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Aldo-Keto Reductase Regulation by the Nrf2 System: Implications for Stress Response, Chemotherapy Drug Resistance, and Carcinogenesis

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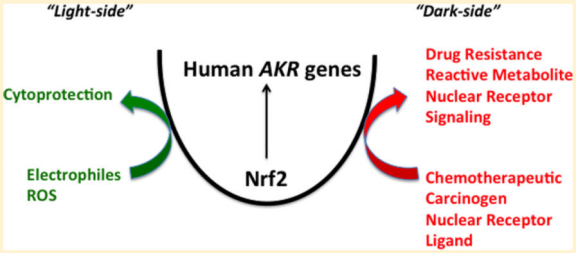
Abstract



Human aldo-keto reductases (AKRs) are NAD(P)H-dependent oxidoreductases that convert aldehydes and ketones to primary and secondary alcohols for subsequent conjugation reactions and can be referred to as "phase 1" enzymes. Among all the human genes regulated by the Keap1/Nrf2 pathway, they are consistently the most overexpressed in response to Nrf2 activators. Although these enzymes play clear cytoprotective roles and deal effectively with carbonyl stress, their upregulation by the Keap1/Nrf2 pathway also has a potential dark-side, which can lead to chemotherapeutic drug resistance and the metabolic activation of lung carcinogens (e.g., polycyclic aromatic hydrocarbons). They also play determinant roles in 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone metabolism to *R*- and *S*-4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol. The overexpression of AKR genes as components of the "smoking gene" battery raises the issue as to whether this is part of a smoking stress response or acquired susceptibility to lung cancer. Human AKR genes also regulate retinoid, prostaglandin, and steroid hormone metabolism and can regulate the local concentrations of ligands available for nuclear receptors (NRs). The prospect exists that signaling through the Keap1/Nrf2 system can also effect NR signaling, but this has remained largely unexplored. We present the case that chemoprevention through the Keap1/Nrf2 system may be context dependent and that the Nrf2 "dose-response curve" for electrophilic and redox balance may not be monotonic.

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1. INTRODUCTION

Aldo-keto reductases (AKRs) belong to a protein superfamily that mainly contains monomeric cytosolic oxidoreductases that catalyze the NAD(P)H-dependent reduction of a large number of endogenous and exogenous carbonyl substrates, e.g., aldehydes and ketones to produce primary and secondary alcohols, respectively, Figure 1.[1–4] In addition, these enzymes can also perform double-bond reduction, e.g., AKR1D1 (steroid 5β-reductase), or nitroreduction, e.g., AKR1C3.[5,6] The alcohol products are often conjugated by sulfonation catalyzed by sulfotransferases (SULTs) or by glucuronidation catalyzed by uridine diphosphate glucuronsyl transferases (UGTs). As such, AKRs are phase 1 enzymes. Because of their ability to detoxify reactive aldehydes and ketones, it is not surprising to find that these enzymes can be cytoprotective, are evolutionary conserved, and are found in archaebacteria, prokaryotes, and eukaroytes. The AKR genes encoding these enzymes can be considered stress response genes because they are regulated by oxidative, electrophilic,[7–10] and osmotic stress[11] and heat shock.[12] Thus, these primordial genes are regulated by primordial signals.[13]

In humans, there are 15 different AKRs that play key roles in the metabolism of sugar and lipid aldehydes, e.g., advanced glycation end products[14,15] and 4-hydroxy-2-nonenal (4-HNE), respectively;[8,16] chemical carcinogens, e.g., aflatoxin dialdehyde;[17] polycyclic aromatic *trans*-dihydrodiols;[18,19] nicotine-derived nitrosaminoketones (NNK);[20,21] as well as cancer chemotherapeutic agents.[22–25] The human AKR genes are involved in the metabolism of prostaglandins[26–29] and natural and synthetic steroid hormones,[30–34] all of which contain carbonyl functionalities (Table 1).

The NF-E2 p45-related factor (also known as nuclear factor (erythroid-derived 2)-like 2 (Nrf2)–Kelch-like ECH-associated protein 1 (Keap1) system[35,36]) induces many of the human AKR genes to respond to oxidative and electrophilic stress.[7,9,10,37] In this perspective, I will discuss the roles of these enzymes and present the case that, depending on the context, upregulation of the AKR genes by the Keap1/Nrf2 system can represent a “dark-side” to Nrf2 activation.

2. THE KEAP1/NRF2 SYSTEM

Levels of the Cap'n'Collar basic leucine zipper transcription factor Nrf2 are kept in check by binding to the cytosolic protein Keap1.[36,38] Keap1 targets Nrf2 for ubiquintination and proteasomal degradation by acting as a substrate adaptor protein for the cullin 3-dependent ubiquitin ligase.[39] Keap1 is decorated with cysteine residues, which permits the protein to sense reactive oxygen species, electrophiles, Michael acceptors, and heavy metals.[40] Once the cysteine residues are oxidized and form adducts with electrophiles or complexes with metals, dimeric Keap1 undergoes a conformational change to bind Nrf2 more tightly. This permits newly translated Nrf2 to evade Keap1-mediated ubiquintination and translocate to the nucleus.[41] Keap1 is not the only repressor of Nrf2. Glycogen synthase kinase 3 also creates a phosphodegron on Nrf2 to reduce endogenous Nrf2 protein.[42,43] Once Nrf2 is translocated to the nucleus, it forms heterodimers with small Maf.[35] The heterodimer binds to the antioxidant response element (ARE), also known as the electrophilic response element

(EpRE), in responsive genes,[44,45] which belong to the ARE-gene battery. Human genes in this battery include those involved in xenobiotic detoxication (e.g., NADPH:quinone oxidoreductase (*NQO1*)), detoxication of reactive oxygen species (e.g., *SOD1*, *CAT*), heme detoxication (e.g., heme oxygenase), and glutathione synthesis (e.g., γ-glutamyl cysteine ligase light chain).[39,46] The human AKR genes *AKR1B1*, *AKR1B10*, *AKR1C1*, *AKR1C2*, *AKR1C3*, *AKR7A2*, and *AKR7A3* are all regulated by the Keap1/Nrf2 system.[7,9,10,47–52] Although many studies measure *NQO1* induction as a read out of Nrf2 activation, often the level of induction of *NQO1* is a quite modest 2–3 fold by comparison to the induction seen in the AKR genes, which can be 1–2 orders of magnitude greater.[52,53] In fact, the AKR gene response can be one of the most robust signatures of Nrf2 activation in humans, yet less attention is placed on the regulation of these genes and its consequences.

3. EVIDENCE THAT HUMAN AKR GENES ARE REGULATED BY ARES

Ciaccio and Tew were the first to report the induction of an AKR gene in human colon (HT29) cells that were resistant to ethacrynic acid and induced by dimethyl maleate, *t*-butyl hydroquinone, and hydroquinone, which are classical Nrf2 activators.[54] The gene regulated was identified as a dihydrodiol dehydrogenase (DD) and was ultimately identified as *AKR1C1*.[9,55] Subsequent studies revealed that exposure of HepG2 cells to benzo[*a*]pyrene and other polycyclic aromatic compounds (bifunctional inducers), and electrophilic Michael acceptors, phenolic antioxidants (monofunctional inducers), and reactive oxygen species (ROS) led to a 3–10 fold increase in *AKR1C* mRNA.[7] In this context, bifunctional inducers bind to the aryl hydrocarbon receptor (AhR) and are metabolically activated to electrophiles and/or ROS to subsequently activate the Keap1/Nrf2 pathway. By contrast, monofunctional inducers act only as direct acting electrophiles or produce ROS to activate the same pathway. The distinction between bifunctional and monofunctional inducers was originally made by Prochaska and Talalay.[56] However, this terminology is no longer used because there are Nrf2 activators that are neither electrophilic nor ROS producing.

In HepG2 cells, induction of *AKR1C* mRNA by compounds that activate AhR and Nrf2 (e.g., benzo[a]pyrene, β-naphthoflavone) was delayed with respect to the induction of *CYP1A1* mRNA, indicating their need to undergo metabolism. By contrast and *AKR1C* mRNA was not induced by the nonmetabolizable AhR ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These data suggest that, in contrast to *CYP1A1*, induction of *AKR1C* member(s) by polycyclic aromatic hydrocarbons (PAHs) is mediated indirectly via an ARE by their electrophilic metabolites rather than a xenobiotic response element regulated by the AhR. RNase protection assays identified *AKR1C1* (DD1) mRNA as the transcript, which was upregulated by β-naphthoflavone and Nrf2 activators (ethacrynic acid and *tert*-butyl hydroquinone) and ROS in both human hepatoma (HepG2) and colon carcinoma (HT29) cells.[7] Subsequently, siRNA against Keap1 and *R*-sulforaphane treatment of human HaCAT keratinocytes resulted in induction of *AKR1C1/2*.[57] Promoter analysis of the *AKR1C1* and *AKR1C2* genes located a distal consensus ARE regulated by Nrf2.[10] Concurrently, studies on AKR7A1 identified this enzyme as an ethoxyquin-inducible aldehyde reductase in rat liver that was induced by Nrf2.[58,59] Human aflatoxin dialdehyde reductases AKR7A2 and AKR7A3 protect against products of lipid peroxidation and aflatoxin dialdehyde in cell lines, respectively.[50,60] Each of the genes for these enzymes contained AREs. Knockdown of

Nrf2 in HepG2 cells led to a reduction of *AKR7A3* mRNA and AKR7A3 protein and increased sensitivity to acetaminophen-induced cytotoxicity.[47] Analysis of the human AKR gene promoters identified the existence of multiple AREs in 13/15 human genes, and many have subsequently been found to be functional, Table 2.[46]

Agyeman et al. conducted transcriptomic and proteomic analysis of gene expression in MCF10A cells treated with either *R*-sulforaphane as an Nrf2 activator or with si-RNA for Keap1 so that Nrf2 would be constitutively active. Transcriptomic studies were performed by microarray and quantitative proteomic analysis was performed by stable-isotope labeling of amino acids in cell culture (SILAC).[53] Transcriptomic analysis showed that *AKR1B10*, *AKR1C1/2*, and *AKR1C3* were highly induced by *R*-sulforaphane where the fold changes were 302-, 15-, and 27-fold, respectively; by contrast, *NQO1* was induced 4.4-fold. SILAC analysis showed that AKR1C1/2 and AKR1C3 proteins were highly induced by *R*-sulforaphane where the changes were 31 and 39, respectively; by contrast, *NQO1* was induced 3.7-fold. Thus, the expression of the *AKR1* genes was consistently more robust than *NQO1*, and this is now considered an important biomarker of the antioxidant response in humans.

4. CHANGES IN HUMAN AKR GENE EXPRESSION WITH DISEASE STATE

Changes in *AKR1* gene expression have been observed in a number of disease states, especially those related to tobacco carcinogenesis. Most notable was the overexpression of *AKR1C1/2* in non-small cell lung carcinoma (NSCLC), where a dramatic increase in *AKR1C1* expression was observed in tumor versus adjacent normal tissue in 317/381 NSCLC patients using differential display;[61] *AKR1B10* and *AKR1C1* were found to be two of the seven most overexpressed of the 30,000 genes displayed on an Affymetrix microarray in NSCLC;[62] *CYP1A1, CYP1B1, AKR1B10, AKR1C1*, and *AKR1C3* were also increased 15–30 fold in expression in oral squamous carcinoma and induced by cigarette smoke condensate in oral dysplasic cells.[63] In addition, *AKR1B10*, *AKR1C1*, and *ALDH3A1* were three of the ten most overexpressed genes in tobacco-exposed bronchial epithelial cells,[64] and *CYP1A1*, *CYP1B1*, *AKR1C1*, *NQO1*, and *ALDH3A1* were part of a gene battery upregulated in buccal oral specimens of smokers.[65] It was also found that *CYP1B1*, *AKR1B10*, *AKR1C1*, and *AKR1C2* were the most upregulated genes in bronchial epithelial cell brushes of smokers and were downregulated in smokers who quit.[66,67] The consistent finding is that, in either smoking-related cancer or upon smoke exposure, *AKR1B10* and *AKR1C1* are consistently overexpressed. Interestingly, A549 cells, which are derived from a human lung adenocarcinoma patient, constitutively express Nrf2 due to a somatic mutation in Keap1 and, as a result, have high expression of *AKR1C1–AKR1C3.*[68]

Dysfunction of the Keap1/Nrf2 system has been observed in NSCLC patients where a high frequency of mutations (19% in 50 cases) were observed in a region of Keap1 that would attenuate its repressive effects on Nrf2.[68] *Keap1* can also become hypermethylated to reduce its expression in lung cancer. Methylation of the *Keap1* promoter was observed in 22/47 NSCLC patients.[69] Thus, *AKR1* genes are clearly upregulated as part of the stress response to tobacco smoke, and at some point in the oncogenic process, *Keap1* becomes either mutated or is epigenetically silenced, leading to high constitutive expression of Nrf2 and

hence induction of *AKR1* genes. This raises the question as to whether the high overexpression of *AKR1* genes is a protective stress response or whether it contributes to disease pathogenesis. To address this question, it is important to consider the roles of these enzymes and in what context their overexpression is protective or harmful or may contribute to oncogenesis. It is known that AKR1B10 prevents retinoic acid signaling by its all-*trans*-retinaldehyde reductase activity[70,71] and that *AKR1C* genes are implicated in the metabolism of tobacco carcinogens,[18–20,72] see sections 8 and 9.

5. AKRS AND DETOXICATION OF LIPID PEROXIDES AND REACTIVE ALDEHYDES

4-HNE and 4-oxo-2-nonenal are by products of lipid peroxidation, which result from the free radical attack of polyunsaturated fatty acids and are a hallmark of oxidative stress.[73] These reactive lipid aldehydes are bifunctional electrophiles because they contain an α,β-unsaturated carbonyl and are therefore Michael acceptors, and through their aldehyde group, they can form Schiff bases. Thus, they have the potential to act as cross-linking agents with proteins and form DNA adducts that are mutagenic.[74] In considering this bifunctionality, Michael addition is favored due to the presence of the α,β-unsaturated carbonyl, but once this occurs, the aldehyde is able to react with lysine residues.

There are redundant mechanisms that can detoxify these reactive aldehydes. AKRs involved in this process are AKR1B1, AKR1B10, AKR1C1, AKR1C2, and AKR7A2.[8,16,50,75,76] Evidence for their critical role comes from si-RNA studies and the use of chemical probes (isoform-selective AKR inhibitors) to show that, in the absence of an AKR, cells become sensitized to the cytotoxic effects of these lipid peroxidation byproducts. Which AKRs are the most important is often determined by differences in substrate preference and their cellular distribution. 4-HNE can be rapidly conjugated by glutathione, and 4-HNE-glutathionyl conjugates are the preferred substrates for AKR1B1.[16] By contrast, AKR1B10, AKR1C1, and AKR7A2 reduce 4-HNE to 4-hydroxy-1,2-nonenol, eliminating the bifunctionality of 4-HNE in a single step. AKR1B10, AKR1C1, and AKR7A2 play this cytoprotective role in the small intestine and colon,[75] lung,[8] and neuronal cells,[77] respectively.

6. AKRS AND CHEMOTHERAPEUTIC DRUG RESISTANCE

Overexpression of AKR genes in cancer cells that are resistant to cancer chemotherapeutic agents is commonplace. This is observed with cisplatin,[22,23] anthracyclins (doxorubicin and duanorubciin),[24] mitomycin,[78] Temozolomide,[79] cyclophosphosphamide,[80–82] and oracin,[25] see Table 3. This overexpression could be associated with a counter-response to the stress generated by these cytotoxic agents or it could be causative in the resistance phenotype. Simpkins was the first to demonstrate that ovarian cancer cells grown in the presence of cisplatin become resistance to the drug and that this resistance is associated with the overexpression of AKR1C1. Stable transfection of AKR1C1 into nonresistant ovarian cancer cells endowed the cancer chemotherapeutic drug resistant phenotype, showing that it was causative.[22,23] Felsted et al.[83] was among the first to show that rat daunorubicin reductase was aldehyde reductase and that this enzyme likely increased the cardiotoxicity of the agent.

AKRs also reduce the cytotoxicity of cyclophosphamide, Table 3. Cyclophosphamide is activated by P450-mediated hydroxylation to yield 4-hydroxy-cyclophosphamide, the immediate precursor of aldophosphamide, which gives rise to phosphoramide mustard and the reactive aldehyde acrolein. AKR1B1 catalyzes the reduction of acrolein to allyl alcohol (K_m = 80 μM, k_{cat} = 87 min^{-1}). Acrolein also causes a time-dependent 7–20-fold increase in the activity of AKR1B1.[81] In medulloblastoma, AKR1B10 is implicated in drug resistance by also reducing aldophosphamide.[80]

AKR1B10 is recognized as a diagnostic tumor marker in lung cancer and in the proliferation of cancer cells.[57] AKR1B10 is also overexpressed in mitomycin-resistant colon cancer cell lines (HT29 cells), where it is implicated in protecting against ROS and the production of lipid peroxidation by products generated by mitomycin treatment.[78] The drug resistance phenotype could be reversed by the AKR1B10 selective inhibitor (*Z*)-2-(4-methoxyphenylimino)-7-hydroxy-*N*-(pyridin-2-yl)-2*H*-chromene-3-carboxamide and si-RNA.[78]

AKR1C1–3 were significantly increased in both U373 and T98G human glioblastoma cells (at both the mRNA and protein levels) after long-term treatment with the alkylating agent Temozolomide.[79] How overexpression mediates resistance to this agent is unknown, but AKR1C1–3 can all stimulate a growth proliferative phenotype in these different cell models.

Oracin is a DNA intercalating anticancer agent. AKR1C1–4 mediate the carbonyl reduction of 6-[2-(2-hydroxyethyl)-aminoethyl]-5,11-dioxo-5,6-dihydro-11*H*-indeno[1,2-*c*]-isoquinoline (oracin) to its inactive metabolite dihydrooracin, leading to drug resistance.[25]

Resistance to enzalutamide is mediated by AKR1C3 through an entirely different mechanism. Enzalutamide is a potent androgen-receptor antagonist used in androgen deprivation therapy in castrate-resistant prostate cancer. Median survival seen with this agent is 3–4 months before drug resistance emerges. This resistance phenotype is caused by overexpression of AKR1C3, which through its 17β-hydroxysteroid dehydrogenase activity can produce potent ligands for the androgen receptor to surmount the effect of this AR antagonist.[88]

In these instances, human AKRs appear to mediate drug resistance, and inhibitors of either the AKRs or the Keap1/Nrf2 system would be desirable. Thus, Nrf2 activation in terms of cancer chemotherapeutic drug resistance represents a “dark-side” to Nrf2 activation.

7. AKRS AND CANCER CHEMOTHERAPEUTIC DRUG ACTIVATION

PR104A is a new anticancer drug developed for the treatment of solid hypoxic tumors. Recently, PR104 was found to be effective under aerobic conditions, increasing its broad anticancer spectrum.[5] The drug contains a nitro group, which is metabolically activated by nitroreduction to the hydroxylamino compound, which is then activated to a DNA damaging species by either acetylation or sulfonation, Figure 2. In comparison with NQO1, AKR1C3 was found to be the superior nitroreductase for this reaction, Figure 1.[5] Induction of AKR1C3 with *R*-sulforaphane led to enhanced bioactivation of the pro-drug and

antiproliferative effects.[5,48] Thus, with cancer chemotherapeutic drug response, whether induction of AKRs is deleterious or beneficial may be drug and tumor dependent.

8. NRF2-REGULATED GENES AND CARCINOGEN METABOLISM

8.1. Aflatoxin

Many of the human AKR genes are implicated in carcinogen metabolism. The hepatocarcinogen aflatoxin is activated by P4503A4 to aflatoxin epoxide, which undergoes hydrolysis and ring opening to form the dialdehyde. Ethoxyquin-inducible aflatoxin dialdehyde reductase (AKR7A1) was originally described by Ellis et al. in rat liver,[59] where the enzyme metabolizes aflatoxin dialdehyde to the mono- and dialcohols, preventing the dialdehyde from forming Schiff bases with proteins. This activity has also been assigned to human AKR7A3.[60] Using liver specific *AKR7A1* transgenic Sprague–Dawley rats, two lines were developed, $AKR7A1^{Tg2}$ and $AKR7A1^{Tg5}$. Both overexpressed AKR7A1 to different degrees, and rates of formation of aflatoxinB1 alcohols were increased. However, neither Tg line protected against acute aflatoxin-induced bile acid proliferation, suggesting that upregulation of *AKR7A* genes is insufficient to protect against protein adduct formation from the dialdehyde and attenuate aflatoxin B1 tumorigenicity.[89] These data suggest that different outcomes in cell-based experiments and whole animals can exist when considering the protective effects of AKR7A3 and perhaps other AKRs as well. Thus, using in vitro experiments to predict in vivo acquired phenotypes resulting from Nrf2 activation need to be interpreted with caution.

8.2. Tobacco Carcinogens

Human AKRs play important roles in the metabolism of two major classes of tobacco carcinogens, polycyclic aromatic hydrocarbons (PAH) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), which may account for their overexpression as part of the “smoking gene” battery.

AKR1A1, AKR1C1–4, and AKR1B10 have all been shown to play a role in the metabolic activation of PAH *trans*-dihydrodiols via their dihydrodiol dehydrogenase activity.[18,19,90] In this reaction *trans*-dihydrodiols are oxidized in the presence of $NAD(P)^+$ to a ketol, which then spontaneously rearranges to a catechol. The catechol undergoes two sequential one-electron oxidation reactions in air to form an *o*-semiquinone anion radical in route to electrophilic *o*-quinones and reactive oxygen species. In the presence of NADPH, NQO1 and AKR enzymes themselves reduce the *o*-quinones back to the catechol, establishing an enzymatic futile redox cycle thereby amplifying the production of ROS. For benzo[*a*]pyrene, the sequence involves the oxidation of benzo[*a*]pyrene-7,8-*trans*-dihydrodiol to form 7,8-dihydroxybenzo[*a*]pyrene and its autoxidation to benzo[*a*]pyrene-7,8-dione (BPQ), Figure 3.[91–93] Subtle differences exist in PAH *trans*-dihydrodiol specificity and stereochemical preference of AKRs. AKR1A1 has the highest catalytic preference for (−)7*R*,8*R*-*trans*-dihydrodiol of benzo[*a*]pyrene, which is the metabolically favored isomer.[19] By contrast, AKR1C1–4 oxidize both the (−)-*R,R* and (+)-*S,S* isomers but prefer bay-region methylated *trans*-dihydrodiols, which are more tumorigenic than their unmethylated counterparts.[18] AKR1B10 has lower specific activities and has a preference for the minor stereoisomers.[90]

The AKR1C1–3 enzymes appear to be the most important in the oxidization of PAH *trans*-dihydrodiols in human lung cells, where they are inducible by the ARE and overexpressed in A549 lung adenocarcinoma cells.[94] In A549 cells, the complete sequence for the activation of benzo[a]pyrene-7,8-t*rans*-dihydrodiol oxidation was observed, resulting in the production of BPQ, ROS, and elevated amounts of mutagenic 8-oxo-dGuo.[94] The production of BPQ and ROS suggests that *AKR1C* genes regulate their own expression via AREs, generating a positive feedback loop that exacerbates the carcinogenicity of PAH tobacco carcinogens, i.e., this would mean that PAH *trans*-dihydrodiols enhance their own genotoxicity by inducing *AKR1C* genes, Figure 3. This feed-forward loop could be attenuated if the intermediate catechol could be either *O*-methylated or conjugated by sulfotransferases or glucuronosyl transferases. In A459 cells treated with B[*a*]P-7,8-*trans*-dihydrodiol, both ROS and 8-oxo-dGuo were increased in the presence of a catechol-*O*-methyl transferase (COMT) inhibitor, showing that some interception of the catechol can occur.[94] To determine the extent by which phase 2-conjugating enzymes can protect against the redox cycling of *o*-quinones, recombinant forms of COMT, SULTs, and UGTs expressed in lung cells were examined for their ability to form catechol conjugates versus the preference of *o*-quinones to redox cycle.[95–97] The specific activities for NQO1 and AKRs to redox cycle BPQ were much greater than those observed for the phase 2 enzymes to conjugate 7,8-dihydroxybenzo[*a*]-pyrene, Table 4.[98] In fact, conversion of the *trans*-dihydrodiols to the *o*-quinones catalyzed by AKRs was 100–1,000-fold slower than their subsequent reduction of the *o*-quinone to produce the catechol. Thus, the redox cycling observed with the *o*-quinones overwhelms the activity of the phase 2-conjugating enzymes. These data support the notion that enzymatic redox cycling of PAH *o*-quinones occurs at an alarming rate, leading to ROS and Nrf2 activation.

The tobacco carcinogen NNK is metabolically activated by P4502A6 and P4502A13 (in the aerodigestive tract)[99] by α-methyl or α-methylene group hydroxylation. α-Methyl hydroxylation results in the formation of formaldehyde and a pyridyloxylbutyl (POB) diazohydroxide, which leads to formaldehyde and POB-DNA adducts,[100,101] respectively. α-Methylene hydroxylation can lead to the formation of an α-methyl-diazohydroxide and a pyridyloxybutylaldehyde. The former collapses to form an α methyl carbonium ion that can form O^6-methyl-guanine DNA adducts.[102] Metabolic activation of NNK can be prevented if it is reduced to *R*-4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (*R*-NNAL) and *S*-NNAL provided the alcohols are glucuronidated by UGTs and eliminated. In the smoking gene battery, AKR1C1–3 reduce NNK to *S*-NNAL, whereas human lung microsomes predominately produce *R*-NNAL.[72] *S*-NNAL is stereoselectively retained in rat lung and has a higher tumorigencity than that of *R*-NNAL.[103,104] *S*-NNAL can still undergo activation by α-methyl and α-methylene hydroxylation. Thus, induction of AKR1C–3 could prevent the formation of *R*-NNAL and its subsequent elimination as *S*-NNAL–*O*-glucuronide and exacerbate DNA-adduct formation. It should be noted that, in the UGT reaction mechanism, there is proposed inversion of stereochemistry at the anomeric carbon.[105]

8.3. Nitroarenes

Nitroarenes are nitrated PAHs, which are unique to diesel exhaust emissions. Diesel exhaust has been recently classified as a Group 1 carcinogen by IARC.[106] Nitroarenes are

metabolically activated by six-electron reduction to form the corresponding amines. The sequence involves formation of nitroso, hydroxylamino, and amine intermediates.[107,108] Following formation of the hydroxylamino, intermediate acetylation by NAT or sulfonation by SULTs[109] introduces a good leaving group so that a nitrenium ion can form, which will result in DNA adducts, Figure 4. This sequence is observed with 3-nitrobenzanthrone (3-NBA), which is the most potent mutagen identified in the Ames test, and with 6-nitrochrysene, which is the most potent tumorigen in a newborn mouse model for lung cancer.[110,111] Enzymes implicated in the metabolic activation of nitroarenes are NQO1 and NADPH:P450 oxidoreductase.[108,109] Using ^{32}P-post-labeling, it was found that the number of DNA adducts derived from NBA[112] was significantly reduced in the presence of dicoumarol, an NQO1 inhibitor.[109] The metabolic activation of nitroarenes to electrophiles and their back oxidation in air in the presence of heavy metals to produce reactive oxygen species[113] indicates that nitroarenes may also induce their own genotoxicity by induction of *NQO1* via the ARE.[109,114]

In these examples, the induction of Nrf2-regulated genes in the context of air pollutants could be deleterious, leading to an enhancement of their genotoxicity; this may be observed with PAH, NNK, and nitroarenes and represents another “dark-side” of Nrf2 activation.

9. NRF2 REGULATION OF AKR GENES AND NUCLEAR RECEPTOR SIGNALING

Human AKR genes metabolize retinoids, prostaglandins, and steroid hormones and play a pivotal role in the prereceptor regulation of nuclear receptors (NR) by regulating the amount of ligand that can bind to these receptors, Table 5. Thus, the prospect exists that the same AKR genes that are regulated by the Keap1/Nrf2 pathway may also influence nuclear receptor action.

AKR1B10 is part of the “smoking gene battery”,[62,71,115] and this enzyme is a highly efficient retinal reductase that reduces all-*trans*-retinaldehyde to all-*trans*-retinol and thus prevents the formation of retinoic acid, Figure 4.[70,116] Retinoic acid binds to the retinoic acid receptor (RAR), which signals cells to differentiate and is thus antiproliferative. The attenuation of this signaling pathway by AKR1B10 suggests that its regulation by the Keap1/Nrf2 system provides a pro-proliferative signal. In NSCLC, where AKR1B10 is highly constitutively expressed due to a Keap1 mutation, or with Keap1 silencing, this could lead to the promotion of lung cancer.

AKR1C3 is also known as prostaglandin (PG) $F_2\alpha$ synthase, converts PGH_2 to $PGF_2\alpha$, and also possesses 11-ketoprostaglandin reductase activity, where it converts PGD_2 to 11β-$PGF_2\alpha$.[28,29] Both $PGF_2\alpha$ and 11β-$PGF_2\alpha$ bind to the FP1 receptor, which activates MAPK pathways to stimulate cell growth and inactivate PPARγ by phosphorylation. Additionally, conversion of PGD_2 to 11β-PGF2α prevents the formation of PGJ_2 ligands (15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; 15d-PGJ_2) for the peroxisome proliferator-activated receptor (PPARγ).[117] By forming heterodimer complexes with RXR, cell differentiation can proceed. Thus, AKR1C3 promotes cell proliferation by forming FP1 ligands and by depriving PPARγ of its ligands.[26,118] This mechanism has been shown to be an important signaling

mechanism in acute myeloid leukemia, and AKR1C3 inhibitors are being sought for their antineoplastic effects.[119]

AKR1C1-AKR1C3 display NADPH dependent 3-, 17- and 20-ketosteroid activity.[32] Thus, AKR1C1 is the primary 20-ketosteroid reductase in humans responsible for the conversion of progesterone to 20α-hydroxyprogesterone and terminates progesterone action at the progesterone receptor.[120] Progestin therapy is used for the treatment of precancerous endometrial conditions (e.g., atypical hyperplasia or endometrial intraepithelial neoplasia), but a portion of patients are resistant to progestin therapy. It has been shown that Nrf2-driven AKR1C1 expression mediates this progestin resistance.[121]

AKR1C2 is the primary 3-ketosteroid reductase responsible for the conversion of 5α-dihydrotestosterone to 5α-androstan-3α,17β-diol and can terminate androgen action at the androgen receptor.[33] By contrast, AKR1C3 is also a major peripheral 17-ketosteroid reductase, is responsible for the formation of potent androgens in the prostate, and is a source of testosterone, the substrate for aromatase, in the breast.[26,31,122] Extensive drug discovery programs exist for the development of AKR1C3 inhibitors for hormone-dependent malignancies.[123–130] The fact that each of these genes are regulated by the Keap1/Nrf2 pathway suggests that Nrf2 activators may have unintended consequences for steroid hormone receptor occupancy and hence steroid hormone action in both physiology and hormone-dependent malignancies.

10. “U”-SHAPED DOSE RESPONSE CURVE FOR NRF2 IN THE CONTEXT OF ELECTROPHILIC AND OXIDATIVE STRESS

Induction of AKR genes is clearly cytoprotective against the formation of lipid peroxidation by products and ROS.[7,50,51,75,76] However, this induction can also mediate cancer chemotherapeutic drug resistance, regulate NR action, and may exacerbate hormonal or chemical carcinogenesis based on context. There is no question that Nrf2 activators can be chemopreventive in rodent tumor models, where studies have been extensive.[131–133] There is also evidence that the principle of chemoprevention can be translated into humans, e.g., the prevention of hepatocarcinogenesis initiated by aflatoxin.[134] However, the animal studies on which the clinical studies are based were performed in species that lack orthologues of the human *AKR1C* genes. As indicated above, there are scenarios in which induction of the *AKR1B10* and *AKR1C* genes may contribute to tumor initiation and promotion. The ability of Nrf2 activation to have both beneficial and harmful consequences suggests that context is important and begs the question as to the shape of the Nrf2-dose response curve, the exposure paradigm and whether epigenetic regulation of the Nrf2 system influences the outcome.

We propose that, even for its cytoprotective effects, the shape of the dose response curve for Nrf2 activation is “U”-shaped and would mirror that seen for either an essential nutrient or trace metal, Figure 5. This hormetic dose–response curve for Nrf2 has also been proposed by other investigators to account for the role of Keap1/Nrf2 in oncogenesis.[135,136] Here, the dose is Nrf2 expression, and the response is oxidative or electrophilic stress. In the absence of Nfr2 expression, exposure to electrophilic or redox active compounds would increase

cellular stress and would be cytotoxic; as Nrf2 is activated by increased exposure to electrophiles or redox-active compounds, redox balance is addressed, and redox homeostasis is achieved; however, at high levels of exposure to electrophiles and redox active compounds, the Keap1/Nrf2 system induces AKR genes that may exacerbate the stress, especially when the compounds are lung carcinogens.

Many of the in vitro experimental systems that have been used to activate the Keap1/Nrf2 pathway use single acute exposures. However, real world exposure to Nrf2 activator inducers are likely to be to low dose and chronic. Important in vitro an in vivo experiments in which repeated single doses of Nrf2 activator are given with concurrent measurement of Nrf2 response were conducted using the chemoprevention agent Oltipraz, where the Nrf2 response was reported as induction of glutathione-S-transferase (GST) levels in rats.[137] Daily, twice weekly, and even weekly administration produced a prolonged response of GST activity. Thus, intermittent exposure to an Nrf2 inducer can have a prolonged effect. In other studies, long-term dietary administration of Oltipraz led to an induction of AKR7A1 that was less pronounced than that of shorter exposures.[138] Both studies indicate that adaptive responses to Nrf2 expression can occur after exposure to inducers. Low dose chronic exposures may also lead to permanent epigenetic changes in gene expression of the Cul3/Nrf2/Keap1 system. The system is known to be epigenetically regulated by DNA methylation, histone modification, and microRNAs.[139] These are important questions that need to be addressed when considering the pharmacokinetic/pharmacodyamic correlates of chemopreventive reagents targeting the Keap1/Nrf2 system.

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Biography

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ABBREVIATIONS

AhR	aryl hydrocarbon receptor
AKR	aldo-keto reductase
ARE	antioxidant response element
B[*a*]P	benzo[*a*]pyrene

BPQ	benzo[*a*]pyrene-7,8-dione
CAT	catalase
COMT	catechol-*O*-methyl transferase
Keap1	Kelch-like ECH associate protein
P450	cytochrome P450
PAH	polycyclic aromatic hydrocarbons
PPARγ	peroxisome proliferator-activated receptor γ
3-NBA	3-nitrobenzanthrone
NNK	4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone
NNAL	4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol
NR	nuclear receptor
NSCLC	non-small cell lung carcinoma
NQO1	NAD(P)H-quinone oxiodreductase
Nrf2	NF-E2 p45-related factor (also known as nuclear factor (erythroid-derived 2)-like 2)
RAR	retinoic acid receptor
ROS	reactive oxygen species
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
SOD1	superoxide dismutase
SULTs	sulfotransferases
UGTs	uridine diphosphate glucuronsyl transferases

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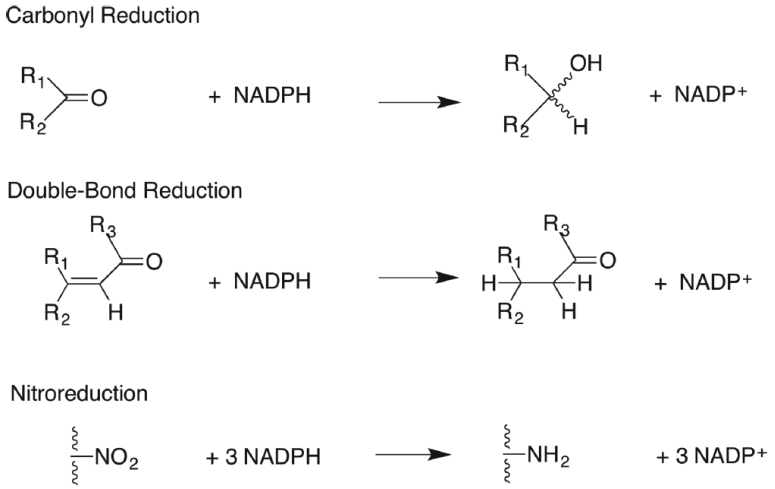


Figure 1.

Reduction reactions catalyzed by human AKRs; steroid double-bond reduction is catalyzed by AKR1D1, and nitroredution is catalyzed by AKR1C3.

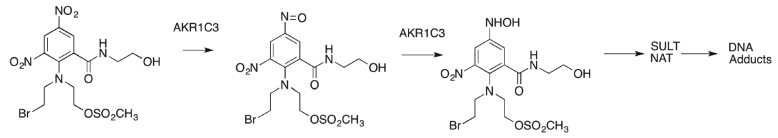


Figure 2.

Bioactivation of PR104A by AKR1C3. Steps involve the sequential formation of the nitroso and hydroxylamino products.

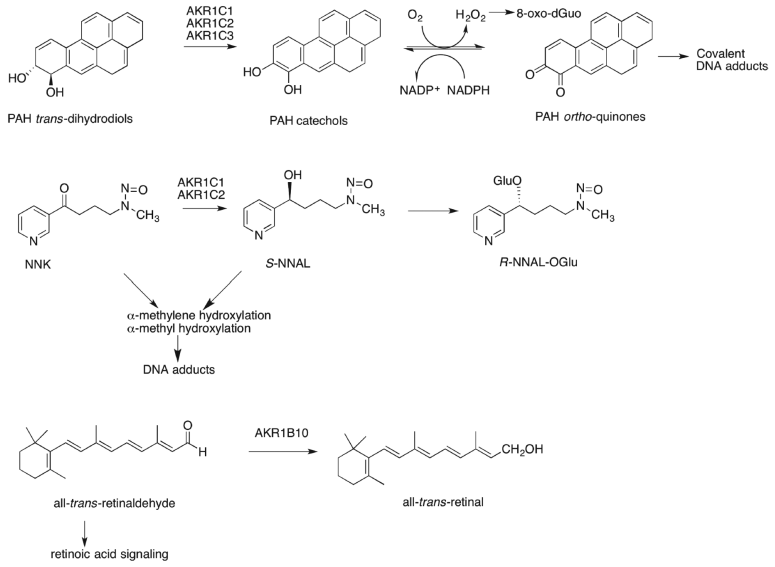


Figure 3.

Role of AKRs in human lung cancer: function of AKRs induced by the smoking gene battery. AKR1C1–3 are involved in the metabolic activation of PAH; AKR1C1 and AKR1C2 show a preference for forming *S*-NNAL, which can be retained in lung tissues for bioactivation, and AKR1B10 prevents retinoic acid signaling. Acting together, these reactions could contribute to lung cancer initiation and promotion.

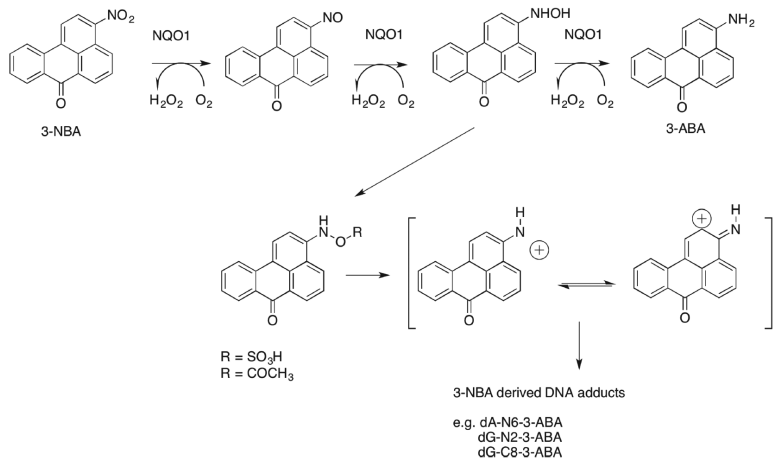


Figure 4.

Nrf2-regulated genes and carcinogen metabolism in humans: Role of NQO1 in the metabolic activation of nitroarenes. NQO1 plays a pivotal role in the metabolic activation of 3-NBA, yet it is the signature gene measured to demonstrate Nrf2 activation.

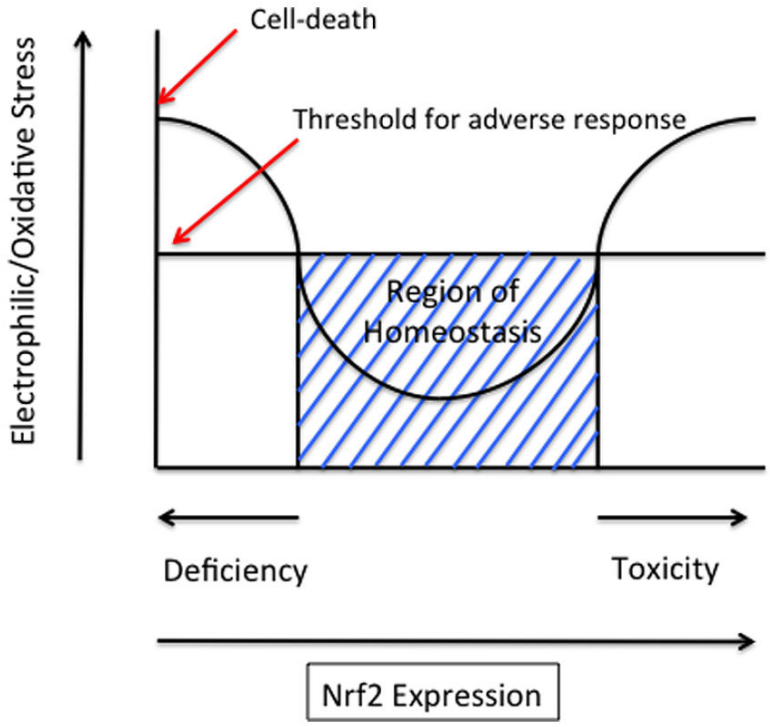


Figure 5.
"U"-shaped dose response for Nrf2. This hermetic dose–response curve is based on data from refs 135, 136, 140, and 141.

Table 1

Human Aldo-Keto Reductases

gene	chromosomal localization	nonsystematic name	physiologic reaction(s)	associated disease	sp
AKR1A1	1p33-p32	aldehyde reductase; dihydrodiol dehydrogenase (DD) 3	glyceraldehyde ➔ glycerol; mevaldic acid➔mevalonic acid		
AKR1B1	7q35	aldose reductase	glucose ➔ sorbitol	diabetic complications (caratogenesis; retinopathy; nephropathy)	so fi
AKR1B10	7q33	aldose reductase “small intestine like aldose reductase”; retinaldehyde reductase	retinal ➔ retinol	NSCLC	ol
				hepatocarcinogenesis	
AKR1B15	7q33	aldose reductase	3-keto-acyl CoA reduction		
AKR1C1	10p15-10p14	3(20α)-hydroxysteroid dehydrogenase; DD1	progesterone ➔20α-hydroxprogesterone	preterm birth; endometriosis	sa
AKR1C2	10p15-10p14	type 3 3α-hydroxysteroid dehydrogenase; DD2	DHT➔ 3α-androstanediol;	androgen insufficiency	ur
			DHP➔allopregnanolone	prementsural syndrome	
AKR1C3	10p15-10p14	type 5 17β-hydroxysteroid dehydrogenase; prostaglandin F synthase; DDx	Δ^4-AD➔ Testosterone	prostate cancer; breast cancer	in
			5α-androstane-3,17-dione➔ DHT	prostate cancer	
			estrone➔17β-estradiol	breast cancer	
			PGH_2➔ $PGF_2\alpha$; PGD_2➔11β-$PGF_2\alpha$	acute myeloid leukemia	
AKR1C4	10p15-10p14	Type 1 3α-hydroxysteroid dehydrogenase; chlordecone reductase; DD4	5β-cholestan-7α-ol-3-one ➔ 5β-cholestane-3α,7α-diol; 5β-cholestane-7α, 12α-diol-3-one ➔ 5β-cholestane-3α,7α, 12α-triol;		ph
AKR1D1	7q32-7q33	Steroid 5β-reductase	Δ^4-cholesten-3-one-➔5β-cholestan-3-one	bile acid deficiency	
AKR1E1	10p15	1,5-anhydro-D-fructose reductase			
AKR6A3	3q26.1	K^+ voltage gated channel β-subunit 1	NADPH dependent channel opening	aberrant redox regulation of Kv channels and CV disease	
AKR6A5	1p36.3	K^+ voltage gated channel β-subunit 2			
AKR6A9	17p13.1	K^+ voltage gated channel β-subunit 3			
AKR7A2	1p35.1-p36.23	afllatoxin aldehyde reductase	succinic semialdehyde➔γ–hydroxybutyrate	neuromodulation and SSDH deficiency	
AKR7A3	1p35.1-p36.23	aflatoxin aldehyde reductase	aflatoxin dialdehyde➔aflatoxin bis-alcohol	hepatocarcinogenesis	

Table 2

Human AKR Genes Regulated by Nrf2

gene name	number of AREs based on Nrf2 consensus sequence	positional matrix human LD < 6	induced by Nrf2
AKR1A1	6	5	X[49]
AKR1B1	1	3	X[49]
AKR1B10	4	2	X[49,52,53]
AKR1B15	4	5	
AKR1C1	10	16	X[7,49,52–54]
AKR1C2	15	24	X[10,49,52,53]
AKR1C3	4	6	X[49,53]
AKR1C4	2	2	
AKR1D1	11	27	
AKR1E2	6	3	
AKR6A5	26	8	
AKR7A2	9	10	X[51]
AKR7A3	4	4	X[47]

Table 3

Human AKRs and Chemotherapeutic Drug Resistance

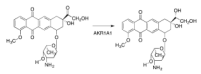
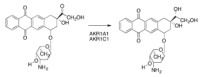
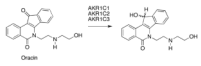
Drug	Reaction	AKR Implicated in Drug Resistance
Cyclophosphamide		AKR1B10
Daunorubicin		AKR1A1[84]
Doxorubicin		AKR1C2[85] AKR1C3[85]
Oracin		AKR1C1[86] AKR1C2[86] AKR1C3[86,87]

Table 4

Enzymatic PAH Catechol Conjugation Relative to PAH *o*-Quinone Redox Cycling

recombinant enzyme^{-1}	**specific activity (nmol min^{-1} mg^{-1}) 10 μM B[*a*]P-7.8-dione[a]**
NQO1 + NADPH	1070[b]
AKR7A2 + NADPH	1270[b]
AKR1C1 + NADPH	64[b]
COMT + S-adenosyl-*L*-methionine	55[c]
SULT1A1 + phosphoadenosine phosphosulfate (PAPS)	0.8[c]
UGT1A3 + UDP-glucuronic acid	>1.0[d]

[a] For catechol conjugation reactions catalyzed by COMT, SULT, or UGT, B[*a*]P-7,8-dione was reduced to the catechol with NADPH under anaerobic conditions.

[b] Mean of six replicates with SD < 10%.

[c] From Michaelis–Menten plots with homogeneous recombinant enzyme.

[d] From commercial microsomes expressing recombinant UGT.

Table 5

Nuclear Receptor Regulation by Human AKRs

human AKR	nuclear receptor ligand regulation	nuclear receptor target	change in gene expression
AKR1B10	all-*trans*-retinaldehyde → all-*trans*-retinol	RAR	decrease
AKR1B15	9-*cis*-retinaldhehyde → *cis*-retinol	RXR	decrease
AKR1C1	progesterone → 20α-hydroxyprogesterone	PR	decrease
AKR1C2	dihydrotestosterone → 3α-androstanediol	AR	decrease
AKR1C3	Δ^4-androstene-3,17-dione → testosterone; 5α-androstane-3,17-dione → dihydrotestosterone	AR	increase
AKR1C3	estrone → 17β-estradiol	ER	increase
AKR1C3	11β-PGD_2 → $PGF_2\alpha$	PPARγ	decrease
AKR1D1	bile-acid biosynthesis	FXR	increase