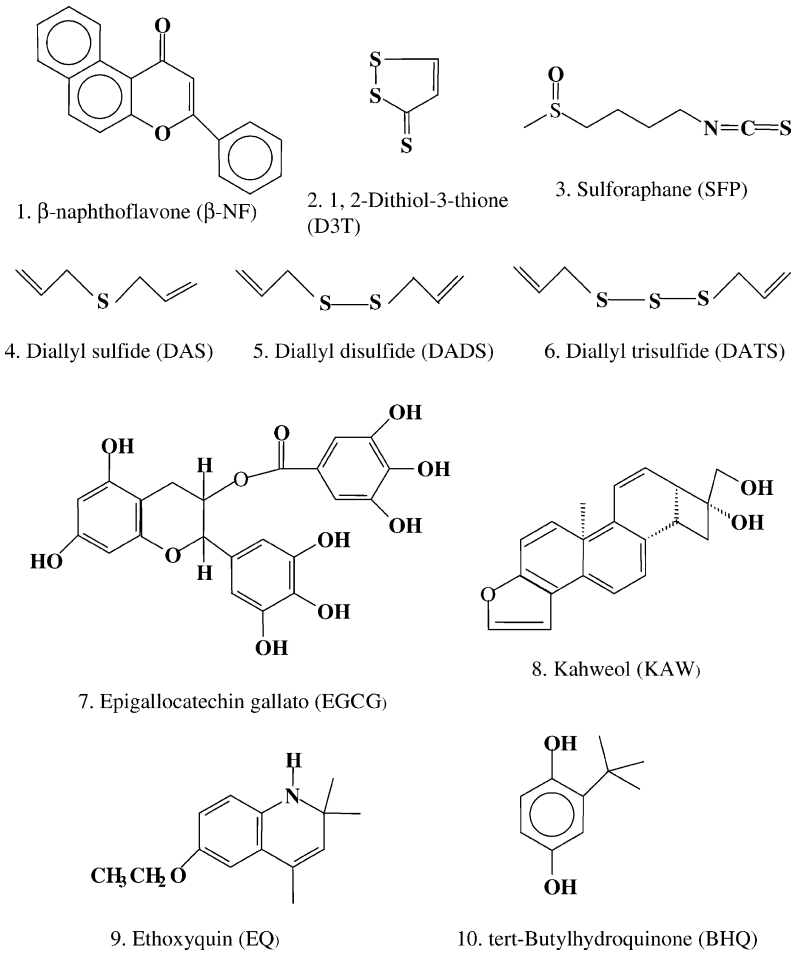

Fig. 1. The chemical structures of 10 representative DME modulators.

prepared with TRIzol reagent (Invitrogen). OneStep RT–PCR was carried out following the manufacturer's instruction (Qiagen). Briefly, each RT–PCR reaction was performed in a 50 μl volume, with 0.5 μg template total RNA, 400 μM of each dNTP, 0.6 μM of each primer, and 10 units of enzyme mix containing omniscript reverse transcriptase, sensiscript reverse transcriptase, and hotstar-Taq DNA polymerase (for details, see Qiagen OneStep RT–PCR kit handbook). The RT–PCR conditions were 50 °C for 30 min, 95 °C for 15 min, followed by 32 cycles of: 94 °C for 40 s, 55 °C for 30 s and 72 °C for 1 min, then an additional extension at 72 °C for 10 min. Ten microliters of PCR product was analyzed by electrophoresis through 1.5% agarose gels. The results were documented on Polaroid instant photographic paper. The primer sequences for *CYP1A1* are AGAATGCCAATGTCCAGCTC/GCCCATAGGCAGGAGTCATA and for *NQO1* are CATGAAGGAGGCTGCTGTG/GAGTGGTGACTCCTCCTCCCAGA.

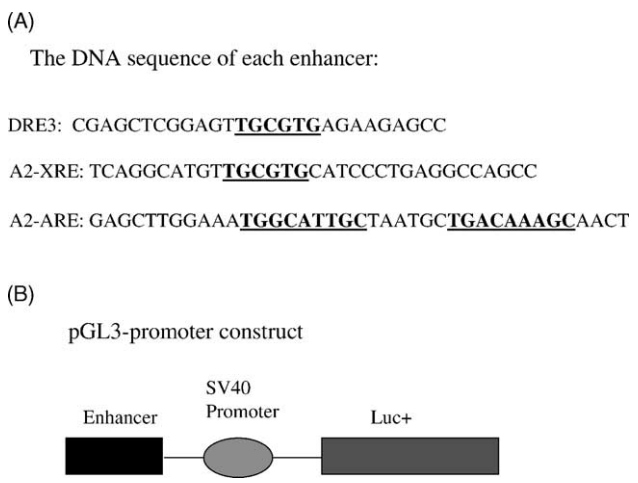

Fig. 2. Construction of pGL3-promoter plasmid containing XRE or ARE enhancer sequence. (A) The DNA sequences of DRE3, A2-XRE and A2-ARE used in this work; the underlined sequences represent the consensus sequences of the ARE or XRE *cis*-acting regulatory elements. (B) A schematic diagram of the pGL3-promoter construct.

2.6. Western blot detection of CYP1A1/2 and GSTA1/2

The hepatoma H4IIE cell line was maintained in DMEM supplemented with 10% FBS. Cells were treated with test compounds and were harvested at 2, 6, 16 and 24 h. Sixty micrograms of cell-lysate protein was loaded into each lane and electrophoresed through a 4–20% gradient SDS–PAGE gel (Bio-Rad), then transferred onto a nitrocellulose

membrane. The membrane was incubated with primary antibody against CYP1A1/2 or GSTA1/2, and subsequently incubated with HRP-conjugated secondary antibody. Finally, ECL detection was carried out and the results were recorded on X-ray film by autophotography.

3. Results

In this work, we have characterized eight classes of known DME modulators in order to test the validity of our cell-based molecular assay system. These include representative compounds of polycyclic aromatic hydrocarbon (β-NF), organosulfur (DAS, DADS, DATS), dithiolthione (D3T), isothiocyanate (SFP), catechin (EGCG), diterpene (KAW) and antioxidant (BHQ and EQ) (Fig. 1).

As shown in Fig. 3A, this cell-based system detected induction of DRE3-regulated promoter activity by three different classes of agents (β-NF induces 3.5-fold at 10 μM; DADS is also potent, inducing 3.4-fold at 10 μM; KAW is moderate, inducing 2.1-fold at 10 μM), while SFP and EQ do not show significant induction of DRE activity. These results are consistent with what is already known, however, our cell-based molecular mechanistic system also provides new information. In contrast to previous concepts, our results demonstrated that D3T and BHQ can also activate DRE3, inducing 3.9- and 2.5-fold at 10 μM, respectively (Fig. 3A). As for the A2-XRE construct, a similar induction profile was obtained compared to the DRE3 constructs (Fig. 3B), suggesting that in our cell-based system the XRE sequence from the *GSTA2* gene responds to inducers in a manner quite similar to the variant of the XRE sequence (DRE3) found in the *CYP1A1* promoter.

In the case of the A2-ARE construct, all compounds except EGCG showed robust activation. At 10 μM, SFP is the strongest activator (4.1-fold induction), and the other compounds induce in a range of 2.8–3.8-fold (Fig. 3C).

As summarized in Table 1, β-NF, D3T, DADS, KAW and BHQ can activate both XRE and ARE, and should be classified as bifunctional inducers; while SFP and EQ exclusively activate ARE, and therefore represent monofunctional inducers. Surprisingly, EGCG activates neither XRE nor ARE in our experiments.

Table 1
Molecular mechanistic classification of DME inducers

	XRE	ARE	Mono	Bi
β-NF	+	+	−	+
D3T	+	+	−	+
SFP	−	+	+	−
DADS	+	+	−	+
EGCG	−	−	N/A	N/A
KAW	+	+	−	+
EQ	−	+	+	−
BHQ	+	+	−	+

Note. Mono, monofunctional inducer; Bi, bifunctional inducer.

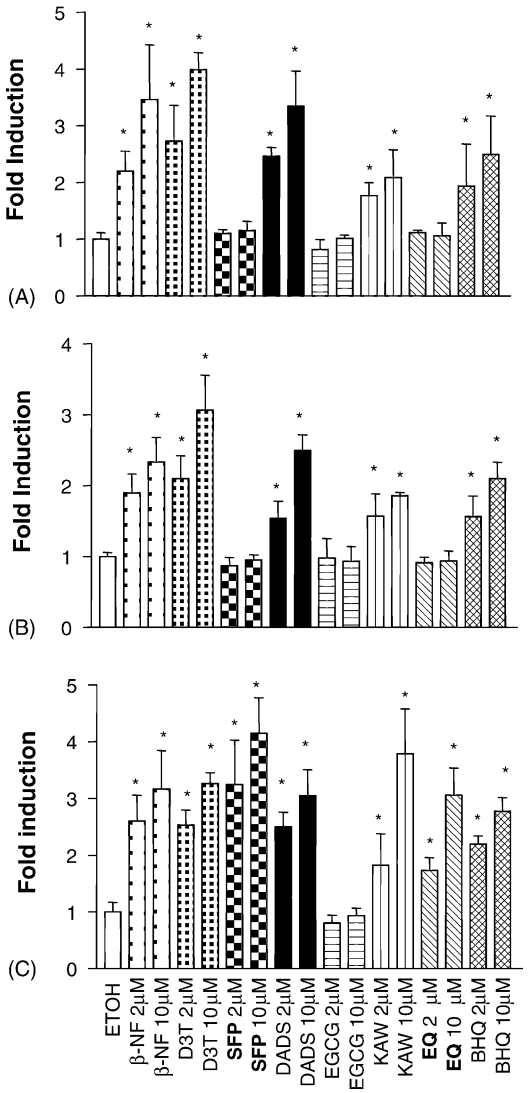

Fig. 3. Characterization of eight representative compounds with XRE- and ARE-based luciferase assays. Rat hepatoma cell line H4IIE was transfected with pGL3-promoter construct containing DRE3 (A), A2-XRE (B) or A2-ARE (C) for 5 h before exposing to designated compounds. The relative luciferase activities reported are expressed as a ratio of the pGL3 reporter activity to that of the control plasmid pRL. The fold induction was recorded as the ratio of relative luciferase activity of treated vs. untreated cells. The values represent the mean ± S.E.M. of three independent measurements. Statistical analysis (Student's *t*-test) was performed by comparison of treated and untreated cells ($^*P < 0.05$). Parts (A)–(C) represent the luciferase assays involving DRE3, A2-XRE and A2-ARE, respectively.

In order to determine whether our system can be used to identify structure–activity relationships, we analyzed DAS, DADS, and DATS, three sibling organosulfur compounds derived from garlic extracts (Fig. 1). The luciferase activity data showed that using the DRE3 promoter sequence, DAS only has a marginal effect, DADS is moderate, inducing 3.4-fold at 10 μM; whereas DATS is the strongest inducer, resulting in almost five-fold induction at 10 μM (Fig. 4A). These results are recapitulated using the A2-ARE, DAS has very little effect; DADS can induce 3.1-fold at 10 μM,

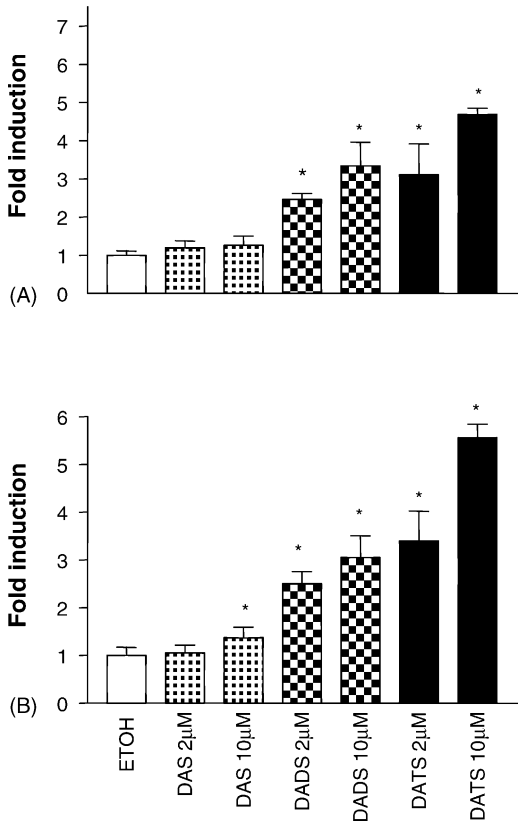

Fig. 4. Comparison of DAS, DADS and DATS on induction of DRE3 or ARE driven luciferase activities. Rat hepatoma cell H4IIE was transfected with pGL3-promoter construct containing DRE3 (A) and A2-ARE (B), before being exposed to DAS, DADS or DATS. The relative luciferase activities reported are expressed as a ratio of the pGL3 reporter activity to that of the control plasmid pRL. The fold induction was recorded as relative luciferase activity of treated vs. untreated cells. The values represent the mean ± S.E.M. of three independent measurements. Statistical analysis (Student's *t*-test) was performed by comparison of treated and untreated cells ($^*P < 0.05$).

and DATS is again the strongest, inducing 5.6-fold at 10 μM (Fig. 4B). The results suggest that the assay is sufficiently robust to detect differences among sibling compounds from the same structural class.

To verify the accuracy of the activation detected, and of the mono-versus bifunctional classification of compounds, RT–PCR experiments were performed to check the mRNA expression induced by D3T, BHQ and EQ. RT–PCR results

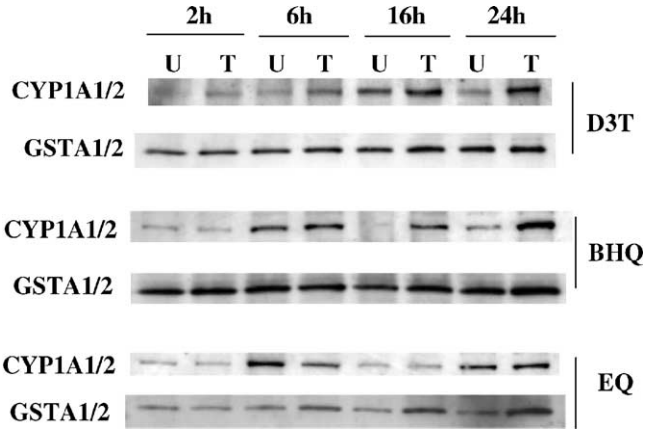

Fig. 6. Comparison of effects of D3T, BHQ and EQ on protein expression of CYP1A1/2 and GSTA1/2. Rat hepatoma H4IIE cells were treated with 10 μM D3T, BHQ, EQ and the vehicle ETOH. The cell lysates were harvested at 2, 6, 16 and 24 h of treatment, and protein expression of CYP1A1/2 and GSTA1/2 was detected by Western blot.

demonstrated that D3T and BHQ can clearly induce both *CYP1A1* and *NQO1*, and on the other hand EQ induces *NQO1* but does not affect *CYP1A1* (Fig. 5). Western blot data gave similar results: D3T and BHQ can induce both CYP1A1/2 and GSTA1/2 (another prototypic phase II enzyme), while EQ can only induce GSTA1/2, and does not affect CYP1A1/2 (Fig. 6). These data are further strong evidence that D3T and BHQ are indeed bifuntional inducers, and validate the cell-based luciferase assay system.

As XRE and ARE represent two very distinct pathways, we wondered about the mechanism of action of bifunctional inducers. Previous hypotheses have suggested an indirect mechanism, whereby some phase I inducing drugs are metabolized by the CYP (or other phase I) enzymes into compounds that can then activate the ARE. To examine this directly we used the murine hepatoma cell 1c1c7 and its mutant tao (deficient in AhR) and c37 (deficient in CYP1A1) to explore the mechanism. The cells were transfected with the A2-ARE construct and then exposed to several bifunctional inducers as well as two monofunctional inducers as controls. The results showed that the monofunctional inducers SFP and EQ can activate the ARE in the absence of a functional AhR receptor and that SFP do not rely upon metabolism by CYP1A1, while EQ is

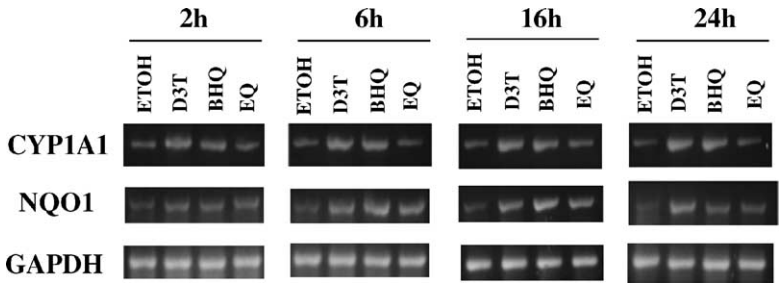

Fig. 5. Effects of D3T, BHQ and EQ on mRNA expression of *CYP1A1* and *NQO1*. Rat hepatoma H4IIE cells were treated with 10 μM D3T, BHQ, EQ and the vehicle ETOH for 2, 6, 16 and 24 h before total RNA being prepared from cells. RT–PCR was carried out to measure the mRNA expression of *CYP1A1* and *NQO1*.

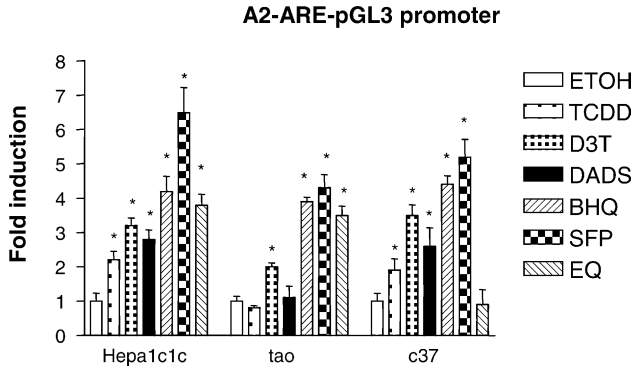

Fig. 7. Effects of AhR- or CYP1A1-deficiency on ARE activation of DME modulators. The murine hepatoma AhR-deficient cell line tao, CYP1A1-deficient cell line c37 and their parent cell line 1c1c7 were transfected with pGL3-ARE promoter construct before being exposed to designated compounds. The ARE driven luciferase activities were measured, and the fold induction was expressed as relative luciferase activity of treated vs. untreated samples. The values represent the mean ± S.E.M. of three independent measurements. Statistical analysis (Student's *t*-test) was performed by comparison of treated and untreated cells ($^{*}P < 0.05$).

dependent on a functional CYP1A1. (Fig. 7). On the other hand, activation of ARE by the bifunctional agents D3T and BHQ occurs in all three cell lines and therefore is not dependent on a either functional AhR or CYP1A1 metabolism (Fig. 7). Curiously, bifunctional agents such as TCDD (a potent XRE inducer) and DADS, activate the ARE-driven construct in the CYP1 expressing and deficient cells, but it is not effective in the AhR deficient Tao cell line (Fig. 7). The demonstration of a potential direct role for the AhR in ARE activation is novel and is under further detailed study. This does demonstrate that our cell-based system can be used to identify novel interactions between the DME pathways.

Fig. 8 is a schematic representation of the classification of model known DME inducers, as well as potential pathways of activation.

4. Discussion

The recent elucidation of molecular mechanisms underlying DME gene modulation provides an opportunity to develop a molecular mechanism-based screening tool. There were two successful reports that used a 41-bp EpRE (electrophile response element, a murine ARE sequence)-based assay to assess the induction potency of different compounds [10,11]. However, the use of ARE-based assay alone for characterization is not sufficient, because XRE is another major *cis*-acting element involved. Furthermore, such a system precludes the possibility of cross-talk between these two regulatory mechanisms. Here we have used both ARE and XRE-based assays in order to construct a quick and efficient characterization tool. The data from analyzing eight classes of known DME modulators showed high efficiency and good accuracy of the tool.

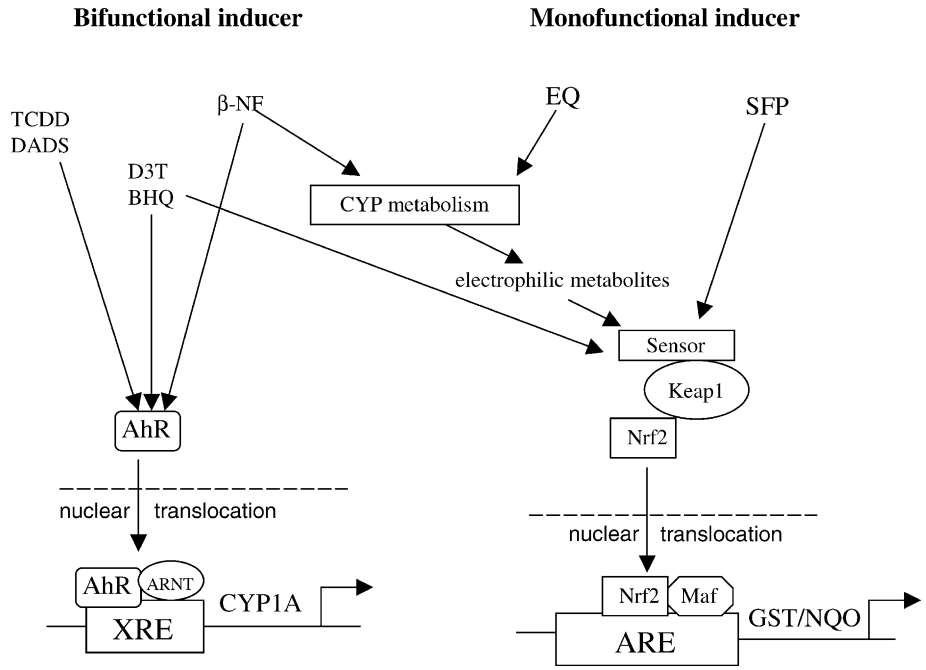

Fig. 8. Schematic diagram showing re-classifications of D3T, and BHQ as bifunctional inducers. The diagram demonstrates that EQ and SFP exclusively activate the ARE pathway, and represent true monofunctional inducers. It also shows that D3T, BHQ activate both XRE and ARE pathways, thus should be reclassified as bifunctional inducers. By using AhR and CYP1A1-deficient cell lines, we further revealed that the activation of ARE-luciferase transgene by D3T, BHQ and β-NF is AhR independent, suggesting that these compounds can activate the XRE and ARE pathways independently and simultaneously. Meanwhile, the activation of ARE-luciferase transgene by TCDD and DADS is dependant on a functional AhR, but not a functional CYP1A1, implicating a more direct role of AhR involved in the activation of the ARE pathway by these compounds.

Our system also provides the opportunity to learn more about some modulators since, for example, it led us to reclassify D3T and BHQ from monofunctional into the category of bifuctionl inducers. D3T is a dithiolthione compound, isolated from cruciferous vegetable, which demonstrates significant cancer chemoprevention effect in several tumorigenesis models [12,13]. D3T and its synthetic derivative oltipraz are generally considered monofunctional inducers, exclusively inducing phase II detoxifying enzymes through activation of the ARE [10,14]. Recently, however, several papers have found that oltipraz can induce *CYP1A1* [15], *UGT6* [16] and *GSTA5* [17] through activation of XRE. In a gene expression microarray experiment, we found that 10 μM oltipraz treatment for 24 h induced *CYP1A1* mRNA expression by six-fold in the human hepatoma HepG2 cells (unpublished results). Together these studies strongly suggest that oltipraz is a bifunctional inducer. Our results in this cell-based mechanistic system adds further confirmation that D3T is a bifunctional inducer like oltipraz, and further suggests that all dithiolthione compounds may be bifunctional inducers.

Another surprising result in our system was obtained with BHQ, which is considered a typical antioxidant agent. Several papers have shown that it can robustly activate the ARE, but no previous data showed that it can also activate the XRE. Buetler et al. in fact did show that BHA, a derivative of BHQ, can induce both mRNA and protein of both phase I and phase II enzymes [18]. More recently, Munzel et al. observed coordinate responses of UGT activity after treatment with BHQ in rat hepatoma 5L cells, mutants without the AhR and with recomplemented AhR. The results suggest a contribution of the AhR pathway to the induction of human UGT1A6 by BHQ [19]. We have additional studies (not shown) that reveal that BHA activates both XRE and ARE, while EQ only activates the ARE in the human HepG2 cell line. In the present study we compared BHQ with EQ in the rat hepatoma H4IIE cell, and the data clearly showed that BHQ can activate both XRE and ARE while EQ only activates the ARE (Fig. 3).

The parallel RT–PCR and Western blots confirmed the validity of using the promoter sequences linked to luciferase to classify DME inducers. The results of measuring mRNA and protein confirm the luciferase results, and furthermore confirm the novel reclassification of BHQ as a bifunctional inducer.

In our experiments, EGCG, an active green tea polyphenol compound, activates neither XRE nor ARE. In previous reports, the induction of DME by EGCG was variable [20], and we consider it likely that other anti-tumor properties are involved in its putative chemopreventive effects, e.g. [21], such as tumor cell growth inhibition [22,23], anti-angiogenesis by inhibition in VEGF induction [24,25], and in VEGF receptor binding [26].

The organosulfur DADS is also a multifunctional compound, which possesses remarkable anti-tumor effects [27–31]. According to results obtained in our system, we can provide this first report that DADS can potently activate both XRE and ARE. In addition we have identified an apparent structure–function relation of the related organosulfur compounds (DAS < DADS < DATS) on XRE and ARE activation, suggesting that the sulfur moiety in the molecule is important in its activity. Our results provide molecular mechanisms for the previous reports that DAS < DADS < DATS for activation of GST subunits Yb1, Yc, Ya [32] and GSTP [33], and that DAS < DADS for rGSTA5 and rAFR1 induction [34]. Thus our system can be used in iterative structure–activity studies of modified chemicals.

Although the XRE and ARE-based luciferase assays cannot entirely replace the traditional assays of enzyme activity, mRNA and protein expression, they have several advantages as an early stage screening tool: (1) As XRE or ARE activation is the major mechanism involved in DME modulation, the majority of DME modulators could be quickly identified using our luciferase assay screening. In this way, substantial time and labor can be saved in related drug discovery projects. (2) Through simultaneous screening for XRE and ARE activations, the candidate compounds can be quickly classified into bifunctional or monofuncional divisions. Monofunctional inducers like sulfurophane and ethoxyquin exclusively activate phase II detoxifying enzymes, which have great potential for a wide clinical application.

Recently, Brook et al. found that in prostate cancer cells, many bifunctional inducers, including oltipraz, do not work for stimulating quinone reductase [35], while monofunctional inducers like sulforaphane can potently induce quinone reductase [36]. The results suggest that there is a possible tissue specificity in response to DME modulators, which justifies the need for a mechanism-based classification of each DME modulators. (3) If this preliminary screening is negative for a novel compound that has chemopreventive efficacy, the mechanism of ARE or XRE activation can be quickly ruled out, so that other possible mechanism of anti-tumor activity can be explored.

In this work, we used a transient transfection approach for luciferase detection in the rat hepatoma H4IIE cell line. While we propose this as potential drug screening tool, it has important potential for more fundamental research. For example, we have provided preliminary data on a novel cross-talk between the pathways. The XRE and ARE gene expression regulation systems are two very distinct and well-studied pathways. These systems are generally thought to function entirely independently, however, our study suggests interesting interactions. By using AhR-deficient cells, we found that some bifunctional inducers, such as D3T and BHQ, can activate the ARE in the absence of a functional AhR, suggesting D3T and BHQ can activate the ARE and the XRE simultaneously and independently. On the other hand we found as well that bifunctional inducers such as TCDD and DADS are unable to activate

the ARE in the absence of a functional AhR, suggesting a possible cross-talk between AhR and ARE pathway.

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