

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/264898544>

Mechanisms Underlying Chemopreventive Effects of Flavonoids via Multiple Signaling Nodes within Nrf2-ARE and AhR-XRE Gene Regulatory Network

Article *in* Current Chemical Biology · October 2013

DOI: 10.2174/2212796811307020008

CITATIONS

4

READS

144

4 authors, including:



[Derek Stewart](#)

James Hutton Institute

41 PUBLICATIONS 124 CITATIONS

[SEE PROFILE](#)



[Yiguo Zhang](#)

Chongqing University

75 PUBLICATIONS 1,387 CITATIONS

[SEE PROFILE](#)

Mechanisms underlying chemopreventive effects of flavonoids *via* multiple signaling nodes within Nrf2-ARE and AhR-XRE gene regulatory networks

Han Xiao^{2,3}, Fenglin Lü¹, Derek Stewart^{2,4} and Yiguo Zhang^{*,1,3}

¹The NSFC-funded Laboratory of Cell Biochemistry and Gene Regulation, College of Medical Bioengineering and Faculty of Life Sciences, University of Chongqing, No. 174 Shazheng Street, Shapingba District 400044, China; ²The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK; ³Jacqui Wood Cancer Centre, Medical Research Institute, Ninewells Hospital & Medical School, University of Dundee, James Arrott Drive, Dundee DD1 9SY, Scotland, UK; ⁴School of Life Science, Heriot Watt University, Edinburgh EH14 4AS, UK

Abstract: Flavonoids, a subclass of polyphenols, are abundant components of fruit and vegetables, prevalent in the diet and having an inverse association with the incidence of various degenerative diseases and cancer. Mechanisms underlying the beneficial effects of flavonoids on the human health are being investigated worldwide. Flavonoids have been found to reduce the risk of carcinogenesis by blocking the initiation and suppressing the promotion and progression of certain cancer cells. The accrued data suggests that the chemopreventive effects of flavonoids are exerted through induction of cytoprotective mechanisms to prevent the activation of pro-carcinogens and oxidants from damaging DNA (genome), and detoxify activated carcinogens by enhancing their conjugation and excretion. The balance of metabolic activation and detoxification of carcinogens is controlled through expression of drug-metabolizing Phase I and Phase II enzymes. If the detoxification pathway is saturated, the AhR-XRE-cytochrome P450s activation pathway produces arene oxides and the consequential additional damage promotes tumorigenesis. Fortunately, such oxidative damages can be prevented by CNC-bZIP transcription factors through differentially regulating antioxidant and detoxification genes, which contain ARE and its homologues in their promoters. Amongst the CNC-bZIP family, Nrf2 is a master regulator of expression of drug-metabolizing enzymes, and its activity is negatively regulated by Keap1 and β -TrCP. Expression of *Nrf2* and downstream genes is tightly controlled by AhR and CNC-bZIP (e.g. Nrf1) family factors, whilst its negator *Keap1* is also regulated by Nrf1 and Nrf2. Such crosstalks between AhR-XRE and Nrf2-ARE regulatory networks indicate that flavonoids trigger multiple signaling pathways to integrally activate cytoprotective genes against cytotoxic insults and oxidative stress. However, the unique chemopreventive role of Nrf1 in regulating antioxidant, detoxification and cytoprotective genes has yet to be fully elucidated and characterised.

Key words: Flavonoids, nuclear factor-erythroid 2 p45 subunit-related factor 2 (Nrf2), Nrf1, aryl hydrocarbon receptor (AhR), antioxidant response element (ARE), xenobiotic response element (XRE), gene regulatory network, chemical biology, cancer chemoprevention, and oxidative stress,

1. INTRODUCTION

It is known that flavonoids belong to a subclass of polyphenols, which are abundant in our diet, and evidence for their roles in the preventive medicine is emerging from research in cancer and other degenerative diseases, such as cardiovascular, Parkinson's and Alzheimer's diseases [1]. There are more than 4000 compounds that have been identified as distinct flavonoids, approximately 900 of which are consumed in the human diet. All flavonoids share a generic structure, consisting of two aromatic rings (A and B rings)

that are linked by 3 carbons' atoms that are usually contained in an oxygenated heterocycle ring (C ring) (Fig. 1). Based on their differences in the C ring, flavonoids are further classified as flavonols, flavones, catechin-tannins, anthocyanidins, and isoflavones (Table 1). An additional number of different sugar combinations, of which more than 80 kinds have been reported, also contribute to the chemical variety of flavonoids. Flavonoids are ubiquitous in fruit and vegetables, existing in nature as conjugates, in either glycosylated or esterified forms, or to a lesser extent, the aglycone form. The conjugates can be converted into aglycones by food-processing [2].

1.1. Bioavailability of Flavonoids and their Metabolisms

Amongst different flavonoids, the bioavailability varies depending on their chemical structures, sugar groups attached and their molecular weights. For instance, direct evidence has been obtained by measuring their concentrations in both the blood plasma and the urine [3, 4], after ingestion

*Address correspondence to this author at the Laboratory of Cell Biochemistry and Gene Regulation, Faculty of Life Sciences and College of Medical Bioengineering, University of Chongqing, Shapingba District 400044, China; Tel: 0086-023-65111632; Fax: 0086-023-65111802; E-mail: yiguozhang@cqu.edu.cn or y.z.zhang@dundee.ac.uk
Division of Cancer Research, Medical Research Institute, Ninewells Hospital & Medical School, University of Dundee, Dundee DD1 9SY, Scotland, UK; Tel: 0044-01382-425617; Fax: 0044-01382-669993; Email: y.z.zhang@dundee.ac.uk

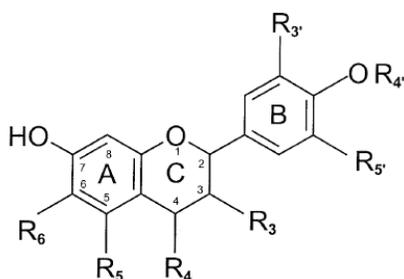


Fig. (1). General structures of common food flavanoids.

of either pure compounds or food stuffs with known contents of the compounds of interest [5]. It is reported that the plasma concentrations of flavonoids are low, usually less than 1 μM and normally in the nM region, and that they reach a maximum level 1-2 h after ingestion. Therefore, the maintenance of a high concentration in plasma requires repeated ingestion of the polyphenols over time [6]. Studies for investigating the extent of polyphenol absorption in humans, after the ingestion of a single dose of polyphenols that are provided as a pure compound, plant extract, whole food or beverage, have shown that the quantities of intact polyphenols in urine vary from one flavonoid to another. Amongst them, inter-individual variations have also been observed, probably due to differences in compositions of the colonic microflora that can affect their metabolisms differently [7].

The absorption and metabolism of polyphenols is routed from the stomach, passed through the gastrointestinal tract into the liver. After crossing those physiological barriers, polyphenols will be circulated in the blood plasma and then transported to various target tissues or excreted in the urine and/or the bile. Flavonoids in the aglycone form can be absorbed by the small intestine, but their most abundant dietary forms as glycosides, esters or polymers in foods are hardly absorbed [8]. However, these conjugates of aglycones can be hydrolyzed by acids in the stomach and by microflora in the intestines, in order to convert to the forms that are readily bioavailable to the body. Only after being hydrolyzed in the gastrointestinal tract, the aglycones are absorbed by the intestinal enterocytes, where they undergo different conjugation reactions, including glucuronidation by UDP-glucuronyl transferase (UGT) and methylation by catechol-*O*-methyl transferase. Once flavonoids reach the liver, the remaining aglycone will further be glucuronidated or sulfated, whilst those methylated polyphenolics may be demethylated [9]. Intriguingly, flavonoids may undergo oxidation of their planar aromatic structures, as part of the role as antioxidants, to

form quinine-like structures that are either detoxified by conjugation with reduced glutathiones or broken down to smaller phenolic compounds [10]. Finally, some of polyphenol metabolites enter the circulation in the blood, where the plasma albumin represents the primary protein responsible for binding and transporting polyphenols [6]. The affinity of polyphenols with the albumin varies according to their chemical structures, but it is not clear whether the binding to albumin affects their biological activities.

1.2. Beneficial Properties of Flavonoids

Collectively, dietary flavonoids have various reported beneficial properties, including antioxidant properties, chelation of metals, and oestrogenic, anti-viral, anti-bacterial, anti-inflammatory and anti-mutagenic activities, along with a dual opposing role in either activation or inhibition of various enzymes. For the antioxidant activity possessed by flavonoids, there are three basic requirements [11, 12]: i) free hydroxyl groups on the 5 and 7 positions of the A ring; ii) the presence of orthodihydroxyl (catechol) groups on the B ring; iii) the presence of a 2,3-double bond in the C ring (Table 1). It has been reported that quercetin, the anthocyanin aglycone and cyanidin have antioxidant potentials 4-fold higher than that of Trolox™, an analogue of vitamin E [13], and that these antioxidant activities are suggested to be the derivation of their roles in cancer prevention [14, 15]. Quercetin, cyanidin and procyanidin are identified as good chelators of metals, such as iron, zinc and copper [16, 17]. As these flavonoids could inhibit platelet aggregation and leukocyte adhesion by chelating iron and scavenging of the relevant radicals, they can thus potentially contribute to the prevention of cardiovascular disease [18]. Furthermore, quercetin and kaempferol have been reported to increase the activity of thioredoxin reductase in the normal human keratinocytes [19].

Epidemiologically, the increase in the intake of flavonoids helps decreasing the risk of developing cardiovascular disease, age-related disease such as Alzheimer's disease, and various types of cancer [9]. However, the mechanisms responsible for their beneficial effect are still under intensive investigation. One of the mechanisms that has been proposed is that flavonoids are protective through their antioxidant properties. Since elevated levels of reactive oxygen species are clearly associated with various neoplastic diseases, the antioxidant property, together with the ability of flavonoids to induce cytoprotective enzymes and regulatory proteins, can hence contribute to their chemopreventive effects. Another possible mechanism is anti-inflammation, since dietary flavonoids can inhibit the NF- κ B signaling pathway that is

Table 1. Chemical characteristics of each subclass of flavonoids.

Class	Flavonols	Flavones	Isoflavones	Catechins	Anthocyanins
Carbon atom in ring C attached to B	2	2	3	2	2
C-ring unsaturation	2-3 double bond	2-3 double bond	2-3 double bond	None	1-2, 3-4 double bond
C-ring functional groups	3-hydroxy, 4-Oxo	4-Oxo	4-Oxo	3-hydroxy; 4-gallate	3-hydroxy

involved in the induction of inflammation [20]; this process contributes to the initiation and progression of neoplastic tumours [21]. Moreover, an additional number of cellular response signaling pathways towards the regulation of cell cycle, proliferation and apoptosis [22, 23], are reportedly induced by flavonoids, suggesting, at least in part, a basis for their chemopreventive effects.

2. ROLES OF FLAVONOIDS IN CANCER CHEMOPREVENTION

Collectively, numerous mechanisms have been implicated in the development of cancer [24]. Carcinogenesis is a polygenic-evolved pathological process that is complex with components that are multifactorial, multievent, and multistep, and hierarchical from initiation, promotion, progression and angiogenesis to invasion and metastasis. By blocking the initiation of carcinogenesis and/or suppressing the later stages, flavonoids can potentially reduce the risk of carcinogenesis and thus serve as chemopreventive agents. Regarding the initiation of carcinogenesis, it always starts with DNA adducts, gene mutation and other genetic alterations. In order to avoid these initiation and deteriorative consequences, a number of direct and indirect intrinsic strategies have evolved for the host to prevent DNA attack from electrophiles, free radicals, reactive oxygen/nitrogen/sulphur species, to enhance the repair of damaged DNA, to inhibit the uptake of pro-carcinogens into cells, and to reduce the toxicity of activated carcinogens in cells by enhancing their biotransformation, conjugation and excretion [25]. For instance, quercetin has been reported to protect the cell and DNA from being damaged by hydrogen peroxide and benzo[a]pyrene (BaP) [26, 27].

The progression of cancer could also be halted by activation of cell cycle arrest or apoptosis. A number of flavonoids either as individuals or in combination, which have been found to suppress cell proliferation or induce apoptosis of carcinoma cells, include quercetin [28], epigallocatechin gallate (EGCG), resveratrol [29, 30], kaempferol [31], procyanidin and pomegranate extracted ellagitannins [32]. Also, cell growth has been inhibited by EGCG through induced cell cycle arrest in the G_0/G_1 phase [33]. Furthermore, another flavonol found in rice bran, tricetin, an *O*-methylated flavanone, was shown to inhibit the growth of breast tumour cells through the G_2/M arrest [34]. Therefore, distinct flavonoids have various potentials to exert chemopreventive effects through different mechanisms. In addition, the isoflavone genistein has also an inhibitory effect on the growth of human ovarian cancer cells (OvC1 and SKOV3) and prostate cancer cells (LNCaP) through up-regulating antioxidant and detoxification genes [35, 36].

3. INDUCIBLE EXPRESSION OF DRUG-METABOLIZING ENZYMES BY FLAVONOIDS

Drug-metabolizing enzymes have been designated, amongst other things, as xenobiotic transformation enzymes. Xenobiotics include a broad spectrum of chemicals: manufactured or natural drugs (e.g. flavonoids and isoflavone genistein), pollutants, alkaloids and pyrolysis products found in food or environments. Most of such xenobiotics are toxic and, if accumulated in the body, may cause cell damage and

eventually kill an organism. To defend against those xenobiotics to which the body is constantly exposed, as well as endobiotics in the body and even toxic products from the cell metabolisms, the human body system has evolved a large number of drug-metabolizing enzymes with various functional specificities, which can biotransform, detoxify and eliminate potential exogenous and endogenous toxicants. The following examples of reactions and relevant enzymes involved in the detoxification include: i) oxidation reaction catalyzed by cytochrome P450 (CYP) enzymes, alcohol dehydrogenase, aldehyde dehydrogenase and glutathione peroxidase; ii) reduction reaction catalyzed by aldo-keto reductases (AKR), short chain dehydrogenase and/or reductase, and NAD(P)H:quinone oxidoreductase 1 (NQO1); iii) hydrolysis catalyzed by epoxide hydrolase; iv) conjugation reactions catalyzed by glutathione transferase (GST), sulfotransferase (SULT), UGT, methyl transferase and *N*-acetyl transferase (NAT) [37].

Generally, the above first three reactions (i.e. oxidation, reduction and hydrolysis) can introduce a functional group to the substrate, such as -OH, -NH₂, -SH or -COOH, leading to a modest increase in the hydrophilicity of the end products. By contrast, glutathionylation, glucuronidation, sulfonation, acetylation, methylation, and other conjugations require cofactors, such as glutathione, other amino acids or sugars, in the reaction with cognate functional groups in the substrates originally, or introduced through the other types of detoxification reactions. As compared with other reactions, such conjugation reactions can result in a significant increase in the hydrophilicity of the substrates, therefore promoting the excretion of foreign chemicals and metabolites from the host cells and the organism [38]. Based on these classic biochemical reactions, the concept of Phase I and Phase II drug metabolism was proposed early in the 1970's [39]. The Phase I enzymes include those responsible for hydrolysis, oxidation, and reduction of xenobiotics, whilst the Phase II enzymes catalyze the conjugation of xenobiotics with sugars, glutathione and other amino acids. Since Phase I and II enzymes are up-regulated by pretreatment with flavonoids they can therefore be preventive or therapeutic beneficial in the case of drugs. Conversely, modification of xenobiotics by drug-metabolizing enzymes can also change their biological effects thereby either attenuating or antagonising cytotoxicity. Overall, drug-metabolizing enzymes play a vital role in determining the intensity and duration of action of drugs (or here dietary Flavonoids), their chemical toxicity and chemical tumourigenesis [40].

Taking the drug-metabolizing gene *Nqo1* as an example, its chemical inducers are collectively classified into nine diverse classes (Fig. 2) [41, 42]: i) diphenols, phenylenediamines and quinones; ii) michael reaction acceptors; iii) isothiocyanates, dithiocarbamates and related sulfur compounds; iv) 1,2-dithiole-3-thiones, oxathiole oxides, and other organosulfur compounds; v) hydroperoxide; vi) trivalent arsenicals; vii) heavy metals; viii) vicinal dimercaptans, and ix) carotenoids and related polyenes. Although these chemicals are structurally distinct, they share common properties of electrophilicity and the capacity to modify sulfhydryl groups. Notably, it has been shown that certain of these inducers are administered to up-regulate *NQO1* responsible

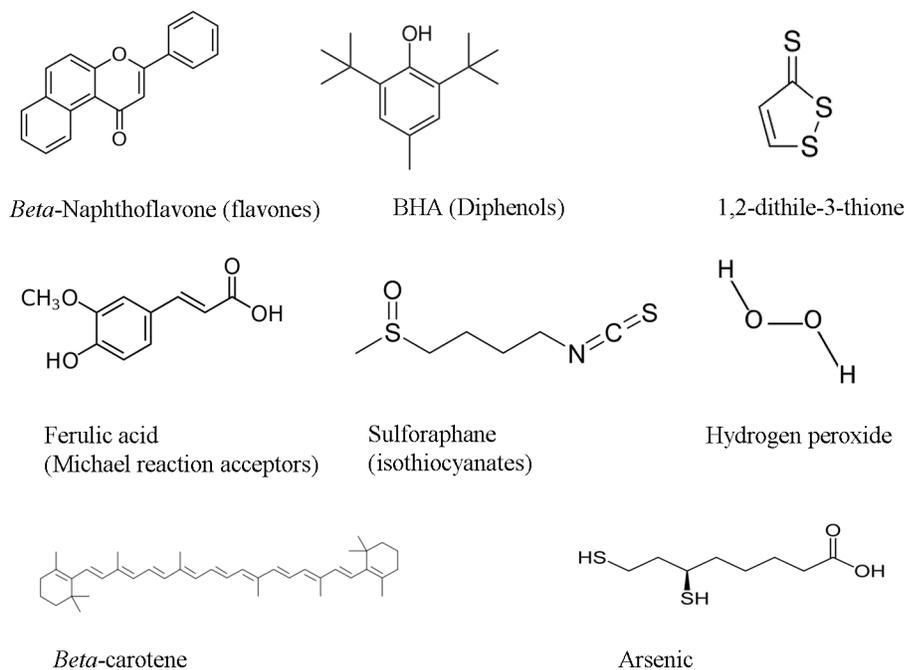


Fig. (2). Structures of typical *Nqo1* inducers selected from each class.

for the detoxification of electrophilic toxicants, in order to block the initiation of tumours in various tissues, such as liver, colon, mammary gland and pancreas [43].

4. MECHANISM FOR INDUCTION OF DRUG-METABOLIZING ENZYMES BY NRF2 BINDING TO THE ARE

The accruing substantial evidence has revealed that drug-metabolizing enzymes (e.g. NAT, GST, SULT, UGT and NQO1) play important roles in the detoxification of electrophilic toxicants, and induction of these genes can protect the cells against carcinogenesis and mutagenesis. Such genes encoding these drug-metabolizing enzymes are regulated by the family of cap'n'collar (CNC) basic-region leucine zipper (bZIP) transcription factors (Fig. 3) through differentially binding to antioxidant response elements (AREs) and its homologous consensus sequences in their promoter regions (Fig. 4) [44]. The first isolated CNC-bZIP protein in mammals was designated the nuclear factor-erythroid 2 (NF-E2) p45-subunit [45]. Subsequently, three closely related transcriptional activators Nrf1 (including a long form TCF11 and a short LCR-F1 isoform) [46-48], Nrf2 [49] and Nrf3 [50] were cloned in succession, along with two distantly related repressors Bach1 and Bach2 [51]. The vertebrate members of this family share two highly conserved structural domains, i.e. the 'CNC' domain and bZIP domain (Fig. 3), with the *Drosophila* Cnc proteins [44, 46, 49, 52]. The *Caenorhabditis elegans* protein skinhead-1 (Skn-1) also belongs to this superfamily due to its CNC domain situated just N-terminal to the basic region [53-55], but it lacks the leucine zipper subdomain. Beyond Skn-1, all other CNC-bZIP proteins form a functional heterodimer with one of small Maf proteins (e.g. MafK, MafF and MafG) or another bZIP protein (e.g. c-Jun), and thus can differentially bind to various ARE/AP-1-like DNA consensus sequences (Fig. 4),

which are contained in distinct subsets of target genes [56]. Therefore, both CNC and bZIP domains determine the property of the family proteins to differentially bind ARE-driven genes with specificity.

4.1. The ARE and its Homologues Confer Differential Gene Regulation by Nrf2 and Other CNC-bZIP Transcription Factors

The ARE was also designated as an electrophile response element, which thus represents a *cis*-acting enhancer sequence that mediates transcriptional activation of those genes in the intracellular responses to electrophiles and oxidative stress. Such proteins that are members of the ARE-gene battery include those associated with glutathione biosynthesis, redox proteins with active sulfhydryl moieties and drug-metabolizing enzymes [57, 58] (Tables 2 and 3). This regulatory element was first identified within the 5'-flanking region of the rat *GSTA2* containing a 41-bp DNA motif, and later was designated as the ARE based on its responsiveness to phenolic antioxidants [59]. Deletion and mutational analysis defined that the core nucleotide sequence, 5'-TGACnnnGC-3' (Fig. 4), is essential for the response to these chemicals [59]. Furthermore, the nucleotides situated at 5'-end immediately to the core ARE are also required for both the basal and inducible expression of the gene regulated, but are not sufficient for induction when its upstream TCA sequence or downstream A/T-rich region was mutated [60]. Consistent with this finding, 5'-TMAAnRTGAY-nnnnGCR www-3' (M = A/C, R = A/G, Y = C/T, W = A/T) is the extended ARE core sequence, demonstrating the importance of the flanking sequences for the context-specific regulation of gene transcription [60]. However, the 3'-flanking 'www' tetra-nucleotide is not required for basal and inducible gene expression; this was found in experiments of a series of point mutations across the whole ARE in

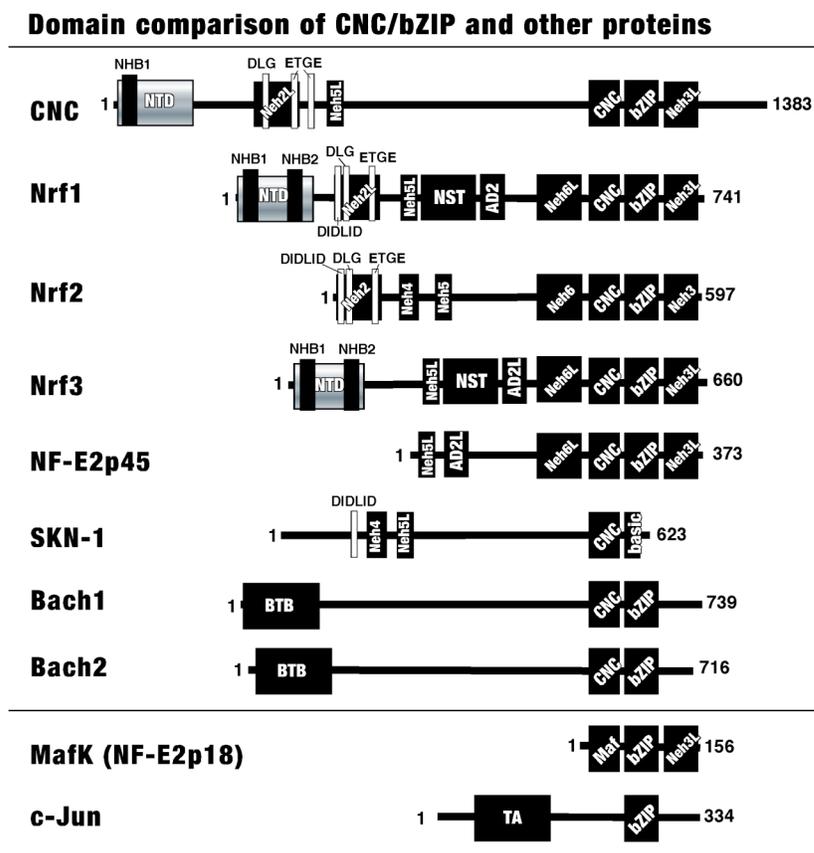


Fig. (3). Schematic diagrams illustrating structural domains of Nrf2 and other CNC-bZIP proteins. Amongst the family members, the CNC and bZIP domains are highly conserved, and thus integrally called the Neh1 domain in Nrf2. The term Neh indicates Nrf2-ECH homology. The Neh2 domain of Nrf2 is represented by the Neh2L in Nrf1 and CNC proteins. Keap1 negatively regulates Nrf2, and possible CNC proteins, through binding to DLG and ETGE motifs. Although these two Keap1-binding motifs are contained in Nrf1, it is not regulated by Keap1. The Neh3 domain shares sequence similarity between the Nrf, Cnc and small Maf proteins, but it is absent in Bach1, Bach2 and Skn-1. The Neh4 and Neh5 domains, along with the DIDLID element, are responsible for transactivation (TA) of target genes. The Neh6 domain is homologous in all four Nrf proteins and is responsible for the β -TrCP-mediated degradation. The N-terminal domain (NTD) of Nrf1 contains N-terminal homolog box 1 (NHB1, which serves as an ER targeting signal) and NHB2, and shares conservation with Nrf3 and Cnc proteins. The BTB domain is responsible for dimerization in Bach1 and Bach2. Each member of the CNC-bZIP family can form a functional dimer with a small Maf or another bZIP protein (e.g. c-Jun).

the mouse *Nqo1* promoter [61]. In addition, this study also revealed that the nucleotides that had previously been suggested to be redundant (which was shown as 'n' in the sequence mentioned above) are considered as a requirement for the gene induction; by contrast, the core sequence that previously had been shown to be essential before was found to be dispensable in the case of mouse *Nqo1* [61]. Taken together, these studies indicate that there are distinct ARE sequences in the promoter regions of different genes. In addition to genes that encode the rat *GSTA2* and mouse *GstA1*, genes encoding the rat and human *NQO1* [62, 63], γ -glutamyl cysteine ligase catalytic (*GCLC*) and modifier (*GCLM*) subunits [64-66], and haeme oxygenase 1 (*HO-1*) [67] were transcriptionally regulated *via* the ARE sites (Table 2). By contrast, an ARE-like sequence was found in some antioxidant and detoxifying gene promoters (e.g., 5'-TGCCattGC-3' in rat *GstA2*, Fig. 4) [68], but its function has not clearly been characterized.

The core ARE shares a striking sequence similarity with the recognition sites for either NF-E2 or small Maf family

factors [69, 70]. The antisense sequence of the NF-E2 binding site is likely to be considered as a type of ARE. Both include either the TPA-response element (TRE, 5'-TGAC/GTC/AA-3', also called AP-1 binding site), or its 5'-TGAC-3' tetranucleotide motif. In addition, the ARE requires a 5'-GC(A/G)-3' trinucleotide at its 3'-end. Both of these motifs also exist in the MARE (Maf recognition element) [71, 72]. Within the 'core' ARE sequences the 5'-TGAC-3' motif is represented in other *cis*-regulatory DNA consensus sequences, that are recognized by members of the AP-1, the ATF, and the cAMP-response element binding protein (CREB) families (Fig. 4). The motif is also present in the unfolded protein response element (UPRE) [73] and the recognition site of the Skn-1, a *Caenorhabditis elegans* transcription factor with a C-terminal CNC-basic region [54, 74, 75]. The 5'-GC-3' motif has been shown to be critical for the ARE-mediated inducibility [59-61], but it is also embedded in other consensus sequences, such as the amino acid response element and the p53 binding site [76]. In addition, the 5'-GAC-3' portion is present in the consensus binding sites of transcription factors p53 and NF- κ B [77, 78]. Transcrip-

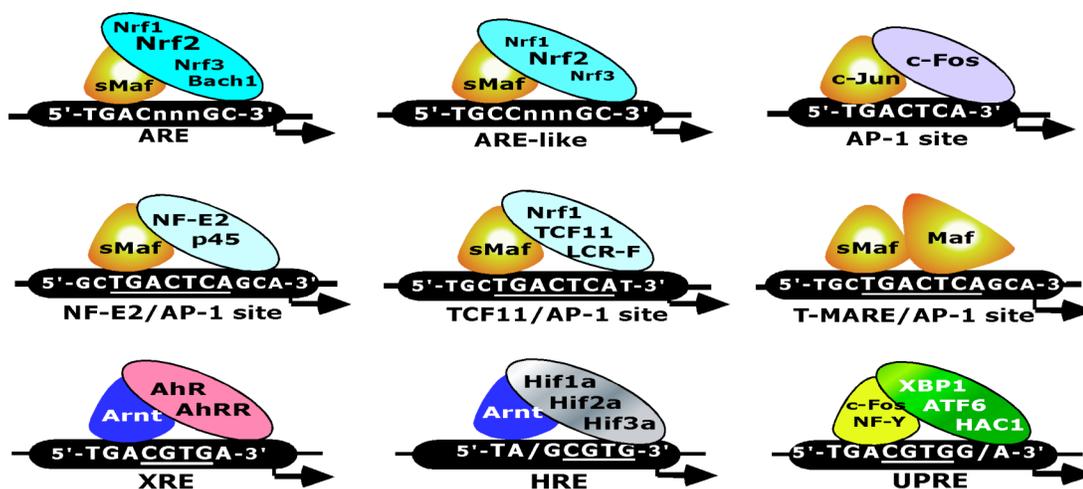


Fig. (4). Consensus sequences of ARE, XRE and other homologues found in the promoter regions of different stress responsive genes. The ARE and ARE-like elements share sequence conservation with the NF-E2, TCF11, AP-1 binding sites and T-MARE (TRE-type Maf recognition element). Amongst these sequences, these three motifs TGAC, GC and TCA are highly conserved. The functional dimers of CNC-bZIP proteins with small Maf (sMaf) or other bZIP proteins can differentially bind to the ARE, ARE-like, NF-E2, TCF11, MARE and AP-1 consensus sites, but it is not know whether they can bind to UPRE competitively with its canonical bZIP factors (XBP-1, ATF6 and HAC1). Expression of XRE-driven genes is regulated positively by AhR and negatively by its repressor AhRR. The CGTG motif essential for functioning of XRE is embodies in the HRE and UPRE response to hypoxia and ER stress, and both the XRE and UPRE also contain the TGAC motif that shares identity with the equivalent of ARE/AP-1 site. These homologous *cis*-elements and their cognate canonical and non-canonical transcription factors can form an all-potent gene regulatory network, which can integrate from multiple signaling towards distinct response gene expression.

tion of *GCLC* can occur indirectly through an NF- κ B-recognized site [79, 80], as well as through the ARE. Collectively, the evolutionary conservation of these consensus sequences suggests that the ARE and the *cis*-elements with homologous sequences, together with their cognate DNA-binding transcription factors, comprise the infrastructure of a large gene regulatory network. A possibility cannot therefore be ruled out that, under certain pathophysiological conditions, there may be some promiscuity between *trans*-acting factors and *cis*-elements or dual regulation of certain genes. This suggests that exposure of cells to a variety of severe distinct stresses could activate an overlapping spectrum of genes controlling redox homeostasis and relevant physiologies. Conversely, if this gene regulatory network is out of control, it would turn on to enter a pathological response process.

The observation that the ARE sequence resembles that of the TRE has raised the possibility that members of the AP-1 family regulate certain ARE-driven genes. Despite their similarities, the ARE has unique features that sets it apart from AP-1 binding sites. For example, there exists a GC dinucleotide motif at the 3' end of the ARE core sequence. This difference suggests that activation of gene expression via the ARE and the TRE are mediated through different signaling pathways. It appears that AP-1 makes little contribution to ARE-driven gene expression as a reporter construct based on the mouse *gsta1-ARE* was active in mouse F9 embryonal carcinoma cells which lack significant TRE-binding activity [81]. However, it should be noted that the ARE found in some genes contains an embedded TRE sequence [68, 82-84], suggesting that such genes may be controlled through both the ARE and AP-1 binding sites. In fact, super-

shift assays have shown that a number of transcription factors can bind to the ARE, notably the CNC/bZIP family including Nrf1, Nrf2, Nrf3, NF-E2 p45, Bach1 and Bach 2 (Fig. 3), the AP-1 family such as c-Jun, c-Fos and ATF4, and the small Maf proteins [85-90]. This is explained by the fact that many of these bZIP proteins can potentially dimerize with each other to generate a diverse array of functional protein complexes that bind to DNA with unique and/or overlapping specificity [91, 92].

Collectively, CNC-bZIP family transcription factors play important roles in development and the regulation of expression of cytoprotective genes involved in various biological processes, including proliferation, apoptosis, differentiation, and stress responses. Amongst this family, Nrf2 is thought as a master regulator of the basal and inducible expression of ARE-driven genes through its functional heterodimer with small Maf proteins. The most compelling evidence that Nrf2 makes a major contribution to the regulation of ARE-driven genes has been obtained from the study of *Nrf2* knockout mice. In particular, the basal and inducible expression of *Gst* and *Nqo1* is substantially reduced in *Nrf2*^{-/-} mice, when compared with their wild-type counterparts [71, 93]. Besides Nrf2 and small Maf proteins, other CNC-bZIP transcription factors may influence ARE-driven gene expression [44, 76].

In vitro DNA-binding studies using antibody supershift assays have shown that Nrf1 and the AP-1 family members can bind the ARE [68]. In fact, Nrf2 is a dispensable factor and cannot compensate the loss of Nrf1 function, because the *Nrf2* knockout mice exhibited normal growth and development, without the spontaneous development of cancer [94]. By contrast, global disruption of *Nrf1* leads to mouse embryonic lethality [52, 95], and conditional knockout of *Nrf1* spe-

Table 2. A list of the ARE sequences in different gene promoters from different species. The sequences shown are from the genes for antioxidant, metal-binding, and detoxification proteins from human, mouse and rat. As AP-1 binding site share some similarities in the sequence of ARE, their sequences in the genes were also shown in the table. The nucleotides in bold capital letters are those that share identity with the extended 16-bp ARE consensus sequences (5'-TMAnnRTGAYnnnGCR-3', M = A/C; R = A/G; Y = C/G/T). The TGAC motif is identical with an equivalent portion of AP1-binding consensus site, whilst the GC motif is essential for the functioning of ARE. Both TGAC and GC motifs are placed in white bold letters on the black backgrounds. Some data are adapted from Hayes *et al.* [57].

Function	Species	Gene	Element	Sequence
Antioxidant enzymes	Human	<i>GCLC</i>	ARE-4/AP1	TCCCCG TGAC TCAG GC G
		<i>GCLM</i>	EpRE ARE(var)	AGACAA TGAC TAA GCA TAACGG T T AC GAA GCA
		<i>GPX2</i>	ARE-1 ARE-2	CCAGGA TGAC TTA GCA GTACAG TGA GAGG GCA
		<i>PRDX1</i>	EpRE-1 EpRE-2	TGTAAC TGA ATCA GC C TTCTCC TGC CTCA GC C
		<i>PRDX6</i>	ARE	GCAACG TGAC CGA GC C
		<i>TRX</i>	ARE/AP1	TCACCG T T AC TCAG GCA
		<i>TXNRD1</i>	ARE	TCAGAA TGAC AAA GCA
	Mouse	<i>Gsr1</i>	ARE-1 ARE-2	TCGCCG TGAC TAA GCA TCACAG TGAC CAA GCG
		<i>Slc7a11</i>	EpRE-2	CCAGCT TGA GAAA GCG
	Rat	<i>SRXN1</i>	ARE-1/AP1	TCACCC TGA GTCA GC G
Metal-binding proteins	Human	<i>FTL</i>	MARE/ARE	TCAGCA TGAC TCAG GCA
		<i>MT1B</i>	ARE	GAGCAG TGAC CTG GCG
	Mouse	<i>Fth1</i>	FER1 API/NF-E2	CCTCCA TGAC AAA GCA CCACCG TGAC TCAG GCA
		<i>Ftl1</i>	EPRE	TCAGCG TGAC TCAG GCA
		<i>Mt1</i>	ARE	GCGCG TGAC CTG GCG
		<i>Mt2</i>	ARE/AP1	GGGGTG TGAC TCAG GCG
Detoxification proteins	Mouse	<i>AKR1C2</i>	ARE	TCAGGG TGAC TCAG GCA
		<i>MGST1</i>	EPRE	ACATCG TGAC AAA GCA
		<i>NQO1</i>	ARE/AP1	TCACAG TGAC TCAG GCG
		<i>UGT1A1</i>	ARE	AAACCCG GACT TTG GCG
	Mouse	<i>Akr1b3</i>	ARE-1	GGAGCA TGAC CCA GCA
		<i>Gsta1</i>	EpRE	TAATGG TGAC TCAG GCA
		<i>Gsta3</i>	EpRE	CAGGCA TGAC ATT GCA
		<i>Mrp2</i>	ARE	CTGGGA TGAC CTC GCA
		<i>Nqo1</i>	ARE	TCACAG TGA GTCC GCA
		Rat	<i>Gsta2</i>	ARE
	<i>GStp1</i>		GPE1/AP1	TCACTA TGAT TCAG GCA
<i>Nqo1</i>	ARE		TCACAG TGAC TTG GCA	
			ARE 'core'	TGAC NNN GC
			ARE consensus	TMANNR TGA YNNN GCR
			AP1-binding site	TGA STCA

cifically in the liver, bone and brain of neonatal mice results in non-alcoholic steatohepatitis and hepatic neoplasia [96, 97], reduced bone size [98] and neurodegenerative disease [99, 100], respectively. This obvious discrepancy between the phenotype of *Nrf1*^{-/-} and *Nrf2*^{-/-} mice clearly indicates that the two CNC/bZIP proteins are functionally distinct: Nrf1 fulfils a unique and indispensable function, that cannot be substituted compensatively by Nrf2 and other CNC-bZIP factors, in regulating a subset of ARE-battery genes respon-

sible for cellular homeostasis and organ integrity during normal development and healthy growth. In addition, induction of those Phase II enzymes by flavonoids occurs upstream through several intracellular signal transduction pathways, involving mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), or phosphatidylinositol-3 kinase [101-103]. These signaling pathways are integrated to activate antioxidant, detoxification and cytoprotective genes regulated by Nrf2 and other transcription factors.

Table 3. A list of selected inducers to activate drug-metabolizing genes regulated by Nrf2.

Species	Gene	Selected inducer
Human	<i>NQO1</i>	B-NF, tBHQ
	<i>GCL</i>	BHA
	<i>UGT1A6</i>	tBHQ
	<i>UGT1A8</i>	EGCG
	<i>UGT1A10</i>	EGCG
Mouse	<i>GSTA1</i>	t-BHQ, SFN, 3-MC, catechol
	<i>GSTP1</i>	
	<i>GCL</i>	
	<i>NQO1</i>	BHA, SFN, I3C
	<i>UGT1A6</i>	EQ, OTZ
	<i>UGT2B5</i>	Curcumin
Rat	<i>Nqo1</i>	B-NF, t-BHQ
	<i>GSTA</i>	
	<i>GSTP1</i>	
	<i>UGT1A6</i>	EQ, OTZ
	<i>UGT1A7</i>	EQ, OTZ
	<i>UGT2B1</i>	EQ, OTZ
	<i>UGT2B3</i>	EQ, OTZ
	<i>UGT2B12</i>	

4.2. The Structure of Nrf2 with its Biological and Physiological Functions

Among the CNC/bZIP family members, Nrf2 acts as a central transcription factor in the ARE-driven gene response [68, 104], and regulates the expression of those genes encoding antioxidant enzymes, metal-binding and detoxification proteins (Table 2), as well as drug-metabolizing enzymes (Table 3). The function of Nrf2 is determined by its six structural domains, namely Neh1 to Neh6, which are conserved amongst species [105]. The Neh1 domain comprises the CNC region fused to bZIP region and confers its ability to dimerize with small Maf proteins and its ability to bind DNA as an obligate heterodimer. The N-terminal Neh2 domain is required for redox-sensitive negative control of the CNC-bZIP factor [105], whilst the C-terminal Neh3 domain interacts with chromodomain helicase DNA-binding protein 6 and therefore might associate with the transcriptional apparatus [106]. Both the central Neh4 and Neh5 are two transactivation domains that interact with CREB-binding protein [107]. The following Neh6 domain contributes to redox-independent negative control of Nrf2 [108]. Some of these domains are also homologous with the equivalents of other CNC-bZIP factors (Fig. 3).

Nrf2 enables the cellular adaptation to oxidants and electrophiles by stimulating the transcriptional activation of around 100 cytoprotective genes [82, 109-114], each containing at least one ARE in their promoters [59, 61]. Such genes whose expression is regulated by Nrf2 include those encoding: i) antioxidant and redox buffer proteins, and other oxidoreductases; ii) enzymes involved in regeneration of NADPH, synthesis of glutathione and other cofactors and their modulation; iii) enzymes for DNA repair to remove oxidative damage; iv) drug-metabolizing enzymes responsible for the Phases I and II detoxification; v) drug-efflux pumps (e.g. multidrug resistance associated proteins) required for the Phase III drug metabolism; vi) heat shock proteins and other molecular chaperones; vii) the 26S proteasomal α - and β -subunits for proteolytic degradation; viii) some growth factors, growth factor receptors, and various transcription factors involved in cell survival, anti-inflammatory and other protective responses. It has been shown that the up-regulated expression of these ARE-battery genes can increase the capacity of cells to scavenge electrophiles, free radicals, and reactive oxygen, nitrogen and sulphur species, and thus this protective effect enables the cells to defend against oxidative damage to lipids, DNAs and RNAs so as to prevent the initiation of tumorigenesis. The increased levels of drug-metabolizing enzymes and drug-efflux pumps allows the detoxification of a wide variety of toxic compounds, including those containing α , β -unsaturated carbonyl, epoxide, halide, hydroperoxide and quinone moieties, and further removal of their inactive conjugated metabolites from cells [115]. Overall, up-regulation of ARE-driven genes by activated Nrf2 enables the cells to adapt to the increased concentration of electrophiles, free radicals, and reactive oxygen, nitrogen and sulphur species and to cope with or address the effects of oxidative stress. Conversely, knock-out of *Nrf2* in the mouse markedly increased the hypersensitivity to hyperoxia [116], and the susceptibility to various forms of chronic lung diseases produced by exposure to cigarette smoke [117, 118]. Importantly, it has been shown that Nrf2 can protect the cells against the formation of DNA adducts and/or gene mutations resulted from aflatoxin B₁, BaP and diesel exhaust fumes [119-121]. Thereby, Nrf2 has been considered as a target of chemopreventive agents against carcinogenesis.

Notably, studies have also revealed that activation of Nrf2 and its downstream ARE-driven genes potentiates the prevention of neurodegenerative, neovascular, cardiovascular diseases and diabetes [122-125]. In the pathogenesis of all these diseases, oxidative stress is a common etiological factor, as implicated in the development of cancer. These, and other studies, have shown that the cytoprotection exerted by up-regulation of Nrf2 is ultimately due to an increase in the expression of ARE-driven genes transactivated by Nrf2 in the antioxidant responses to combat oxidative insults.

4.3. Negative Regulation of Nrf2 by Keap1

The activity of Nrf2 is negatively regulated by kelch-like ECH-associated protein 1 (Keap1) through binding to its Neh2 domain [105], and thus Keap1 was also designated as an inhibitor of Nrf2 (iNrf2) [126], which retains this CNC-bZIP protein in the cytoplasm under normal conditions (Fig. 5). Clearly, Nrf2 is a highly unstable protein with a short

half-life ($t_{1/2} \sim 15$ min), subject to the proteolytic degradation catalyzed by the 26S proteasomal complex *via* the ubiquitin-dependent pathway [127, 128]. Studies by different groups have showed that the association of Keap1 with Nrf2 promotes ubiquitylation of this CNC-bZIP protein in a normal constitutive manner [129, 130] through the cullin 3 (Cul3)-dependent pathway [131, 132]. Keap1 is thereby identified as an adaptor protein of the ubiquitin E3 ligase Cul3 complex with ring-box protein 1 (Rbx1). This adaptor protein is composed of three main structure domains: i) the Broad-complex, Tramtrack, Bric-à-brac (BTB) domain for its homodimerization; ii) the intervening region (IVR) for associated with the Cul3 ligase; and iii) the six Kelch repeats and its C-terminal region for docking the Neh2 of Nrf2 [133-137]. Moreover, studies by genetic knockdown of the cellular Keap1 protein [114, 138] and using *Keap1* knockout animals [139-141] revealed that, upon interaction with Keap1/Cul3, Nrf2 is targeted directly for ubiquitylation and degradation.

Interestingly, Keap1 is also thought of as a redox-sensing metalloprotein, because it is enriched with cysteine (Cys) residues; for example, 25 and 27 Cys residues are contained in the mouse and human Keap1 proteins, respectively [105]. Experimental evidence has shown that these Cys residues play a vital role in regulating the substrate adaptor function of Keap1, and approximately half of the Cys residues are likely to be highly reactive, and hence able to form a thiolate anion under normal physiological conditions [113, 142-144]. These Cys residues present Keap1 as an attractive target for potential regulation by thiol-reactive chemical species and, hence, inhibitory modulation of its activity was suggested to be an important mechanism for Nrf2 activation [105, 130, 145, 146]. Ectopic over-expression of recombinant Keap1 in various cell lines has shown that Cys23, Cys273 and Cys288 are required for its repression of Nrf2 [130, 131, 147]. By contrast, Cys151 appeared to be required for inhibition of the substrate adaptor activity of Keap1 by inducing agents indicated [130]. Further detailed chemical and functional analyses, combined with molecular modeling and phylogenetic comparison, has showed that Keap1 can directly recognize NO, Zn^{2+} and alkenals through three distinct Cys sensors, respectively [148]. The C288 alkenal sensor is of ancient origin, having evolved in a common ancestor of bilaterians. The Zn^{2+} sensor has a minimal construction comprising H225, C226, and C613. The NO sensor, which had a co-emergence with an expansion of the NOS gene family in vertebrates, comprises a cluster of basic amino acids (H129, K131, R135, K150, and H154) that facilitate *S*-nitrosation of C151. The authors suggest that Keap1 is a specialized sensor that quantifies stress by monitoring the intracellular concentrations of NO, Zn^{2+} , and alkenals, which collectively serve as second messengers that may signify danger and/or damage associated with prolonged exposure to harmful environmental conditions.

To explain how Keap1 recruits with Nrf2 and assists in ubiquitination of this CNC-bZIP protein by Cul3-Rbx1, a two-site substrate recognition model, also called the hinge and latch model, was presented [149-151]. In this proposed model, each of the Kelch-repeat domains from a Keap1 homodimer binds to one Nrf2 protein through either a weak-

binding DLG motif (residues 29-31) or a strong-binding ETGE motif (residues 75-84), both located in the N-terminal Neh2 domain of Nrf2 (Fig. 5). The binding affinity of Kelch to the ETGE motif is approximately 100-fold higher than that of Kelch to the DLG motif [152, 153]. Structural biology studies [135, 151-154] suggest that the forked-stem homodimer of Keap1 binds both the DLG and ETGE motifs in Nrf2 to align the seven ubiquitin-accepting lysine residues between these two motifs into a conformation suitable for ubiquitin conjugation.

Consistent with the two-site structural model, stress-induced modification of Keap1 at the Cys residues, such as C151, C273, or C288 in the BTB and linker domain, imposes a conformational change that disrupts the weak Kelch-DLG binding [105, 129-132]. The resulting dissociation of Nrf2 from Keap1 diminishes the CNC-bZIP protein ubiquitination and degradation in the cytoplasm, but increases the level of Nrf2 protein localized in the nucleus, resulting in the activation of Nrf2 signaling pathway. Besides the inhibition of Nrf2 ubiquitination [155], it can be stabilized through another model for ubiquitylation of Keap1 triggered by its Cys modification or other induction mechanisms. It has been reported that certain xenobiotics can trigger the ubiquitylation of Keap1 [156, 157]. Besides Keap1, β -transducin repeat containing protein (β -TrCP) has also been identified to be involved in the 'Ying-Yang' regulation of Nrf2 protein stability through binding to the DSGxS motif in the Neh6 domain (Fig. 5) [158, 159].

4.4. Differential Regulation of Nrf2 Through Distinct Upstream Signaling Pathways

The above description has suggested that any mechanism that can disrupt the interaction between Keap1 and Nrf2 targeted for their ubiquitylation would lead to the activation of Nrf2-ARE gene regulatory network. For this reason, several upstream signaling protein kinases, such as PKC, MAPK, and PRKR-like endoplasmic reticulum (ER) kinase (PERK), have been implicated directly or indirectly in the modification of Nrf2, resulting in its activation. Upon oxidative stress, phosphorylation of Nrf2 at serine 40 by PKC has been reported to release this CNC-bZIP protein from Keap1 [102]. An additional study by Cullinan *et al* suggested that the membrane-bound ER stress sensor PERK can mediate phosphorylation of Nrf2, trigger dissociation of Nrf2 from the Keap1/Cul3/Rbx1 complex, and inhibit *in vitro* re-association of this complex with Nrf2 [160]. However, Nrf2 is not an ER-resident protein [161], and thus it is required to ascertain whether or how Nrf2 is recruited to the inner nuclear envelope membrane associated with PERK. Besides, activation of several upstream MAPKs, such as extracellular signal-regulated kinase 2 (ERK2), ERK5, c-Jun NH₂-terminal kinase 1 (JNK1), can transduce differential signaling responses to the phosphorylation of Nrf2 and its transcriptional activation [162]. Further research found that phosphorylation of Nrf2 at serines 215, 408, 558, 577 and threonine 559 by MAPKs, including ERK2, JNK1/2 and p38 kinases, could moderately affect the activity of Nrf2. [163], and thus proposed that the direct phosphorylation of Nrf2 contributes limitedly to the regulation of Nrf2 activity. Conversely, it has been reported that phosphorylation of Nrf2 by p38 kinase caused an increase in the interaction between

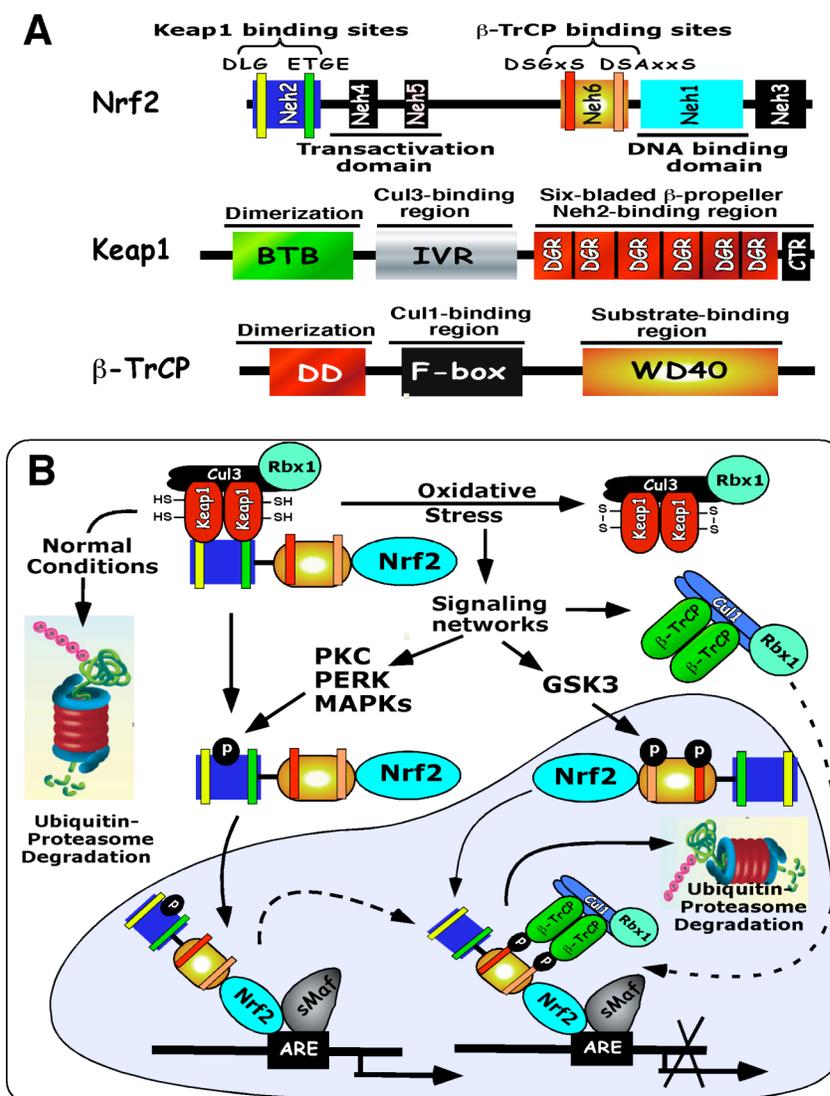


Fig. (5). Nrf2 is negatively regulated by Keap1 and β -TrCP within distinct signaling pathways. (A) Shows structural domains of Nrf2, Keap1 and β -TrCP. Nrf2 contains two Keap1-binding DLG and ETGE motifs in its Neh2 domain, and also two β -TrCP-binding DSGxS and DSxSxS degress in the Neh6 domain. The adaptor Keap1 is homodimerized through its BTB domain, and binds Cul3 E3 ubiquitin ligase and Nrf2 through its IVR and doubleglycine-repeat (DGR) domains, respectively. The β -TrCP dimer, formed through its D-domain (DD) binds Cul1 E3 ubiquitin ligase and Nrf2 through the F-box and WD40 domains, respectively. (B) Shows a model for negative regulation of Nrf2 by Keap1 or β -TrCP. Under normal redox conditions, a homodimer of Keap1 can bind to the DLG and ETGE motifs of Nrf2 through its six-bladed β -propeller structure formed by both the DGR and the CTR (C-terminal region). In addition interacting with Nrf2, Keap1 also binds the Cul3- Rbx1 complex and this association allows ubiquitination of Nrf2 to target for proteosomal degradation. Upon oxidative stress, repression of Nrf2 by Keap1 is antagonized when reactive cysteines of this adaptor protein, particularly within its IVR are modified, and/or Nrf2 at Ser⁴⁰ located between the DLG and ETGE motifs is phosphorylated (P) by protein kinase C (PKC) or other kinases (e.g. PERK). Then phosphorylated Nrf2 is translocated into the nucleus through its nuclear localization signal within the basic region. In the nucleus Nrf2 binds to the ARE-driven genes after it forms a functional heterodimer with a sMaf protein through the interaction between their ZIP regions. The β -TrCP dimer can bind Nrf2 through the DSGxS and DSxSxS degress, which is phosphorylated by GSK-3 β , and this also allows the association of this CNC-bZIP protein with the β -TrCP-mediated Cul1-Rbx1 complex targeted for the nuclear degradation.

Nrf2 and Keap1, which consequently attenuates both the constitutive and inducible Nrf2 activity [162, 164].

Recently, glycogen synthase kinase-3 β (GSK-3 β) was reported to catalyze phosphorylation of the DSGxS to become a DSGxpS phosphodegron for the binding to β -TrCP, enabling Nrf2 to be targeted for the ubiquitin E3 ligase Cul1/Rbx1 complex-mediated degradation pathway inde-

pendent of Keap1 (Fig. 5) [158, 159, 165]. An additional study showed that, in response to oxidative stress, the cyclin-dependent kinase inhibitor p21 is regulated through its ¹⁵⁴KRR motif interacting directly with both the DLG and ETGE motifs in Nrf2, and that the interaction can competitively inhibit Keap1 with respect to binding to Nrf2, compromising its ubiquitination [166]. This study, by using *p21*-

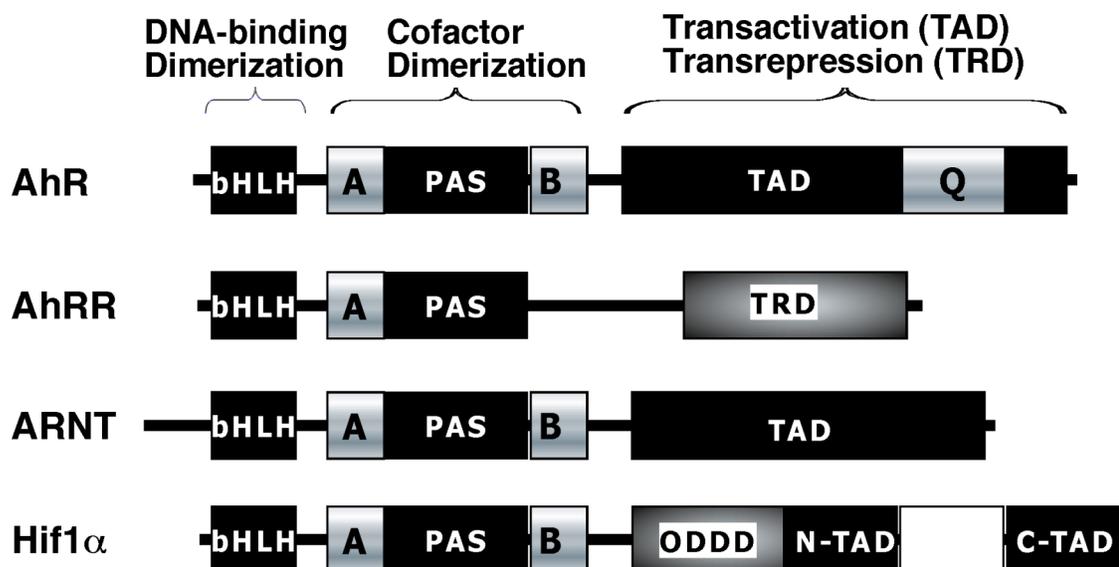


Fig. (6). Schematic representation of the mouse bHLH-PAS domain proteins AhR, AhRR, ARNT and Hif1 α . Their structural domains are characterized as the basic helix-loop-helix (bHLH), Per-Arnt-sim (PAS), transactivation (TAD) or trans-repression (TRD). The basic region of bHLH contributes primarily to its DNA binding activity and the nuclear localization signal, whereas the HLH portion is also responsible for binding to target genes and dimerization with its partner. The PAS is a signal-sensing domain, and also contributes to binding for cofactors and other proteins. In addition to TAD, Hif1 α contains a VHL-recognized ODDD that mediates the oxygen-regulated stability.

deficient mice, demonstrated that p21 up-regulates Nrf2 under both basal and induced conditions.

4.5. Induction of the Nrf2 Gene Itself Through Both ARE and XRE

Besides the predominant regulation of Nrf2 by Keap1/Cul3-mediated ubiquitination and other various signaling pathways, it may also be regulated at its transcriptional level on the basis that within the *Nrf2* gene promoter region there are two ARE sequences starting at -579 nt, 5'-TGACTCCGC-3', and -317 nt, 5'-TGACTCCGC-3' [111, 167], together with one xenobiotic response element (XRE) beginning at -712 nt, 5'-GCGTG-3', and additional two XRE-like sequences starting at +755 nt, 5'-CACGC-3' and +870 nt, 5'-CACGC-3', respectively [168]. In fact, it has also been shown that treatments with either ³H-1,2-dithiole-3-thione, or isothiocyanate sulforaphane as an ARE inducer, can modestly increase expression of *Nrf2* mRNA in keratinocytes [111]. The presence of functional XRE in the gene promoter is also proved by the evidence that the XRE-specific inducer, 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD) increases the *Nrf2* mRNA levels in hepatoma 1c1c7 cells [168]. Furthermore, there exist multiple single nucleotide polymorphisms (SNPs) in the promoter of human *Nrf2*, and one of these SNPs (-617C/A) significantly reduces the gene expression [169]. However, it is not known whether such polymorphisms can prevent the variant allele from being transcriptionally activated by thiol-active agents. In addition, there contains functional ARE sequences in the *Keap1* gene promoter, and thus its transcriptional expression is finely tuned by an auto-regulatory feedback loop within Nrf2 [170] or another CNC-bZIP factor Nrf1 [171]. Together, the feedback controlling expression of both *Keap1* and *Nrf2* should be integrated with multiple signaling responses to the

Keap1-Nrf2-ARE gene regulatory network, which talks with another XRE-pivoting network.

4.6. The Dark Side of Nrf2 Involved in Cancer Promotion and Drug Resistance

The beneficial aspects of Nrf2 are described above, but the CNC-bZIP factor also possesses several deleterious properties. This two-sided conclusion is drawn from several studies showing that Nrf2 can promote tumorigenesis and chemoresistance. The first evidence that Nrf2 was involved in cancer promotion was obtained from northern blotting and chromatin immunoprecipitation revealing that *Nrf2*, and placental *GSTP1* (that is not expressed in the normal liver) were specifically up-regulated in parallel with development of precancerous lesions and hepatocellular carcinoma [172]. Later studies identified *Keap1* mutation or loss of heterozygosity in the *Keap1* locus in lung cancer cell lines or cancer tissues [152, 173]; the ultimate result of *Keap1* mutation is the increase in the constitutive activity of Nrf2 and the transactivation of its downstream genes. An investigation of 65 Japanese patients with lung cancer suggested that there was a high incidence of somatic mutations of *Keap1* with lung adenocarcinoma [174]. Consistently, another report indicated that *Keap1* expression is reduced in lung cancer cell lines and tissues, compared to that expressed in normal bronchial epithelial cell line [175]. The reduced expression of *Keap1* is accompanied by Nrf2 over-expression at the later stage of lung cancer [176]. Moreover, the mutation of Keap1C23Y, leading to its inability to repress Nrf2, was also found in breast cancer [147]. Collectively, these findings suggest that loss of function of *Keap1* results in the prolonged activation of Nrf2 activity. Such a consequence of prompting the survival of cancer cells is likely due to the up-regulation of a subset of the downstream genes, of which are involved in anti-apoptosis and/or anti-senescence. The permanently hy-

Table 4. A list of drug-metabolizing enzymes regulated by AhR. Such a list of genes regulated by AhR through the XRE is still growing, some of which were described [180, 181].

Species	Gene
Mouse	<i>Cyp1a1</i>
	<i>Cyp1a2</i>
	<i>Cyp1b1</i>
Rat	<i>Cyp1a1</i>
	<i>Aldh3a1</i>
	<i>Ugt1a1</i>
	<i>GSTya</i>
	<i>Nqo1</i>

peractive Nrf2 can thus act as an unrecognized mediator of oncogenesis and promote tumourigenesis [174, 177, 178].

Nrf2 also contributes to the resistance of cancer cells to chemotherapy. It was recently highlighted in a study that the prognosis in patients with lung cancer that contain mutant *Keap1* or *Nrf2* was worse than that in patients with lung tumours lacking such mutations [179]. Although the homeostatic activation of Nrf2 protects the normal cells against cytotoxic agents, it is possible that in the malignant cells in human tumours permanently hyperactive Nrf2 may confer resistance against chemotherapeutic drugs. In fact, the *in vitro* studies by Wang *et al* [176] investigated the role of Nrf2 in determining drug responses in lung carcinoma, breast adenocarcinoma and neuroblastoma, revealing that up-regulation of Nrf2 enhanced chemoresistance whereas its down-regulation sensitizes cells to chemotherapeutic agents (e.g. cisplatin, doxorubicin and etoposide). It is therefore desirable to overcome drug resistance caused by up-regulation of Nrf2. For this reason, Hayes *et al* have reviewed the means to solve this problem, either by antagonizing Nrf2 directly or exploiting up-regulated ARE-drive genes to activate cytotoxic pro-drugs [113].

5. MECHANISM FOR INDUCTION OF DRUG-METABOLIZING GENES BY AhR BINDING TO THE XRE

Although carcinogenesis is a complex and protracted multistage process, the entire pathological course can be initiated by a single event wherein a cellular macromolecule is damaged by one of many endogenous or exogenous cytotoxic agents or carcinogens. Such initiatory events can be defended against by cytoprotective strategies, such as up-regulation of drug-metabolizing enzymes that are involved in promoting the conjugation and excretion to reduce carcinogen toxicity. For example, reduction of electrophilic quinones by NQO1 has proved an important detoxification pathway, which converts quinones to hydroquinones and reduces oxidative cycling. Such chemicals that can increase the expression of NQO1 or its activity (Fig. 2) are helpful in preventing the initiation of cancer. Besides Nrf2, the aryl

hydrocarbon receptor (AhR) also plays a pivotal role in the transcriptional regulation of NQO1 and other drug-metabolizing genes, e.g. those encoding cytochrome P450 (CYP) enzymes (Table 4) [180, 181].

5.1. Regulation of Drug-Metabolizing CYP Genes by AhR Through Binding to the XRE

Collectively, CYP enzymes play important roles in drug, carcinogen, and steroid hormone metabolism [182]. It has been identified that four (i.e. CYP1 to CYP4) of 18 mammalian CYP gene families are mainly responsible for metabolism of foreign compounds, including drugs, food additives and environmental pollutants [183]. Some of the CYP enzymes are substrate inducible, a property that allows the cell to adapt to changing chemical environments. Induction of CYPs has both advantages and disadvantages. On the one hand, the enzyme induction inhibits chemical carcinogenesis because it increases the rate of carcinogen detoxification to prevent the accumulation of lipophilic compounds to damaging levels. On the other hand, since CYP enzymes have broad substrate specificities, enzyme induction by one compound may lead to increased metabolism of another compound, potentially leading to the loss of the beneficial drug effects. These points should be considered seriously: that enzyme induction of CYPs produces an imbalance between bioactivation and detoxification, leading to adverse effects of drugs administered on the organism. In the case of polycyclic aromatic hydrocarbons, such as those found in cigarette smoke, the metabolism by cytochromes P450 can generate arene oxides, which are electrophiles that bind covalently to cellular components. Besides arene oxides, other reactive species are also produced during the bioactivation process mediated by CYPs and other Phase I drug-metabolizing enzymes [184]. Therefore, at high drug concentrations biotransformation and detoxification pathways can become saturated and induction of CYPs may also increase the production of reactive metabolites beyond the capacity of cellular defenses, thereby leading to potential toxicity or neoplasia [185, 186].

Expression of CYP1 to CYP4 families responsible for biotransformation of xenobiotics are tightly regulated through different mechanisms [183]. The expression of CYP1 family members is principally regulated by AhR and its heterodimer partner called AhR nuclear translocator (ARNT) (Fig. 6) [187], whilst the expression of CYP2, CYP3 and CYP4 family enzymes is regulated by three distinct nuclear factors, i.e. constitutive androstane receptor, pregnane X receptor and peroxisome proliferator-activated receptor, respectively [188]. Typically, some inducers of CYP1A1 include halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons and the environmental contaminant TCDD. To gain insights into the mechanism of CYP1A1 induction, TCDD was employed as the xenobiotic inducer. Since CYP1A1 is clearly involved in both the metabolism of polycyclic aromatic hydrocarbons and the production of reactive genotoxic metabolites that may initiate carcinogenesis, it is important to understand the basis of CYP1A1 induction. As expected, the study using *AhR*-defective and *ARNT*-defective cells revealed that induction of CYP1A1 is dependent on AhR/ARNT [189]. Later studies of the protein-DNA interaction showed that an AhR/ARNT complex binds the

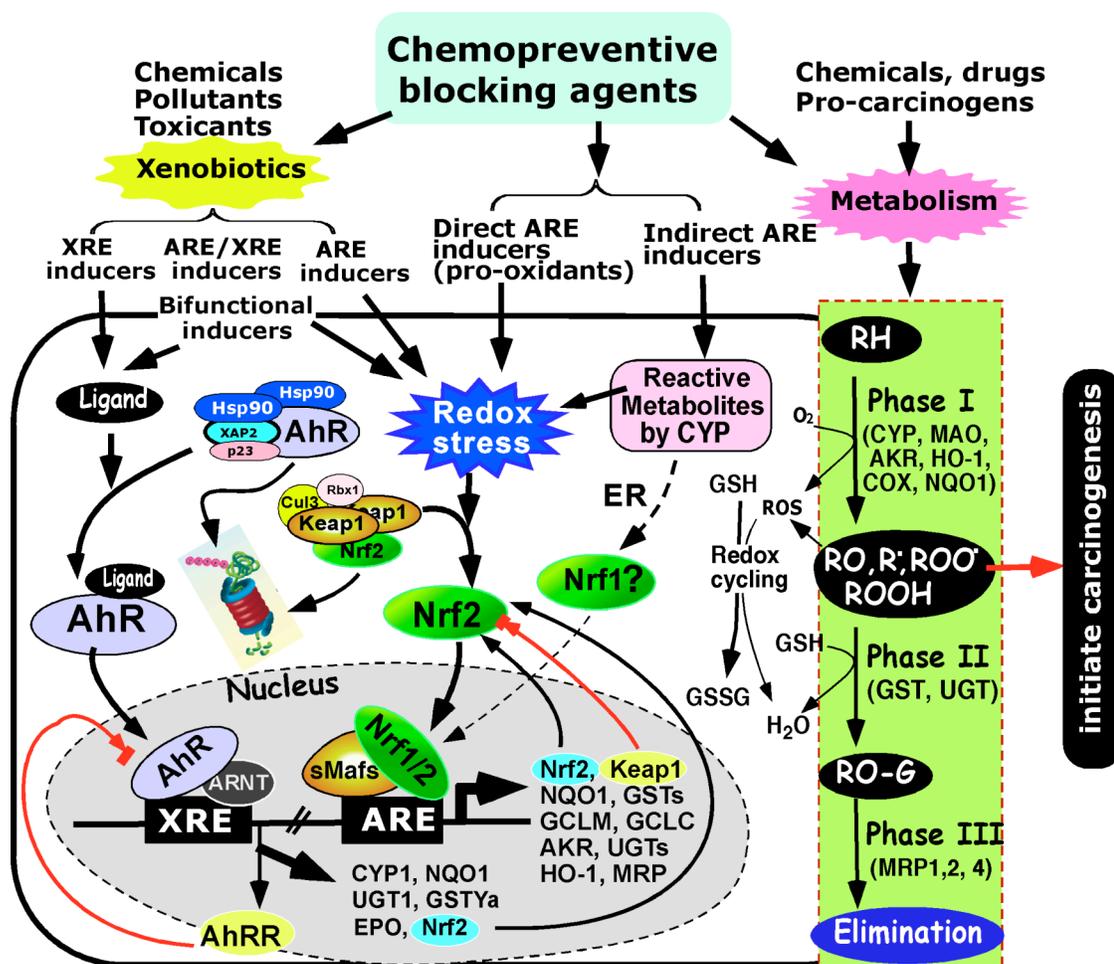


Fig. (7). Multiple signaling crosstalks between Nrf2-ARE and AhR-XRE gene regulatory networks. Based on induction of the responsive genes, xenobiotics including chemicals, pollutants and toxicants are divided into ARE-, XRE- and ARE/XRE-inducers. As ARE-inducers triggers redox stress response, the resulting Nrf2 will be released from Keap1, translocate into nucleus, heterodimerize with small Maf, and bind to ARE in the promoter region, leading to target gene activation. By contrast, AhR is kept inactive in cytoplasm by binding to a complex of Hsp90, XAP2 and p23 protein. Once XRE-inducers as ligands bind AhR, this receptor will be released from the complex and translocated into nucleus, wherein it heterodimerizes with ARNT. This dimer subsequently binds to XRE-driven genes; this binding activity can be inhibited competitively by AhRR, but its gene transcription is positively regulated by AhR. The mouse *Nrf2* and *Nqo1* genes contains both XRE and ARE in their promoters, and thus they are regulated by AhR and CNC-bZIP family factors (e.g., Nrf1 and Nrf2 itself). The transcriptional activity of Nrf2 is negatively regulated by Keap1 and β -TrCP, and in turn the negator *Keap1* gene is positively regulated by Nrf1 and Nrf2. Such crosstalks between AhR-XRE and Nrf2-ARE networks, along with their respective negative feedback loops, finely control antioxidant, detoxification and drug-metabolizing genes. These two signaling response networks have been portrayed as targets of chemopreventive blocking agents (e.g. flavonoids). The bifunctional inducers activate transcription of ARE-driven genes after they are biotransformed by CYPs largely in the ER into reactive intermediate metabolites, that have characteristic of the monofunctional inducers. Such antioxidant, detoxification and cytoprotective genes are induced by nontoxic chemopreventive agents in order to block Phase I enzymes-mediated bioactivation toxicants and pro-carcinogens (RH) into reactive intermediates (e.g. RO, R', ROO' and ROOH represent radical, alkoxy, peroxy and hydroperoxide, respectively). These possible intermediates can further be detoxified by Phase II enzymes to become glutathione-conjugated compounds (RO-G) and then be excreted by Phase III efflux pumps.

cis-regulatory element 5'-TnGCGTG-3', which is present in multiple copies within the enhancer of *CYP1A* [190]. This element was designated the XRE (Fig. 4), but it is also called the dioxin responsive element or the aryl hydrocarbon-responsive element [191]. Further mutational analysis of the core sequence indicates that 5'-CGTG-3' is essential for the functional XREs [192].

Notably, the tetranucleotide 5'-CGTG-3' from the XRE is embedded in either the hypoxia response element (HRE, 5'-

T^A/_GCGTG-3') or the UPRE (5'-TGACGTC^G/_A-3') (Fig. 4). This evolutionary conservation suggests possible crosstalk between XRE-, HRE- and UPRE-battery gene regulatory networks. On the other hand, since these three homologous *cis*-elements are so very much alike that it is hard to distinguish between them, they are likely to be recognized by cognate canonical and non-canonical transcription factors and partners. As a consequence, the *cis*-element-specific binding of canonical factors could be either competitively inhibited

or unexpectedly imposed by non-canonical misrecognized factors, in particular during pathological stress conditions.

5.2. The Structure of AhR with its Functional Regulation

The AhR belongs to the family of eukaryotic Per-ARNT-Sim (PAS) domain proteins (Fig. 6), that function as sensors of extracellular signals and environmental stresses affecting growth and development [193]. Amongst this family, AhR regulates adaptive and toxic responses to a variety of chemical pollutants, including polycyclic aromatic hydrocarbons and polychlorinated dioxins, and TCDD serves as a classic inducer of the receptor. In the early 1990s, the mouse *AhR* cDNA was first cloned [194, 195], followed by the human and rat *AhR* homologues [196, 197]. Later, additional cDNA of *AhR* has also been isolated from other species such as birds, fish, amphibians, but the rodent and human *AhR* have been employed in the most extensive studies [198]. The comparative study demonstrated that AhR is significantly evolutionarily conserved amongst distinct species [198]. The early studies to analyze the *AhR* cDNA revealed that the translated protein contains two structural domains, i.e. the basic helix-loop-helix (bHLH) and PAS domains, in the N-terminal half of the molecule [194, 195]. The bHLH domain contributes to DNA binding and also to protein-protein dimerization through the HLH portion. It is important to note that just a nuclear localization signal is contained within bHLH, whilst one or more nuclear export signals are present in both bHLH and PAS domains. The PAS domain is further divided into two subdomains PAS-A and PAS-B. A study using the yeast Gal4 fusion protein system provided evidence that the C-terminus of the AhR harbours a potent transactivation domain, consisting of proline/serine/threonine (P/S/T)-rich, glutamine (Q-rich) and acidic subdomains, each of which exhibits varying levels of activation and functions independently [199-201]. In addition, the AhR shares structural similarity with its nucleus dimerization partner ARNT and its repressor AhRR (Fig. 6).

It is clear that the unliganded AhR is held in the cytoplasm as an inactive protein in a complex with the chaperone proteins HSP90, HSP23, and an immunophilin-like protein XAP or p23 (Fig. 7). The binding of HSP90 is essential to retain AhR in the cytoplasm, because this interaction can mask the nuclear localization signal of AhR. Upon ligand binding, the HSP90-bound AhR is released from the cytoplasmic complex before translocating into the nucleus, whereupon it heterodimerizes with another bHLH-PAS protein ARNT. This heterodimer subsequently binds to XREs in the regulatory region of target genes. [187]. It has been reported that a number of co-activators and various general components form the transcriptional complex with the AhR/ARNT heterodimer [202], but the specific interaction and order of the complex formation still needs to be fully elucidated.

After ligand binding, phosphorylation of both AhR itself and the HSP90 complex on several residues are required for transformation of the unliganded AhR into the fully functional form [203]. Subsequently, the fully functional AhR induces the expression of many detoxification genes, which contain XREs in their promoter regions. These genes include CYP enzymes, e.g. *CYP1A1*, *CYP1A2*, *CYP1B1*, and

CYP2S1, and other drug-metabolizing enzymes such as *UGT1A6*, *NQO1*, *ALDH3A1*, and several *GST* isoenzymes (Table 4) [203].

Collectively, distinct possible mechanisms by which AhR is down-regulated either before or after its activation, include the 26S proteasome-mediated degradation of AhR, competitive inhibition of AhR by its repressor AhRR, and binding to its antagonists. *In vitro* experimentation has shown that AhR is rapidly depleted after exposure to its ligands [204-206]. This event is most likely to occur after the transcriptional activation of its target genes, but can be blocked by the proteasome inhibitor MG132. Such degradation occurs through the 26S proteasome complex present in both the cytoplasm and the nucleus. Further studies have revealed that AhR degradation also occurs after the receptor translocates into nucleus, wherein it forms a complex with the Cul4B E3 ubiquitin ligase, damaged-DNA-binding 1, ransducin β -like 3 and Rbx1. The Cul4B E3 ligase can catalyze ubiquitylation of AhR and other nuclear receptors, e.g. estrogen receptor α and β subunits, and androgen receptor [207]. The ubiquitin labeling targets AhR to 26S proteasome-mediated degradation.

There is a negative feedback loop of AhR signaling with its repressor AhRR, which can in turn be transcriptionally induced by activated AhR [208]. The promoter region of *AhRR* contains a functional XRE sequence, enabling the expression of *AhRR* gene upon ligand activation of AhR. As it contains two bHLH and PAS-A domains that are structurally similar with AhR, followed by a C-terminal transcription repression domain, AhRR also forms a heterodimer with ARNT [208]. This heterodimer binds competitively to the XRE sequence with the AhR/ARNT heterodimer and subsequently recruits co-repressors [209]. Overall, the ultimate activation of AhRR leads to the inhibition of AhR [208, 210]. In addition, it should be noted that the hypoxia inducible factor 1 α (Hif1 α) is another bHLH-PAS transcription factor, and can also form a functional heterodimer with ARNT (also called Hif1 β). They regulate target genes through the HRE, which contains 5'-CGTG-3' identical with the essential XRE for binding to AhR or AhRR (Fig. 4). However, it has yet to be determined whether the Hif1 α -HRE gene regulatory pathway is involved in the drug metabolism or xenobiotic response or if AhR regulates some genes in the response to hypoxia.

5.3. Ligands of AhR Regulate Expression of XRE-Driven Genes

The transcription factor AhR acts as a soluble ligand-activated nuclear receptor. Such ligands of AhR include exogenous and endogenous compounds, and exhibit structural diversity, though their binding affinities differ to a great extent. Exogenous ligands consist of not only synthetic ones but also normal dietary components. Amongst those AhR ligands identified and characterized, exogenous synthetic ones that show the highest affinity include planar, hydrophobic halogenated aromatic hydrocarbons (e.g. polyhalogenated dibenzo-p-dioxins, dibenzofurans, and biphenyls) and polycyclic aromatic hydrocarbons (e.g. 3-methylcholanthrene, BaP, benzanthracenes and benzoflavones), as well as related compounds. Between halogenated and polycyclic aromatic hydrocarbons, the former ligands are more metab-

olically stable and act as the most potent class of AhR inducers, within the pM to nM range of binding affinities, whereas the latter ligands are the more metabolically labile ones with the relatively lower binding affinity in the nM to μ M range [211].

Dietary chemicals acting as ligands of AhR have been described in numerous studies, showing that those chemicals can either activate or inhibit the AhR signaling pathway. In 1978, Watternberg and Loub reported that indoles occurring in edible cruciferous vegetables can inhibit the formation of neoplasia induced by AhR in mice, indicating they can inhibit the activity of AhR [212]. In 1991, another group showed that indole-3-carbinolcan, one of the aforementioned indoles, acts as AhR agonist and increases the *CYP1A1* activity [213]. Besides indole-3-carbinolcan, other dietary plant compounds such as curcumin [214], quercetin and keampferol [215], have been reported to be able to competitively bind to the AhR. On the other hand, some dietary plant chemicals, such as resveratrol [216], have also been identified as inhibitors of AhR. It is noteworthy to mention that many dietary chemicals themselves have no or little ligand-binding activity of AhR; however, once these chemicals enter the mammalian digestive tract, they may undergo the conversion into significantly more potent AhR ligands. Examples of such chemicals include indole-3-carbinolcan, which itself is a weak inducer of gene expression, whereas indole-[3,2-b]-carbazole, an acidic condensation product from indole-3-carbinolcan, has relatively high affinity of AhR (~0.2-3.6 nM) [211].

The evidence for, and characterization of, endogenous ligands of AhR, in addition to exogenous ligands, has been provided in various studies. Firstly, the existence of endogenous ligands is postulated from the identification of the nuclear AhR complexes in unexposed cells in culture and tissue slices. Secondly, the effect of endogenous ligands is deduced from the fact that AhR-deficient cells had altered cell cycle progression [217, 218]. Thirdly, activation of AhR by endogenous ligands occurs in the *AhR* knockout animals, that exhibit numerous physiological changes and developmental abnormalities [219, 220]. A number of the candidates have been suggested as endogenous ligands of AhR including indigoids, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester, equilenin, arachidonic acid metabolites, heome metabolites, tryptophan metabolites, and ultraviolet photoproducts of tryptophan [221]. Taken together, these studies indicate that AhR can bind many different chemicals, including environmental contaminants, therapeutic agents, naturally occurring chemicals and small molecules isolated from tissues. These chemicals have diverse structures and distinct affinities of ligand binding to the AhR.

5.4. Physiological Functions of AhR

Some of the aforementioned AhR inducers are environmental pollutants that cause acute and chronic toxicity and some of these are carcinogens. They induce the AhR-mediated expression of genes responsible for xenobiotic-metabolizing enzymes, such as cytochrome P450 families. Besides its involvement in xenobiotic metabolism, AhR plays crucial roles in distinct physiological processes [222], which range from reproduction, development, immunity, cell

cycle, cell proliferation, to cell adhesion and migration [223]. The physiological functions of AhR vary with its distinct expression in diverse cells, tissues and organs. The constitutive AhR is highly expressed in liver, but is also abundant in placenta, thymus, lung, kidney, small intestine, heart and pancreas [224].

The involvement of AhR in normal physiological processes has been proven by evidence showing that it was activated in a xenobiotic-independent way [225-228]. The *AhR*-null mouse also provided a deeper insight into the physiological processes dependent on its transcriptional activity. These animal models not only demonstrated that this receptor is essential for dioxin-induced cytotoxicity [229] and carcinogenesis [230], but also revealed the existence of an *AhR*-deficient phenotype. Different studies showed that genetic deletion of *AhR* in the mouse caused either early death or pathological changes by 13 months, which was accompanied by a wide variety of phenotypic alteration in major organ systems [231, 232]. These phenotypes include progressive cardiac hypertrophy, gastric hyperplasia that progressed into polyps with age, T cell deficiency in the spleen, and abnormalities in skin such as hyperkeratosis, and marked dermal fibrosis.

Besides the above effect on the cardiovascular system, the immune system and skin, the *AhR*-null females also showed difficulties in maintaining pregnancy, and their pups exhibited a poor survival rate during lactation and weaning [233]. A significant impact of its function on the development of liver is supported by the facts that *AhR*-null mice had smaller livers and also show portal fibrosis and early lipid accumulation in this organ [219, 234]. Comparison of the liver mRNA profiles from between wild-type and *AhR*-null mice revealed that the expression patterns of 392 genes were changed due to the absence of AhR. The mechanisms underlying these physiological functions of AhR include its effect on the cell cycle, which can in turn affect the progress of cell proliferation, either inhibiting or promoting it depending on the cell phenotypes [223]. Also, the AhR is involved in cell adhesion and migration, in addition to developmental processes.

5.5. Dual Apposing Roles of AhR in the Progress of Tumourigenesis

As AhR can promote and inhibit cell proliferation, there has been some discussion as to whether it is a tumour promoter or suppressor. The AhR cooperates with signaling molecules involved in cell survival pathways, which allow cells to sustain proliferation. An example is NF- κ B [235], with which AhR can physically interact, leading to its activation in human breast cancer MCF-7 cells. Activation of NF- κ B causes the transactivation of the *c-Myc* proto-oncogene. By this mechanism, the AhR may contribute to increased cell proliferation and carcinogenesis in the breast. Another study also showed that AhR induces the proliferation of human lung carcinoma A549 cells, due to the over-expression of the nuclear receptor. Transgenic mice expressing a constitutively active *AhR* have showed spontaneous tumours in the glandular stomach [236], and also increased frequency of the formation of hepatocarcinomas induced by N-nitrosodiethyl [237]. Such facts that over-expression and activation of AhR

can stimulate cell proliferation and even promote carcinogenesis indicate that the receptor has oncogenic activity.

Conversely, several studies found that activation of AhR can halt the cell cycle at different stages and also inhibit cell proliferation. In non-proliferating 5L-heptoma cells, induction of AhR by exogenous ligands activates transcription of the *p27^{kip1}* tumour suppressor, and consistent induction of *p27^{kip1}* by dioxin in fetal thymus was accompanied by inhibition of cell proliferation [238]. Although AhR can stimulate proliferation of MCF-7 cells without exogenous ligands, the presence of exogenous ligands allows this receptor to synergize and interact with the Rb tumour suppressor, resulting in the inhibition of Rb-mediated E2F-dependent transcription and ultimately leading to cell cycle arrest [239]. Another study showed that cell cycle was blocked by dioxin at the G₁ in MCF-7 and mouse hepatoma Hepa-1 cells [240]. The blockage was due to the fact that the interaction between activated AhR and the p300 co-activator leads to a displacement of p300 from the E2F-dependent promoter and proliferation arrest. The arrest of cell cycle by constitutively activated AhR has also been found in a few of other cell lines, transgenic mice, and mouse thymus in organ culture through different mechanisms [223]. These findings suggest that constitutive or ligand-induced activation of AhR may act as a tumour suppressor by inhibiting cell proliferation. Overall, depending on the phenotypes of cells and inducers of the receptor, AhR can either inhibit or promote cell proliferation, and thus have dual tumour suppressor or oncogenic activity.

6. AhR CROSSTALKS WITH MULTIPLE SIGNALING PATHWAYS

The AhR mediates dioxin-induced toxicity and also influences many of physiological functions. The mechanisms that underlie the wide diversity of AhR activity are established by its cross talks with multiple signal transduction pathways (e.g. MAPKs), cell cycle progression and apoptosis, and transcriptional factors such as Nrf2 and Hif-1 [203, 241, 242]. The MAPKs, including three families: ERK1/2, JNK/SAPK and p38 kinases, mediate important intracellular signaling transduction [243]. MAPKs and their downstream protein kinases can phosphorylate a large panel of substrates (e.g. AhR and Nrf2) on serine and threonine residues, which enable them to regulate gene expression and protein functions. Generally, ERK1/2 are involved in regulating both mitogenic and developmental events, four p38 kinases play important roles in the inflammatory response, apoptosis and cell cycle, and three JNK isoforms play essential roles in multiple cellular signaling towards the immune system, stress-induced and developmentally programmed apoptosis, carcinogenesis, and pathogenesis of diabetes [244].

Although the well-known AhR ligand TCDD activates ERK and JNK, such activation occurred similarly in both the *AhR*-expressing and *AhR*-null cells, suggesting induction of MAPK by this ligand in an AhR-independent manner [245]. However, TCDD-stimulated MAPKs appear critical for the induction of AhR-dependent gene transcription and *CYP1A1* expression. TCDD and another ligand 3-methylcholanthrene induced morphological changes that modulate epithelial cell plasticity [246]. Such dioxin-induced events were mimicked by constitutive expression and activation of AhR. In addition,

a correlated event was the activation of JNK, which is reversible using a JNK inhibitor, indicating the effect of AhR on cell plasticity is in an JNK dependent pathway. Therefore, these novel effects on cell plasticity support a mechanistic role for the AhR in cancer progression as mediated by many of its ligands. Activation of p38 kinases by the AhR ligand TCDD seems to be a cell-specific consequence [246], because p38 kinase activated by TCDD in an AhR-independent mechanism occurred in RAW 264.7 macrophages but not in embryonic fibroblasts.

Another signaling pathway AhR interacts with is the Rb-E2F axis, which is responsible for several cell cycle checkpoints at G₁ and S phases. Direct interactions of ligand-activated AhR with either the hypophosphorylated Rb or E2F have been found [239, 240]. Such an interaction between AhR and Rb blocks the phosphorylation of Rb leading to the repression of S-phase specific gene transcription. Alternatively, AhR activation can induce CDK inhibitors that arrest the cell cycle at G₁ phase. Additional study using *AhR*-expressing and *AhR*-null fibroblasts showed that the proliferation rate is faster in *AhR*-expressing fibroblasts compared with that in *AhR*-null fibroblast in a ligand independent manner [247]. Growth-promoting genes were significantly down-regulated in *AhR*-null fibroblast, whereas growth-arresting genes were up-regulated. These results suggested that AhR plays an intrinsic role in regulating cell proliferation independent of either exogenous or endogenous ligands. In contrast, AhR-dependent promotion of cell proliferation occurred through induction of JunD and cyclin A [248]. On the other hand, activation of E2F1 can activate apoptosis, but there is also evidence suggesting that E2F1 acts as a tumour suppressor due to its ability to initiate apoptosis in cells that lose the normal cell cycle control [249]. It has been found that AhR and E2F1 can physically interact *in vitro* and *in vivo*, so as to result in the repression of the transcriptional activity of E2F, and ultimately the inhibition of apoptosis.

6.1. Cross Talks Between AhR and Nrf2

Besides the physical interaction, AhR can modulate expression of several key genes, which contain the XRE sequence in their promoter regions, at the transcriptional level (e.g. *Nqo1*, *Nrf2* and *AhRR*). Clearly, the AhR has been shown to be able to bind directly to the promoter region of *Nrf2* [168] and its target drug-metabolizing genes (e.g. *Nqo1*) through the XRE sequences (Fig. 7). As AhR and Nrf2 regulate expression of Phase I and Phase II detoxification enzymes, i.e. NQO1, an enzyme catalyzing BaP-quinone detoxification, knockdown of *AhR* by RNA interference (RNAi) diminished BaP-induced expression of *Nrf2* and *Nqo1*, and knockdown of Nrf2 significantly decreased *NQO1* mRNA and protein levels in cells treated with or without BaP [250]. Mutation of the Nrf2-binding ARE site abrogated the *Nqo1* promoter activity, but this activity was unaffected by mutation of the AhR-binding XRE site, suggesting a role for the signaling of AhR-XRE to activate Nrf2-ARE gene regulatory network in enhanced expression of *Nqo1*. The chemopreventive potential of functionalized auronones plant-derived flavonoid analogues and related compounds as inducers of NQO1, may facilitate exploitation of the proposed crosstalk between the AhR and Nrf2 gene bat-

teries [251]. Recently, the antifungal agent ketoconazole was identified as an inducer of AhR signaling and the Nrf2 antioxidant response in human keratinocytes [252]. Ketoconazole stimulated the nuclear translocation of Nrf2, and its cytoprotective effects against oxidative stress strongly depend on a functional AhR [252]. Sustained activation of the AhR induced by TCDD results in oxidative stress, DNA damage and subsequent steatohepatitis in *Nrf2*-null mice [253]. The aggravated hepatosteatosis is due to increased lipogenesis in the liver, as accompanied by higher expression of *Fgf21* and triglyceride-synthesis genes, and activation of *c-Jun* and *NF-κB*, but by down-regulation of bile-acid-synthesis genes and cholesterol-efflux transporters, and attenuated induction of phase-II enzymes *Nqo1*, *Gsta1/2*, and *Ugt2b35*. In addition, as a response to low-glucose, endogenous compounds are recruited as AhR ligands to induce various gene expression, of which *CYP1* and *Nrf2* induction was abolished by RNAi for *AhR* [254], suggesting a relationship between drug-metabolizing enzymes and mechanisms of the anti-stress response against tumor angiogenesis. These findings demonstrate that the AhR-Nrf2 pathway opens up new opportunities to prevent and treat cancers and other diseases [255].

As phytochemicals have the potential to counteract adverse effects of carcinogens, the impacts of flavonoids on expression of AhR-Nrf2 pathway components in BaP-stimulated colon cancer Caco-2 cells were investigated [256]. In contrast to kaempferol, quercetin and BaP efficiently induced *CYP1A1*, *CYP1A2* and *CYP1B1* mRNA. The BaP up-regulated *AhR*, but down-regulated *AhRR*. By contrast, the flavonoids quercetin and kaempferol did not affect *AhR* expression but counteracted repression of *AhRR* induced by BaP. Only quercetin was found to induce *AhRR*, whilst *ARNT* appeared to be down-regulated by BaP, as well as flavonoids. Activation of the Nrf2 pathway by either BaP or the flavonoids was revealed by induction of *Nrf2* and target genes such as *NQO1*, *GSTP1*, *GSTA1* and *GCLC*. Importantly, the flavonoids can abolish the induction of *Nrf2*, *GSTP1* and *NQO1* by BaP. The authors suggested that quercetin acts a dual ARE-inducer and XRE-inducer, whilst kaempferol acts just an ARE-inducer (Fig. 7). Similarly, we also found that Quercetin and kaempferol up-regulated the Nrf2-ARE-*Nqo1* signaling pathway through stabilizing this CNC-bZIP protein (data unpublished). Furthermore, ARE/XRE-driven reporter mutagenesis experiments showed that the ARE is required for both the basal and inducible expression of *Nqo1*, whereas the XRE is involved in the basal *Nqo1* expression but not in its induction by these two flavonoids, although they can act as AhR agonists because the expression of *CYP1A1* is up-regulated in both experimental cells and the mouse small intestine. The chemopreventive effect of youngiasides, isolated from *Crepidiastrum denticulatum*, is elicited through induction of quinone reductase activity in hepatoma Hepa-1c1c7 cells, with a relatively high chemoprevention index [257]. Youngiasides up-regulated the expression of *CYP1A1* and *quinone reductase* in Caco-2 cells through activation of both the Nrf2-ARE and AhR-XRE pathways, suggesting a bifunctional inducer of quinone reductase for potential chemopreventive agents. In addition, flavonoid-contained coffee induces expression of *UGTs*, e.g. *UGT1A8* to *UGT1A10*, in liver and stomach by the AhR-XRE and Nrf2-ARE [258, 259], in order to protect

against the pathologies of chronic liver disease, hepatocellular carcinoma and diabetes. Both AhR and Nrf2 are also key regulators of human multidrug resistance protein 4 induced by TCDD, 3 methylcholanthrene or oltipraze [260]. It should be noted that Nrf2 can regulate expression of *AhR* and modulate its several downstream events [261]. In addition, additional crosstalk events between AhR and Nrf2 have been reviewed by Hayes *et al* [242].

6.2. Cross Talks Between AhR and Hif1

The hypoxia inducible factors Hif1 α , Hif2 α , and Hif3 α are bHLH-PAS proteins that heterodimerize with ARNT; these complexes preferentially bind to HRE and activate the transcription of genes, e.g. *erythropoietin (Epo)*, that regulate adaptation to hypoxia [241]. The HRE are homologous with the XRE with respect to binding of the AhR/ARNT complex (Fig. 4), and thus it is postulated that activation of one pathway would inhibit the other due to competition for ARNT or other limiting factors through binding to the HRE/XRE. For example, the promoter region of *Epo* also contains five functional XREs, besides HRE, immediately upstream of transcriptional start site [241]. Activation of the hypoxia response pathway inhibited up-regulation of *Cyp1a1*, but activation of the AhR actually enhanced the induction of *Epo* by hypoxia. This suggests crosstalks between Hif1 α -HRE and AhR-XRE in response to hypoxia and xenobiotics. This is also supported by the evidence that hypoxia inhibited induction of AhR activity and also down-regulated expression of its target drug-metabolising enzymes in an ARENT-dependent manner [262, 263]. Activation Hif1 α attenuated induction of AhR-regulated gene expression by BaP, leading to increased genetic instability and malignant progression in response to hypoxia and exogenous genotoxins [264]. However, an AhR ligand, aminoflavone (which is an active component of a novel anticancer agent AFP464 in phase I clinical trials) inhibited activity of Hif1 α and protein accumulation in an AhR-independent pathway in MCF-7 cells [265].

Interestingly, major expression of *Cyp2s1* in epithelial tissues is inducible by TCDD *via* the AhR pathway. Its promoter contains three overlapping HREs embedded within the trimeric XRE segment [266]. Each of the trimeric XRE sequence can bind the AhR/ARNT dimer and also mediate dioxin-dependent transcription of *Cyp2s1*, whilst each HRE within this segment can bind the Hif1 α /ARNT dimer and contributes toward hypoxia inducibility. These two dimers differentially bind to the region containing the trimeric XRE segment of *Cyp2s1* in a dioxin- or hypoxia-dependent fashion. In addition to the HRE, UPRE also contains a portion essential for the functional XRE (Fig. 4). It was recently reported that activation of the AhR pathway and induction of the unfolded protein response are involved in suppression of adipocyte differentiation and adipogenesis by cigarette smoke, but AhR was neither activated by ER stressors and AhR agonists did not induce an ER stress response [267]. This case suggests no crosstalk between the XRE and UPRE gene regulatory networks, but it remains to be further identified using distinct experimental systems within other pathophysiological stress conditions.

7. CONCLUDING REMARKS

Collectively, a number of cytoprotective mechanisms have evolved to defend against toxic electrophiles, chemical carcinogens and oxidative stress. Some of these cytoprotective mechanisms have been portrayed as targets of cancer chemopreventive agents (e.g. phytochemicals) [268-270] and this has been addressed via research with a particular focus on the intrinsic antioxidant and detoxification mechanisms [271-273]. Amongst the well studied are multiple signaling nodes and branches within both AhR-XRE and Nrf2-ARE gene regulatory networks (Fig. 7) [272, 274, 275]. These two reciprocally interactive signaling networks, along with their feedback regulatory loops, finely controlled expression of drug-metabolizing enzymes are involved in biotransformation at Phase I (e.g. CYPs, NQO1), detoxification at Phase II (e.g. GSTs) and drug-efflux excretion at Phase III (MRPs). It has also been shown that Nrf1 and Nrf2 are two important CNC-bZIP proteins involved in regulating both the basal and inducible expression of antioxidant, detoxification and cytoprotective genes, in addition to those encoding drug-metabolizing enzymes. Clearly, Nrf2 has been identified as a master regulator of drug-metabolizing enzymes and antioxidant cytoprotective proteins and is also considered as a target for cancer chemoprevention [276-278]. The activity of Nrf2 is negatively regulated by Keap1 and β -TrCP in different subcellular compartments (Fig. 5). These two adaptor proteins can respectively recruit the ubiquitin E3 ligases Cul3 and Cull1 complexes to the Neh2 and Neh6 domains of Nrf2 targeted for the 26S proteasome-mediated degradation. This negator *Keap1* gene expression is regulated by Nrf1 and Nrf2, whilst transcription of *Nrf2* and downstream genes (e.g. *Nqo1*), which contain two *cis*-elements XRE and ARE, is tightly controlled by AhR and CNC-bZIP (e.g. Nrf1) family factors. Such crosstalks between AhR-XRE and Nrf2-ARE regulatory networks indicate that multiple signaling pathways are integrated to activate antioxidant, detoxification and cytoprotective genes against cytotoxic insults and oxidative stress.

In targeted cells, toxic chemicals, drugs, pollutants, xenobiotics and pro-carcinogens can be biotransformed to become reactive metabolites, electrophiles or activated carcinogens, as accompanied by free radicals and reactive oxygen species, produced in the activation by AhR-XRE-driven Phase I enzymes, predominantly CYPs (on the right side of Fig. 7). At the same time, reactive metabolites and redox stress can also activate the Keap1-Nrf2-ARE-driven Phase II enzymes for the conjunction of those toxicants and carcinogens to be detoxified and Phase III drug-efflux pumps for elimination of them. Based on these differences in the activation of AhR-XRE and Nrf2-ARE either alone or together, xenobiotics are classified into ARE-, XRE- and ARE/XRE-inducers (on the left side of Fig. 7). Therefore, it is critical to maintain the balance between the AhR-XRE-CYPs activation and Nrf2-ARE-Phase II detoxification in carcinogen-targeted cells. If the detoxification pathway is saturated, activated carcinogens, along with reactive electrophiles and reactive oxygen species produced in the Phase I activation, will remain at potentially dangerously high levels thereby increasing the risk of genomic instability and subsequent carcinogenesis initiation.

In untargeted cells, nontoxic chemopreventive agents, such as dietary flavonoids, can predominantly induce activation of AhR-XRE and Nrf2-ARE either alone or together, and are thereby classified into monofunctional and bifunctional inducers (Fig. 7). Chemopreventive blocking agents either interact directly with reduced glutathione or acquire this ability indirectly as a consequence of biotransformation by Phase I enzymes, suggesting that these compounds produce a type of thiol/oxidative stress [279]. The redox stress may be caused by modification of cysteine residues in proteins [280-282] and also trigger redox signaling dependent on Keap1 [57, 283]. Therefore, up-regulated expression of drug-metabolizing enzymes has been determined as targets of cancer chemoprevention against potential toxicants and carcinogens. Once the host cells are targeted by toxicants and carcinogens, the possible consequence is blocked by chemopreventively-enhanced antioxidant capacity via induction of GCLC, GCLM, glutathione synthase, peroxiredoxin, ferritins, and HO-1, and increased expression of drug-metabolizing enzymes (e.g. NQO1, AKR, UGT and GST). Induction of these cytoprotective genes by chemopreventive agents has now been recognized primarily through the Keap1-Nrf2-ARE pathway. However, constitutive hyperactive Nrf2 has been shown to protect cancer cells against hypoxia stress and even therapeutic drugs, and consequently promotes tumorigenesis and increases drug resistance [174, 177, 178].

To date, there has been a disproportionate focus on Nrf2, but relatively less is known about the function of Nrf1. This consequence is due largely to the fact that *Nrf2* knockout mice are viable [94], whilst global knockout of *Nrf1* in the mouse leads to embryonic lethality and severe oxidative stress [52, 95, 284, 285]. Specifically, conditional knockout of *Nrf1* in the liver and brain of neonatal mice results in non-alcoholic steatohepatitis and hepatic neoplasia [96, 97] and neurodegenerative disease [99, 100], respectively. These facts demonstrate that Nrf1 fulfills an essential function, distinct from Nrf2, in regulating expression of antioxidant, detoxification and cytoprotective genes responsible for maintaining cellular homeostasis and organ integrity. Molecular and cell biology studies have identified that Nrf1 is a membrane-bound glycoprotein spanning across the ER and nuclear envelope membranes [161, 286, 287] and it is activated by a redox inducer tBHQ [288]. It is therefore postulated that the Nrf1-ARE pathway is activated by ER-derived redox stress, in part produced in the ER-based Phase I enzymes-mediated bioactivation, and will be considered as a potential target of chemoprevention. Two reports have showed that quercetin and genistein, both common dietary flavonoids in e.g. onion and soy respectively, up-regulate Nrf1-mediated peroxiredoxins and glutathione peroxidase enabling cytoprotection against oxidative stress-induced ocular disease and endothelial cell injury, [289, 290]. However, the unique role of Nrf1 in chemoprevention in relation to oxidative stress driven tumour-promoting inflammation and the cancerogenesis cascade remains to be elucidated. What is clear, however, is that as a first line defense against cancers per se dietary flavonoids, sourced from many different food crop species, clearly show great promise and further research into their specific mode of action is warranted. In addition the comparative activities of the flavonoids with respect to chemical substitution, such as methylation, glycosylation,

acylation etc., or basal structure (flavonols, flavones, flavanones, flavanols, flavan-3-ols, isoflavones, and anthocyanins) would be hugely informative in developing new crop varieties and products that would further reduce the risk of cancerogenesis.

ABBREVIATIONS

AhR	= Aryl hydrocarbon receptor
AhRR	= AhR repressor
AKR	= Aldo-keto reductases
ARE	= Antioxidant response element
ARNT	= AhR nuclear translocator
BaP	= Benzo[a]pyrene
bHLH	= The basic helix-loop-helix domain
BTB	= The Broad-complex, Tramtrack, Bric-à-brac domain
β-TrCP	= β-transducin F-box/WD repeat containing protein
bZIP	= Basic-region leucine zipper
CREB	= cAMP-response element binding protein
CNC	= Cap'n'collar
Cul	= Cullin
Cys	= Cysteine residue
CYP	= Cytochrome P450 enzyme
EGCG	= Epigallocatechin gallate
EPO	= Erythropoietin
ER	= Endoplasmic reticulum
ERK	= Extracellular signal-regulated kinase
GCL	= γ-glutamyl cysteine ligase
GCLC	= GCL catalytic subunit
GCLM	= GCLmodifier subunit
GSK	= Glycogen synthase kinase
GST	= Glutathione S-transferase
JNK	= c-Jun NH ₂ -terminal kinase
Hif1	= Hypoxia inducible factor 1
HO-1	= Haeme oxygenase 1
HRE	= Hypoxia response element
Keap1	= Kelch-like ECH-associated protein 1
MAPKs	= Mitogen-activated protein kinases
NAT	= N-acetyl transferase
NF-E2	= Nuclear factor-erythroid 2
NQO1	= NAD(P)H:quinone oxidoreductase 1
Nrf	= NF-E2 p45 subunit-related factor

PAS	= Eukaryotic Per-ARNT-Sim domain
PERK	= PRKR-like endoplasmic reticulum kinase
PKC	= Protein kinase C
Rbx1	= Ring-box protein 1
RNAi	= RNA interference
Skn-1	= Kinhead-1
SNP	= Single nucleotide polymorphism
SULT	= Sulfotransferase
TCDD	= 2,3,7,8-tetrachloro dibenzo-p-dioxin
TRE	= PA-response element for binding principally by AP-1
UGT	= UDP-glucuronyl transferase
UPRE	= Unfolded protein response element
XRE	= Xenobiotic response element

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

We are greatly thankful for Professor John D Hayes who provides invaluable comments on this review. This work is supported by the National Natural Science Foundation of China (major programme NO 91129703 awarded to YZ), the Fundamental Research Funds for the Central Universities (Chongqing University, CDJRC11230003 awarded to YZ), and the Association for International Cancer Research (grant 09-0254 awarded to Professor John D Hayes). Dr. Han Xiao's studentship was co-funded by the James Hutton Institute and the University of Dundee. Professor Derek Stewart acknowledges funding from the Scottish Government Rural & Environment Science and Analytical Services (RESAS), Climafruit (Interreg IVB) and EU Berry (EU FP7 KBBE – 2010 -4 265942).

REFERENCES

- [1] Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr* 2004; 79: 727-47.
- [2] Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 2004; 134: 3479S-85.
- [3] Marrugat J, Covas MI, Fitó M, *et al.* Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation: A randomized controlled trial. *Eur J Nutr* 2004; 43: 140-47.
- [4] Tian Q, Giusti MM, Stoner GD, Schwartz SJ. Urinary excretion of black raspberry (*Rubus occidentalis*) anthocyanins and their metabolites. *J Agr Food Chem* 2006; 54: 1467-72.
- [5] Fitó M, Guxens M, Corella D, *et al.* Effect of a traditional Mediterranean diet on lipoprotein oxidation: A randomized controlled trial. *Arch Intern Med* 2007; 167: 1195-203.
- [6] D'Archivio M, Filesi C, Di Benedetto R, *et al.* Polyphenols, dietary sources and bioavailability. *Ann Ist Super Sanita* 2007; 43: 348-61.
- [7] Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* 2005; 81: 230S-42.

- [8] Scalbert A and Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr* 200; 130: 2073S-85.
- [9] Singh M, Arseneault M, Sanderson T, Murthy V, Ramassamy C. Challenges for research on polyphenols from foods in Alzheimer's disease: bioavailability, metabolism, and cellular and molecular mechanisms. *J Agr Food Chem* 2008; 56: 4855-73.
- [10] Corona G, Tzounis X, Dessl MA, *et al.* The fate of olive oil polyphenols in the gastrointestinal tract: Implications of gastric and colonic microflora-dependent biotransformation. *Free Radic Res* 2006; 40: 647-58.
- [11] Apak R, Güçlü K, Demirata B, *et al.* Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 2007; 12: 1496-547.
- [12] Zhang H. Structure-activity relationships and rational design strategies for radical scavenging antioxidants. *Curr Computer-aided Drug Design* 2005; 1: 257-73
- [13] Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Biol Med* 1995; 22, 375-83.
- [14] Akira M, Hitoshi A, Junji T. Multitargeted cancer prevention by quercetin. *Cancer Lett* 2008; 269, 315-25.
- [15] Padhye S, Ahmad A, Oswal N, *et al.* Fluorinated 2'-hydroxychalcones as garcinol analogs with enhanced antioxidant and anticancer activities. *Bioorg Med Chem Lett* 2010; 20:5818-21.
- [16] Ferrali M, Signorini C, Caciotti B, *et al.* Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity. *FEBS Lett* 1997; 416:123-29.
- [17] Quesada IM, Bustos M, Blay M, *et al.* Dietary catechins and pro-cyanidins modulate zinc homeostasis in human HepG2 cells. *J Nutr Biochem* 2011; 22: 153-63.
- [18] Mladěnka P, Zatloukalová L, Filipický T, Hrdina R. Cardiovascular effects of flavonoids are not caused only by direct antioxidant activity. *Free Radic Biol Med* 2010; 49: 963-75.
- [19] Sugahara M, Nakanishi J, Katsuta Y. Kaempferol enhanced the intracellular thioredoxin system in normal cultured Human Keratinocytes. *Biosci Biotech Biochem* 2010; 74: 1701-3.
- [20] Prasad S, Phromnoi K, Yadav V R, Chaturvedi MM, Aggarwal BB. Targeting inflammatory pathways by flavonoids for prevention and treatment of cancer. *Planta Med* 2010; 76: 1044-63.
- [21] Pierini R, Gee JM, Belshaw NJ, Johnson IT. Flavonoids and intestinal cancers. *Br J Nutr* 2008; 99: ES53-9.
- [22] Ong CS, Zhou J, Ong CN, Shen HM. Luteolin induces G1 arrest in human nasopharyngeal carcinoma cells via the Akt-GSK3 β -Cyclin D1 pathway. *Cancer Lett.* 2010; 298: 167-75.
- [23] Zhong Y, Krisanapun C, Lee SH, *et al.* Molecular targets of apigenin in colorectal cancer cells: involvement of p21, NAG-1 and p53. *Eur J Cancer* 2010; 46: 3365-74.
- [24] Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; 100: 57-70.
- [25] Manson MM, Linseisen J, Rohrmann S, *et al.* Bioactive components in foods. Wiley 2007
- [26] Duthie SJ, Collins AR, Duthie GG, Dobson VL. Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidised pyrimidines) in human lymphocytes. *Mutat Res* 1997; 393: 223-31.
- [27] Wilms LC, Hollman PCH, Boots AW, Kleinjans JCS. Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. *Mutat Res* 2005; 582: 155-62.
- [28] Hsieh TC, Wu JM. Targeting CWR22Rv1 prostate cancer cell proliferation and gene expression by combinations of the phytochemicals EGCG, genistein and quercetin. *Anticancer Res* 2009; 29: 4025-32.
- [29] She QB, Huang C, Zhang Y, Dong Z. Involvement of c-jun NH₂-terminal kinases in resveratrol-induced activation of p53 and apoptosis. *Mol Carcinog* 2002; 33: 244-50.
- [30] Hsieh TC, Wu JM. Suppression of cell proliferation and gene expression by combinatorial synergy of EGCG, resveratrol and γ -tocotrienol in estrogen receptor-positive MCF-7 breast cancer cells. *Int J Oncol* 2008; 33: 851-9.
- [31] Leung HWC, Lin CJ, Hour MJ, *et al.* Kaempferol induces apoptosis in human lung non-small carcinoma cells accompanied by an induction of antioxidant enzymes. *Food Chem Toxicol* 2007; 45: 2005-13.
- [32] Syed DN, Afaq F, Mukhtar H. Pomegranate derived products for cancer chemoprevention. *Semin Cancer Biol.* 2007; 17: 377-85.
- [33] Chen J, Ye ZQ, Koo M. Growth inhibition and cell cycle arrest effects of epigallocatechin gallate in the NBT-II bladder tumour cell line. *BJU Int* 2004; 93: 1082-6.
- [34] Cai H, Hudson EA, Mann P, *et al.* Growth-inhibitory and cell cycle-arresting properties of the rice bran constituent tricin in human-derived breast cancer cells *in vitro* and in nude mice *in vivo*. *Br J Cancer* 2004; 91: 1364-71.
- [35] Mann GE, Bonacasa B, Ishii T, Siow RC. Targeting the redox sensitive Nrf2-Keap1 defense pathway in cardiovascular disease: protection afforded by dietary isoflavones. *Curr Opin Pharmacol* 2009; 9:139-145
- [36] Hormann V, Kumi-Diaka J, Durity M, Rathinavelu A. Anticancer activities of genistein- topotecan combination in prostate cancer cells. *J Cell Mol Med* 2012, 16: 2631-2636.
- [37] Zimniak P. Detoxification reactions: Relevance to aging. *Ageing Res Rev* 2008; 7: 281-300.
- [38] Klaassen CD. Casarett and Doull's toxicology: the basic science of poisons. McGraw-Hill Medical, New York, 2001.
- [39] Williams RT. Detoxification mechanisms. Wiley, New York, 1971.
- [40] Parkinson A, Ogilvie BW. Biotransformation of xenobiotics. McGraw-Hill Medical, New York, 2008.
- [41] Russo GL. Ins and outs of dietary phytochemicals in cancer chemoprevention. *Biochem Pharmacol* 2007; 74: 533-44.
- [42] Dinkova-Kostova AT, Liby KT, Stephenson KK, *et al.* Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc Natl Acad Sci USA* 2005; 102: 4584-9.
- [43] Myzak MC, Dashwood RH. Chemoprotection by sulforaphane: keep one eye beyond Keap1. *Cancer Lett* 2006; 233: 208-18.
- [44] Sykiotis GP, Bohmann D. Stress-activated cap'n'collar transcription factors in aging and human disease. *Sci Signal* 2010; 3: re3
- [45] Huang CF, Wang YC, Tsao DA, *et al.* Antagonism between members of the CNC-bZIP family and the immediate-early protein IE2 of human cytomegalovirus. *J Biol Chem* 2000; 275: 12313-20.
- [46] Chan JY, Han XL, Kan YW. Cloning of Nrf1, an NF-E2-related transcription factor, by genetic selection in yeast. *Proc Natl Acad Sci USA* 1993; 90: 11371-5.
- [47] Luna L, Johnsen O, Skartlien AH, *et al.* Molecular cloning of a putative novel human bZIP transcription factor on chromosome 17q22. *Genomics* 1994; 22: 553-62.
- [48] Caterina JJ, Donze D, Sun CW, Ciavatta DJ, Townes TM. Cloning and functional characterization of LCR-F1: a bZIP transcription factor that activates erythroid-specific, human globin gene expression. *Nucleic Acids Res* 1994; 22: 2383-91.
- [49] Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP-1 repeat of the β -globin locus control region. *Proc Natl Acad Sci USA* 1994; 91: 9926-30.
- [50] Kobayashi A, Ito E, Toki T, *et al.* Molecular cloning and functional characterization of a new cap'n' collar family transcription factor Nrf3. *J Biol Chem* 1999; 274: 6443-52.
- [51] Oyake T, Itoh K, Motohashi H, *et al.* Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site. *Mol Cell Biol* 1996; 16: 6083-95.
- [52] Chan JY, Kwong M, Lu R, *et al.* Targeted disruption of the ubiquitous CNC-bZIP transcription factor, Nrf-1, results in anemia and embryonic lethality in mice. *EMBO J* 1998; 17: 1779-87.
- [53] Bowerman B, Draper BW, Mello CC, Priess JR. The maternal gene *Skn-1* encodes a protein that is distributed unequally in early *C. elegans* embryos. *Cell* 1993; 74: 443-52.
- [54] Blackwell TK, Bowerman B, Priess JR, Weintraub H. Formation of a monomeric DNA binding domain by *Skn-1* bZIP and homeodomain elements. *Science* 1994; 266: 621-8.
- [55] Rupert PB, Daughdrill GW, Bowerman B, Matthews BW. A new DNA-binding motif in the *Skn-1* binding domain-DNA complex. *Nat Struct Biol* 1998; 5: 484-91.
- [56] Motohashi H, Shavit JA, Igarashi K, Yamamoto M, Engel JD. The world according to Maf. *Nucleic Acids Res* 1997; 25: 2953-9.
- [57] Hayes JD, McMahon M, Chowdhry S, Dinkova-Kostova AT. Cancer chemoprevention mechanisms mediated through the Keap1-Nrf2 pathway. *Antioxid Redox Signal* 2010; 13: 1713-48.

- [58] Shen G, Kong AN. Nrf2 plays an important role in coordinated regulation of Phase II drug metabolism enzymes and Phase III drug transporters. *Biopharm Drug Dispos* 2009; 30: 345-55.
- [59] Rushmore TH, Morton MR, Pickett CB. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J Biol Chem* 1991; 266: 11632-9.
- [60] Wasserman WW, Fahl WE. Functional antioxidant responsive elements. *Proc Natl Acad Sci USA* 1997; 94: 5361-6.
- [61] Nioi P, McMahon M, Itoh K, Yamamoto M, Hayes JD. Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H:quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. *Biochem J* 2003; 374: 337-48.
- [62] Favreau LV, Pickett CB. Transcriptional regulation of the rat NAD(P)H:quinone reductase gene: identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J Biol Chem* 1991; 266: 4556-61.
- [63] Jaiswal AK. Human NAD(P)H:quinone oxidoreductase (NQO1) gene structure and induction by dioxin. *Biochem* 1991; 30: 10647-53.
- [64] Moinova HR, Mulcahy RT. An electrophile responsive element (EpRE) regulates β -naphthoflavone induction of the human γ -glutamylcysteine synthetase regulatory subunit Gene. *J Biol Chem* 1998; 273:14683-9.
- [65] Wild AC, Gipp JJ, Mulcahy T. Overlapping antioxidant response element and PMA response element sequences mediate basal and β -naphthoflavone-induced expression of the human gamma-glutamylcysteine synthetase catalytic subunit gene. *Biochem J* 1998; 332: 373-81.
- [66] Mulcahy RT, Wartman MA, Bailey HH, Gipp JJ. Constitutive and β -naphthoflavone-induced expression of the human γ -glutamylcysteine synthetase heavy subunit gene is regulated by a distal antioxidant response element/TRE sequence. *J Biol Chem* 1997; 272: 7445-54.
- [67] Inamdar NM, Ahn YI, Alam J. The heme-responsive element of the mouse heme oxygenase-1 gene is an extended AP-1 binding site that resembles the recognition sequences for Maf and NF-E2 transcription factors. *Biochem Biophys Res Commun* 1996; 221: 570-6.
- [68] Nguyen T, Sherratt PJ, Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu Rev Pharmacol Toxicol* 2003; 43: 233-60.
- [69] Miller JL, Walsh CE, Ney PA, Samulski RJ, Nienhuis AW. Single-copy transduction and expression of human gamma-globin in K562 erythroleukemia cells using recombinant adeno-associated virus vectors: the effect of mutations in NF-E2 and GATA-1 binding motifs within the hypersensitivity site-2 enhancer. *Blood* 1993; 82: 1900-6.
- [70] Motohashi H, O'Connor T, Katsuoka F, Engel JD, Yamamoto M. Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors. *Gene* 2002; 294: 1-12.
- [71] Itoh K, Chiba T, Takahashi S, *et al.* An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 1997; 236: 313-22.
- [72] Mignotte V, Wall L, deBoer E, Grosveld F, Romeo PH. Two tissue-specific factors bind the erythroid promoter of the human porphobilinogen deaminase gene. *Nucleic Acids Res* 1989; 17: 37-54.
- [73] Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* 2003; 23: 7448-59.
- [74] Carroll AS, Gilbert DE, Liu X, *et al.* SKN-1 domain folding and basic region monomer stabilization upon DNA binding. *Genes Dev* 1997; 11: 2227-38.
- [75] Walker AK, See R, Batchelder C, *et al.* A conserved transcription motif suggesting functional parallels between *Caenorhabditis elegans* SKN-1 and Cap'n'Collar-related basic leucine zipper proteins. *J Biol Chem* 2000; 275: 22166-71.
- [76] Zhang, Y. Molecular and cellular control of the Nrf1 transcription factor: An integral membrane glycoprotein. *Vdm Verlag Dr. Müller Publishing House Germany*, the first edition, pp1-264, 2009.
- [77] Wang Y, Schwedes JF, Parks D, Mann K, Tegtmeyer P. Interaction of p53 with its consensus DNA-binding site. *Mol Cell Biol* 1995; 15: 2157-65.
- [78] Zabel U, Schreck R, Baeuerle, PA. DNA binding of purified transcription factor NF- κ B: affinity, specificity, Zn²⁺ dependence, and differential half-site recognition. *J Biol Chem* 1991; 266: 252-60.
- [79] Yang H, Magilnick N, Lee C, *et al.* Nrf1 and Nrf2 regulate rat glutamate-cysteine ligase catalytic subunit transcription indirectly via NF- κ B and AP-1. *Mol Cell Biol* 2005; 25: 5933-46.
- [80] Yang H, Magilnick N, Ou X, Lu SC. Tumour necrosis factor alpha induces co-ordinated activation of rat GSH synthetic enzymes via nuclear factor κ B and activator protein-1. *Biochem J* 2005; 391: 399-408.
- [81] Prester T, Talalay P. Electrophile and antioxidant regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci USA* 1995; 92: 8965-9.
- [82] Mathers J, Fraser JA, McMahon M, *et al.* Antioxidant and cytoprotective responses to redox stress. *Biochem Soc Symp*, 2004; 71: 157-76.
- [83] Nioi P, Hayes JD. Contribution of NAD(P)H:quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. *Mutat Res* 2004; 555: 149-71.
- [84] Nerland DE. The antioxidant/electrophile response element motif. *Drug Metab Rev* 2007; 39: 235-48.
- [85] Venugopal R, Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 1998; 17: 3145-56.
- [86] Novotny V, Prieschl EE, Csonga R, Fabjani G, Baumruker T. Nrf1 in a complex with fosB, c-jun, junD and ATF2 forms the AP-1 component at the TNF alpha promoter in stimulated mast cells. *Nucleic Acids Res* 1998; 26: 5480-5.
- [87] Johnsen O, Murphy P, Prydz H, Kolsto AB. Interaction of the CNC-bZIP factor TCF11/LCR-F1/Nrf1 with MafG: binding-site selection and regulation of transcription. *Nucleic Acids Res* 1998; 26: 512-20.
- [88] Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc Natl Acad Sci USA* 1996; 93: 14960-5.
- [89] Johnsen O, Skammelsrud N, Luna L, *et al.* Small Maf proteins interact with the human transcription factor TCF11/Nrf1/LCR-F1. *Nucleic Acids Res* 1996; 24: 4289-97.
- [90] He CH, Gong P, Hu B, *et al.* Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. *J Biol Chem* 2001; 276: 20858-65.
- [91] Vinson C, Myakishev M, Acharya A, *et al.* Classification of human bZIP proteins based on dimerization properties. *Mol Cell Biol* 2002; 22: 6321-35.
- [92] Newman JR, Keating AE. Comprehensive identification of human bZIP interactions with coiled-coil arrays. *Science* 2003; 300: 2097-101.
- [93] McMahon M, Itoh K, Yamamoto M, *et al.* The cap 'n' collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related Factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res* 2001; 61: 3299-307.
- [94] Chan K, Lu R, Chang JC, Kan YW. NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. *Proc Natl Acad Sci USA* 1996; 93: 13943-8.
- [95] Farmer SC, Sun CW, Winnier GE, Hogan BL, Townes TM. The bZIP transcription factor LCR-F1 is essential for mesoderm formation in mouse development. *Genes Dev* 1997; 11: 786-798.
- [96] Xu Z, Chen L, Leung L, *et al.* Liver-specific inactivation of the Nrf1 gene in adult mouse leads to nonalcoholic steatohepatitis and hepatic neoplasia. *Proc Natl Acad Sci USA* 2005; 102: 4120-5.
- [97] Ohtsuiji M, Katsuoka F, Kobayashi A, *et al.* Nrf1 and Nrf2 play distinct roles in activation of antioxidant response element-dependent genes. *J Biol Chem* 2008; 283: 33554-62.
- [98] Kim J, Xing W, Wergedal J, Chan JY, Mohan S. Targeted disruption of nuclear factor erythroid-derived 2-like 1 in osteoblasts re-

- duces bone size and bone formation in mice. *Physiol Genomics* 2010; 40: 100-10.
- [99] Kobayashi A, Tsukide T, Miyasaka T, *et al.* Central nervous system-specific deletion of transcription factor Nrf1 causes progressive motor neuronal dysfunction. *Genes Cells* 2011; 16: 692-703.
- [100] Lee CS, Lee C, Hu T, *et al.* Loss of nuclear factor E2-related factor 1 in the brain leads to dysregulation of proteasome gene expression and neurodegeneration. *Proc Natl Acad Sci USA* 2011; 108: 8408-13.
- [101] Yu R, Lei W, Mandelkar S, *et al.* Role of a mitogen-activated protein kinase pathway in the induction of phase II detoxifying enzymes by chemicals. *J Biol Chem* 1999; 274: 27545-52.
- [102] Huang HC, Nguyen T, Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. *J Biol Chem* 2002; 277: 42769-74.
- [103] Lee JM, Hanson JM, Chu WA, Johnson JA. Phosphatidylinositol 3-kinase, not extracellular signal-regulated kinase, regulates activation of the antioxidant-responsive element in IMR-32 human neuroblastoma cells. *J Biol Chem* 2001; 276: 20011-6.
- [104] Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 2007; 47: 89-116.
- [105] Itoh K, Wakabayashi N, Katoh Y, *et al.* Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Gene Dev* 1999; 13: 76-86.
- [106] Niou P, Nguyen T, Sherratt PJ, Pickett CB. The carboxy-terminal Neh3 domain of Nrf2 is required for transcriptional activation. *Mol Cell Biol* 2005; 25: 10895-906.
- [107] Katoh Y, Itoh K, Yoshida E, *et al.* Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. *Gene Cell* 2001; 6: 857-68.
- [108] McMahon M, Thomas N, Itoh K, Yamamoto M, Hayes JD. Redox-regulated turnover of Nrf2 is determined by at least two separate protein domains, the redox-sensitive Neh2 degron and the redox-insensitive Neh6 degron. *J Biol Chem* 2004; 279: 31556-67.
- [109] Thimmulappa RK, Mai KH, Srisuma S, *et al.* Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res* 2002; 62: 5196-203.
- [110] Lee JM, Calkins MJ, Chan K, Kan YW, Johnson JA. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *J Biol Chem* 2003; 278: 12029-38.
- [111] Kwak MK, Wakabayashi N, Itoh K, *et al.* Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 Pathway. *J Biol Chem* 2003; 278: 8135-45.
- [112] McMillian M, Nie A, Parker JB, *et al.* Drug-induced oxidative stress in rat liver from a toxicogenomics perspective. *Toxicol Appl Pharmacol* 2005; 2007(2suppl): 171-8.
- [113] Hayes JD, McMahon M. NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. *Trends Biochem Sci* 2009; 34: 176-88.
- [114] MacLeod AK, McMahon M, Plummer SM, *et al.* Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. *Carcinogenesis* 2009; 30: 1571-80.
- [115] Klaassen CD. *Toxicology: the basic science of poisons*. McGraw-Hill, 2008.
- [116] Cho HY, Jedlicka AE, Reddy SPM, *et al.* Linkage analysis of susceptibility to hyperoxia. Nrf2 is a candidate gene. *Am J Respir Cell Mol Biol* 2002; 26: 42-51.
- [117] Cho HY, Jedlicka AE, Reddy SPM, *et al.* Role of Nrf2 in protection against hyperoxic lung injury in mice. *Am J Respir Cell Mol Biol* 2002; 26: 175-82.
- [118] Rangasamy T, Cho CY, Thimmulappa RK, *et al.* Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J Clin Invest* 2004; 114: 1248-59.
- [119] Aoki Y, Sato H, Nishimura N, *et al.* Accelerated DNA adduct formation in the lung of the Nrf2 knockout mouse exposed to diesel exhaust. *Toxicol Appl Pharmacol* 2001; 173: 154-60.
- [120] Ramos-Gomez M, Dolan PM, Itoh K, Yamamoto M, Kensler TW. Interactive effects of Nrf2 genotype and oltipraz on benzo[a]pyrene-DNA adducts and tumor yield in mice. *Carcinogenesis* 2003; 24: 461-7.
- [121] Aoki Y, Hashimoto AH, Amanuma K, *et al.* Enhanced spontaneous and benzo(a)pyrene-induced mutations in the lung of Nrf2-deficient *gpt* delta mice. *Cancer Res* 2007; 67: 5643-8.
- [122] Shih AY, Imbeault S, Barakauskas V, *et al.* Induction of the Nrf2-driven antioxidant response confers neuroprotection during mitochondrial stress *in vivo*. *J Biol Chem* 2005; 280: 22925-36.
- [123] Chen PC, Vargas MR, Pani AK, *et al.* Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proc Natl Acad Sci USA* 2009; 106: 2933-8.
- [124] Cano M, Thimmalappa R, Fujihara M, *et al.* Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and Age-related Macular Degeneration. *Vision Res* 2010; 50: 652-64.
- [125] Li J, Ichikawa T, Janicki JS, Cui T. Targeting the Nrf2 pathway against cardiovascular disease. *Expert Opin Ther Targets* 2009; 13: 785-94.
- [126] Dhakshinamoorthy S, Jaiswal, A. K. Functional characterization and role of Nrf2 in antioxidant response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. *Oncogene* 2001; 20: 3906-17.
- [127] Nguyen T, Sherratt PJ, Huang HC, Yang CS, Pickett CB. Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. *J Biol Chem* 2003; 278: 4536-41.
- [128] Stewart D, Killeen E, Naquin R, Alam S, Alam J. Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium. *J Biol Chem* 2003; 278: 2396-2402.
- [129] McMahon M, Itoh K, Yamamoto M, Hayes JD. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem* 2003; 278: 21592-600.
- [130] Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol Cell Biol* 2003; 23: 8137-51.
- [131] Kobayashi A, Kang MI, Okawa H, *et al.* Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol* 2004; 24: 7130-9.
- [132] Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol Cell Biol* 2004; 24: 10941-53.
- [133] Adams J, Kelso R, Cooley L. The Kelch repeat superfamily of proteins: propellers of cell function. *Trends Cell Biol* 2000; 10: 17-24.
- [134] Zipper LM, Mulcahy RT. The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm. *J Biol Chem* 2002; 277: 36544-52.
- [135] Li X, Zhang D, Hannink M, Beamer LJ. Crystal structure of the Kelch domain of human Keap1. *J Biol Chem* 2004; 279: 54750-8.
- [136] Dinkova-Kostova AT, Holtzclaw WD, Wakabayashi N. Keap1, the sensor for electrophiles and oxidants that regulates the phase 2 response, is a zinc metalloprotein. *Biochemistry* 2005; 44: 6889-99.
- [137] Furukawa M, Xiong Y. BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. *Mol Cell Biol* 2005; 25: 162-71.
- [138] Devling TW, Lindsay CD, McLellan LI, McMahon M, Hayes JD. Utility of siRNA against Keap1 as a strategy to stimulate a cancer chemopreventive phenotype. *Proc Natl Acad Sci USA* 2005; 102: 7280-5A.
- [139] Wakabayashi N, Itoh K, Wakabayashi J, *et al.* Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. *Nat Genet* 2003; 35: 238-45.
- [140] Kobayashi A, Ohta T, Yamamoto M. Unique function of the Nrf2-Keap1 pathway in the inducible expression of antioxidant and detoxifying enzymes. *Methods Enzymol* 2004; 378: 273-86.
- [141] Komatsu M, Kurokawa H, Waguri S, *et al.* The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* 2010; 12: 213-23.
- [142] Dinkova-Kostova AT, Holtzclaw WD, Cole RN, *et al.* Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating

- induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci USA* 2002; 99: 11908-13.
- [143] Egger AL, Liu G, Pezzuto JM, van Breemen RB, Mesecar AD. Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. *Proc Natl Acad Sci USA* 2005; 102: 10070-5.
- [144] Hong F, Freeman ML, Liebler DC. Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. *Chem Res Toxicol* 2005; 18: 1917-26.
- [145] Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, *et al.* Protection against electrophile and oxidant stress by induction of the phase 2 response: Fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci USA* 2004; 101: 2040-5.
- [146] Dinkova-Kostova AT, Massiah MA, Bozak RE, Hicks RJ, Talalay P. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. *Proc Natl Acad Sci USA* 2001; 98: 3404-9.
- [147] Nioi P, Nguyen T. A mutation of Keap1 found in breast cancer impairs its ability to repress Nrf2 activity. *Biochem Biophys Res Commun* 2007; 362: 816-21.
- [148] McMahon M, Lamont DJ, Beattie KA, Hayes JD. Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. *Proc Natl Acad Sci USA* 2010; 107: 18838-43.
- [149] McMahon M, Thomas N, Itoh K, Yamamoto M, Hayes JD. Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism. *J Biol Chem* 2006; 281: 24756-68.
- [150] Tong KI, Kobayashi A, Katsuoka F, Yamamoto M. Two-site substrate recognition model for the Keap1-Nrf2 system: a hinge and latch mechanism. *Biol Chem* 2006; 387: 1311-20.
- [151] Ogura T, Tong KI, Mio K, *et al.* Keap1 is a forked-stem dimer structure with two large spheres enclosing the intervening, double glycine repeat, and C-terminal domains. *Proc Natl Acad Sci USA* 2010; 107: 2842-7.
- [152] Padmanabhan B, Tong KI, Ohta T, *et al.* Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. *Mol Cell* 2006; 21: 689-700.
- [153] Tong KI, Katoh Y, Kusunoki H, *et al.* Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. *Mol Cell Biol* 2006; 26: 2887-900.
- [154] Lo SC, Li X, Henzl MT, Beamer LJ, Hannink M. Structure of the Keap1:Nrf2 interface provides mechanistic insight into Nrf2 signaling. *EMBO J* 2006; 25: 3605-17.
- [155] Kern, J. T., Hannink, M. and Hess, J. F. (2007) Disruption of the Keap1-containing ubiquitination complex as an antioxidant therapy. *Curr Top Med Chem* 7, 972-978
- [156] Hong F, Sekhar KR, Freeman ML, Liebler DC. Specific patterns of electrophile adduction trigger Keap1 ubiquitination and Nrf2 activation. *J Biol Chem* 2005; 280: 31768-75.
- [157] Zhang DD, Lo SC, Sun Z, *et al.* Ubiquitination of Keap1, a BTB-Kelch substrate adaptor protein for Cul3, targets Keap1 for degradation by a proteasome-independent pathway. *J Biol Chem* 2005; 280: 30091-9.
- [158] Rojo AI, Medina-Campos ON, Rada P, *et al.* Signaling pathways activated by the phytochemical nordihydroguaiaretic acid contribute to a Keap1-independent regulation of Nrf2 stability: Role of glycogen synthase kinase-3. *Free Radic Biol Med* 2012; 52: 473-87.
- [159] Rada P, Rojo AI, Chowdhry S, *et al.* SCF/ β -TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. *Mol Cell Biol* 2011; 31: 1121-33.
- [160] Cullinan SB, Zhang D, Hannink M, *et al.* Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol Cell Biol* 2003; 23: 7198-209.
- [161] Zhang Y, Crouch DH, Yamamoto M, Hayes JD. Negative regulation of the Nrf1 transcription factor by its N-terminal domain is independent of Keap1: Nrf1, but not Nrf2, is targeted to the endoplasmic reticulum. *Biochem J* 2006; 399: 373-85.
- [162] Xu C, Yuan X, Pan Z, *et al.* Mechanism of action of isothiocyanates: the induction of ARE-regulated genes is associated with activation of ERK and JNK and the phosphorylation and nuclear translocation of Nrf2. *Mol Cancer Ther* 2006; 5: 1918-26.
- [163] Sun Z, Huang Z, Zhang DD. Phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. *PLoS One* 2009; 4: e6588.
- [164] Keum YS, Yu S, Chang PJJ, *et al.* Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. *Cancer Res* 2006; 66: 8804-13.
- [165] Salazar M, Rojo AI, Velasco D, *et al.* Glycogen synthase kinase-3 β inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. *J Biol Chem* 2006; 281: 14841-51.
- [166] Chen W, Sun Z, Wang XJ, *et al.* Direct Interaction between Nrf2 and p21Cip1/WAF1 upregulates the Nrf2-mediated antioxidant response. *Mol Cell* 2009; 34: 663-73.
- [167] Kwak MK, Itoh K, Yamamoto M, Kensler TW. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. *Mol Cell Biol* 2002; 22: 2883-92.
- [168] Miao W, Hu L, Scrivens PJ, Batist G. Transcriptional regulation of NF-E2 p45-related factor (Nrf2) expression by the aryl hydrocarbon receptor-xenobiotic response element signaling pathway. *J Biol Chem* 2005; 280: 20340-8.
- [169] Marzec JM, Christie JD, Reddy SP, *et al.* Functional polymorphisms in the transcription factor NRF2 in humans increase the risk of acute lung injury. *FASEB J* 2007; 21: 2237-46.
- [170] Lee OH, Jain AK, Papusha V, Jaiswal AK. An auto-regulatory loop between stress sensors INrf2 and Nrf2 controls their cellular abundance. *J Biol Chem* 2007; 282: 36412-20.
- [171] Zhao R, Hou Y, Xue P, *et al.* Long isoforms of NRF1 contribute to arsenic-induced antioxidant response in human keratinocytes. *Environ Health Perspect* 2011; 119: 56-62.
- [172] Ikeda H, Nishi S, Sakai M. Transcription factor Nrf2/MafK regulates rat placental glutathione S-transferase gene during hepatocarcinogenesis. *Biochem. J.* 2004; 380: 515-21.
- [173] Singh A, Misra V, Thimmulappa RK, *et al.* Dysfunctional Keap1-Nrf2 interaction in non-small-cell lung cancer. *PLoS Med* 2006; 3: e420.
- [174] Ohta T, Iijima K, Miyamoto M, *et al.* Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. *Cancer Res* 2008; 68: 1303-9.
- [175] Wang R, An J, Ji F, *et al.* Hypermethylation of the Keap1 gene in human lung cancer cell lines and lung cancer tissues. *Biochem Biophys Res Commun* 2008; 373: 151-4.
- [176] Wang XJ, Sun Z, Villeneuve NE, *et al.* Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. *Carcinogenesis* 2008; 29: 1235-43.
- [177] DeNicola GM, Karreth FA, Humpton TJ, *et al.* Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 2011; 475: 106-9.
- [178] Kensler TW and Wakabayashi N. Nrf2: friend or foe for chemoprevention? *Carcinogenesis* 2010; 31: 90-9.
- [179] Shibata T, Ohta T, Tong KI, *et al.* Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. *Proc Natl Acad Sci USA* 2008; 105: 13568-73.
- [180] Beischlag TV, Morales JL, Hollingshead BD, Perdev GH. The aryl hydrocarbon receptor complex and the control of gene expression. *Crit Rev Eukaryot Gene Expr* 2008; 18: 207-50.
- [181] Sun YV, Boverhof DR, Burgoon LD, Fielden MR, Zacharewski TR. Comparative analysis of dioxin response elements in human, mouse and rat genomic sequences. *Nucl. Acids Res* 2004; 32: 4512-23.
- [182] Estabrook RW. The remarkable P450s: a historical overview of these versatile hemoprotein catalysts. *FASEB J* 1996; 10: 202-4.
- [183] Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer* 2006; 6: 947-60.
- [184] Cribb AE, Peyrou M, Muruganandan S, Schneider L. The endoplasmic reticulum in xenobiotic toxicity. *Drug Metab Rev* 2005; 37, 405-42.
- [185] Conney AH. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res* 1982; 42: 4875-917.
- [186] Weisburger EK. Mechanisms of chemical carcinogenesis. *Ann Rev Pharmacol Toxicol* 1978; 18: 395-415

- [187] Hankinson O. The aryl hydrocarbon receptor complex. *Ann Rev Pharmacol Toxicol* 1995; 35: 307-40.
- [188] Kawajiri K, Fujii-Kuriyama Y. Cytochrome P450 gene regulation and physiological functions mediated by the aryl hydrocarbon receptor. *Arch Biochem Biophys* 2007; 464: 207-12.
- [189] Jones PB, Durrin LK, Galeazzi DR, Whitlock JP. Control of cytochrome P1-450 gene expression: analysis of a dioxin-responsive enhancer system. *Proc Natl Acad Sci USA* 1986; 83: 2802-6.
- [190] Denison MS, Fisher JM, Whitlock JP. Inducible, receptor-dependent protein-DNA interactions at a dioxin-responsive transcriptional enhancer. *Proc Natl Acad Sci USA* 1988; 85: 2528-32.
- [191] Whitlock JP. Introduction of cytochrome p450-1A1. *Ann Rev Pharmacol Toxicol* 1999; 39: 103-25.
- [192] Shen ES, Whitlock JP. Protein-DNA interactions at a dioxin-responsive enhancer: Mutational analysis of the DNA-binding site for the liganded Ah receptor. *J Biol Chem* 1992; 267: 6815-9.
- [193] Gu YZ, Hogenesch JB, Bradfield CA. The PAS Superfamily: Sensors of Environmental and Developmental Signals. *Ann Rev Pharmacol Toxicol* 2003; 40: 519-61.
- [194] Burbach KM, Poland A, Bradfield CA. Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proc Natl Acad Sci USA* 1992; 89: 8185-9.
- [195] Ema M, Sogawa K, Watanabe N, *et al.* cDNA cloning and structure of mouse putative Ah receptor. *Biochem Biophys Res Commun* 1992; 184: 246-53.
- [196] Dolwick KM, Schmidt JV, Carver LA, Swanson HI, Bradfield CA. Cloning and expression of a human Ah receptor cDNA. *Mol Pharmacol* 1993; 44: 911-7.
- [197] Carver LA, Hogenesch JB, Bradfield CA. Tissue specific expression of the rat Ah-receptor and ARNT mRNAs. *Nucl. Acids Res.* 1994; 22: 3038-44.
- [198] Hahn ME. Aryl hydrocarbon receptors: diversity and evolution. *Chem Biol Interact* 2002; 141: 131-60.
- [199] Jain S, Dolwick KM, Schmidt JV, Bradfield CA. Potent transactivation domains of the Ah receptor and the Ah receptor nuclear translocator map to their carboxyl termini. *J Biol Chem* 1994; 269: 31518-24.
- [200] Korkalainen M, Tuomisto J, Pohjanvirta R. Restructured Transactivation Domain in Hamster AH Receptor. *Biochem Biophys Res Commun* 2000; 273: 272-81.
- [201] Rowlands JC, McEwan IJ, Gustafsson JA. Trans-activation by the human aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator proteins: direct interactions with basal transcription factors. *Mol Pharmacol* 1996; 50: 538-48.
- [202] Fujii-Kuriyama Y, Kawajiri K. Molecular mechanisms of the physiological functions of the aryl hydrocarbon (dioxin) receptor, a multifunctional regulator that senses and responds to environmental stimuli. *Proc Jpn Acad Ser B Phys Biol Sci* 2010; 86: 40-53.
- [203] Puga A, Ma C, Marlowe JL. The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. *Biochem Pharmacol* 2009; 77: 713-22.
- [204] Davarinos NA, Pollenz RS. Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytoplasmic proteasome following nuclear export. *J Biol Chem* 1999; 274: 28708-15.
- [205] Pollenz RS, Dougherty EJ. Redefining the role of the endogenous XAP2 and C-terminal HSP70-interacting protein on the endogenous Ah receptors expressed in mouse and rat cell lines. *J Biol Chem* 2005; 280: 33346-56.
- [206] Lees MJ, Peet DJ, Whitelaw ML. Defining the role for Xap2 in stabilization of the dioxin receptor. *J Biol Chem* 2003; 278: 35878-88.
- [207] Ohtake F, Baba A, Takada I, *et al.* Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. *Nature* 2007; 446: 562-6.
- [208] Mimura J, Ema M, Sogawa K, Fujii-Kuriyama Y. Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. *Gene Dev* 1999; 13: 20-5.
- [209] Oshima M, Mimura J, Yamamoto M, Fujii-Kuriyama Y. Molecular mechanism of transcriptional repression of AhR repressor involving ANKRA2, HDAC4, and HDAC5. *Biochem Biophys Res Commun* 2007; 364: 276-82.
- [210] Baba T, Mimura J, Gradin K, *et al.* Structure and expression of the Ah receptor repressor gene. *J Biol Chem* 2001; 276: 33101-10.
- [211] Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptors by structurally diverse exogenous and endogenous chemicals. *Ann Rev Pharmacol Toxicol* 2003; 43: 309-34.
- [212] Wattenberg LW, Loub WD. Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. *Cancer Res* 1978; 38: 1410-3.
- [213] Bjeldanes F, Kim JY, Grose KR, Bartholomew JC, Bradfield CA. Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol *in vitro* and *in vivo*: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci USA* 1991; 88: 9543-7.
- [214] Ciolino HP, Daschner PJ, Wang TTY, Yeh GC. Effect of curcumin on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells. *Biochem Pharmacol* 1998; 56: 197-206.
- [215] Ciolino HP, Daschner PJ, Yeh GC. Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. *Biochem J* 1999; 340: 715-722.
- [216] Ciolino HP, Daschner PJ, Yeh GC. Resveratrol inhibits transcription of CYP1A1 *in vitro* by preventing activation of the aryl hydrocarbon receptor. *Cancer Res* 1998; 58: 5707-12.
- [217] Ma Q, Whitlock JP Jr. The aromatic hydrocarbon receptor modulates the Hepa 1c1c7 cell cycle and differentiated state independently of dioxin. *Mol Cell Biol* 1996; 16: 2144-50.
- [218] Wei C, Kolluri SK, Kiefer F, Götlicher M. Complementation of Ah receptor deficiency in hepatoma cells: negative feedback regulation and cell cycle control by the Ah receptor. *Exp Cell Res* 1996; 226: 154-63.
- [219] Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine *Ahr*-null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci USA* 1996; 93: 6731-6.
- [220] Lahvis GP, Lindell SL, Thomas RS, *et al.* Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. *Proc Natl Acad Sci USA* 2000; 97: 10442-7.
- [221] Nguyen LP, Bradfield CA. The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol* 2007; 21: 102-16.
- [222] Henklová P, Vrzala R, Ulrichová J, Dvořák Z. Role of mitogen-activated protein kinases in aryl hydrocarbon receptor signaling. *Chem Biol Interact* 2008; 172: 93-104.
- [223] Barouki R, Coumoul X, Fernandez-Salguero PM. The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. *FEBS Lett* 2007; 581: 3608-15.
- [224] Harper PA, Riddick DS, Okey AB. Regulating the regulator: Factors that control levels and activity of the aryl hydrocarbon receptor. *Biochem Pharmacol* 2006; 72: 267-79.
- [225] Singh SS, Hord NG, Perdew GH. Characterization of the activated form of the aryl hydrocarbon receptor in the nucleus of HeLa cells in the absence of exogenous ligand. *Arch Biochem Biophys* 1996; 329: 47-55.
- [226] Richter CA, Tillitt DE, Hannink M. Regulation of subcellular localization of the aryl hydrocarbon receptor (AhR). *Arch Biochem Biophys* 2001; 389: 207-17.
- [227] Allan LL, Sherr DH. Constitutive activation and environmental chemical induction of the aryl hydrocarbon receptor/transcription factor in activated human B lymphocytes. *Mol Pharmacol* 2005; 67: 1740-50.
- [228] Chang CY, Puga A. Constitutive activation of the aromatic hydrocarbon receptor. *Mol Cell Biol* 1998; 18: 525-35.
- [229] Fernandez-Salguero PM, Hilbert DM, Rudikoff S, Ward JM, Gonzalez FJ. Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. *Toxicol Appl Pharmacol* 1996; 140: 173-9.
- [230] Shimizu Y, Nakatsuru Y, Ichinose M, *et al.* Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* 2000; 97: 779-82.
- [231] Fernandez-Salguero PM, Ward JM, Sundberg JP, Gonzalez FJ. Lesions of aryl-hydrocarbon receptor-deficient mice. *Vet Pathol* 1997; 34: 605-14.
- [232] Lund AK, Goens MB, Nuñez BA, Walker MK. Characterizing the role of endothelin-1 in the progression of cardiac hypertrophy in aryl hydrocarbon receptor (AhR) null mice. *Toxicol Appl Pharmacol* 2006; 212: 127-35.

- [233] Abbott BD, Schmid JE, Pitt JA, *et al.* Adverse reproductive outcomes in the transgenic Ah receptor-deficient mouse. *Toxicol Appl Pharmacol* 1999; 155: 62-70.
- [234] Fernandez-Salguero P, Pineau T, Hilbert DM, *et al.* Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* 1995; 268: 722-6.
- [235] Dong WK, Lee G, Shafat AQ, *et al.* The RelA NF- κ B subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* 2000; 19: 5498-06
- [236] Andersson P, McGuire J, Rubio C, *et al.* A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors. *Proc Natl Acad Sci USA* 2002; 99: 9990-5.
- [237] Moennikes O, Loeppen S, Buchmann A, *et al.* A Constitutively active dioxin/aryl hydrocarbon receptor promotes hepatocarcinogenesis in mice. *Cancer Res* 2004; 64: 4707-10.
- [238] Kolluri SK, Weiss C, Koff A, Göttlicher M. p27Kip1 induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells. *Gene Dev* 1999; 13: 1742-53.
- [239] Puga A, Barnes SJ, Dalton TP, *et al.* Aromatic hydrocarbon receptor interaction with the retinoblastoma protein potentiates repression of E2F-dependent transcription and cell cycle arrest. *J Biol Chem* 2000; 275: 2943-50.
- [240] Marlowe JL, Knudsen ES, Schwemberger S, Puga A. The aryl hydrocarbon receptor displaces p300 from E2F-dependent promoters and represses S phase-specific gene expression. *J Biol Chem* 2004; 279: 29013-22.
- [241] Chan WK, Yao G, Gu YZ, Bradfield CA. Cross-talk between the aryl hydrocarbon receptor and hypoxia inducible factor signaling pathways. *J Biol Chem* 1999; 274: 12115-23.
- [242] Hayes JD, Dinkova-Kostova AT, McMahon M. Cross-talk between transcription factors AhR and Nrf2: lessons for cancer chemoprevention from dioxin. *Toxicol Sci* 2009; 111: 199-201.
- [243] Cobb MH, Goldsmith EJ. Dimerization in MAP-kinase signaling. *Trends Biochem Sci* 2000; 25: 7-9
- [244] Weston CR, Davis RJ. The JNK signal transduction pathway. *Curr Opin Genet Dev* 2002; 12: 14-21
- [245] Tan Z, Chang X, Puga A, Xia Y. Activation of mitogen-activated protein kinases (MAPKs) by aromatic hydrocarbons: role in the regulation of aryl hydrocarbon receptor (AhR) function. *Biochem Pharmacol* 2002; 64: 771-80.
- [246] Diry M, Tomkiewicz C, Koehle C, *et al.* Activation of the dioxin/aryl hydrocarbon receptor (AhR) modulates cell plasticity through a JNK-dependent mechanism. *Oncogene* 2006; 25: 5570-4.
- [247] Chang X, Fan Y, Karyala S, *et al.* Ligand-independent regulation of transforming growth factor β 1 expression and cell cycle progression by the aryl hydrocarbon receptor. *Mol Cell Biol* 2007; 27: 6127-39.
- [248] Andrysik Z, Vondráček J, Machala M, *et al.* The aryl hydrocarbon receptor-dependent deregulation of cell cycle control induced by polycyclic aromatic hydrocarbons in rat liver epithelial cells. *Mutat Res* 2007; 615: 87-97.
- [249] DeGregori J, Johnson DG. Distinct and Overlapping Roles for E2F Family Members in Transcription, Proliferation and Apoptosis. *Curr Mol Med* 2006; 6: 739-48.
- [250] Lin X, Yang H, Zhou L, Guo, Z. Nrf2-dependent induction of NQO1 in mouse aortic endothelial cells overexpressing catalase. *Free Radic Biol Med* 2011; 51: 97-106.
- [251] Lee CY, Chew EH, Go ML. Functionalized auronas as inducers of NAD(P)H:quinone oxidoreductase 1 that activate AhR/XRE and Nrf2/ARE signaling pathways: synthesis, evaluation and SAR. *Eur J Med Chem* 2010; 45: 2957-71.
- [252] Tsuji G, Takahara M, Uchi H, *et al.* Identification of ketoconazole as an AhR-Nrf2 activator in cultured human keratinocytes: the basis of its anti-inflammatory effect. *J Invest Dermatol* 2012; 132: 59-68.
- [253] Lu H, Cui W, Klaassen CD. Nrf2 protects against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced oxidative injury and steatohepatitis. *Toxicol Appl Pharmacol* 2011; 256: 122-35.
- [254] Terashima J, Habano W, Gamou T, Ozawa S. Induction of CYP1 family members under low-glucose conditions requires AhR expression and occurs through the nuclear translocation of AhR. *Drug Metab Pharmacokinet* 2011; 26: 577-83.
- [255] Haarmann-Stemmann T, Abel J, Fritsche E, Krutmann J. The AhR-Nrf2 pathway in keratinocytes: on the road to chemoprevention? *J Invest Dermatol* 2012; 132: 7-9.
- [256] Niestroy J, Barbara A, Herbst K, *et al.* Single and concerted effects of benz[a]pyrene and flavonoids on the AhR and Nrf2-pathway in the human colon carcinoma cell line Caco-2. *Toxicol In vitro* 2011; 25: 671-83.
- [257] Yun JH, Lee SB, Lee HJ, *et al.* Bi-functional induction of the quinone reductase and cytochrome P450 1A1 by youngiasides via Nrf2-ARE and AhR-XRE pathways. *Biol Pharm Bull* 2010; 33: 1650-7.
- [258] Kalthoff S, Ehmer U, Freiberg N, Manns MP, Strassburg CP. Coffee induces expression of glucuronosyltransferases by the aryl hydrocarbon receptor and Nrf2 in liver and stomach. *Gastroenterology* 2010; 139: 1699-710, e1691-2.
- [259] Kalthoff S, Ehmer U, Freiberg N, Manns MP, Strassburg CP. Interaction between oxidative stress sensor Nrf2 and xenobiotic-activated aryl hydrocarbon receptor in the regulation of the human phase II detoxifying UDP-glucuronosyl transferase 1A10. *J Biol Chem* 2010; 285: 5993-6002.
- [260] Xu S, Weerachayaphorn J, Cai SY, Soroka CJ, Boyer JL. Aryl hydrocarbon receptor and NF-E2-related factor 2 are key regulators of human MRP4 expression. *Am J Physiol Gastrointest Liver Physiol* 2010; 299: G126-35.
- [261] Shin S, Wakabayashi N, Misra V, *et al.* NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis. *Mol Cell Biol* 2007; 27: 7188-97.
- [262] Legendre C, Hori T, Loyer P, *et al.* Drug-metabolising enzymes are down-regulated by hypoxia in differentiated human hepatoma HepaRG cells: HIF-1 α involvement in CYP3A4 repression. *Eur J Cancer* 2009; 45: 2882-92.
- [263] Fleming CR, Billiard SM, Di Giulio RT. Hypoxia inhibits induction of aryl hydrocarbon receptor activity in topminnow hepatocarcinoma cells in an ARNT-dependent manner. *Comp Biochem Physiol C Toxicol Pharmacol* 2009; 150: 383-9.
- [264] Schults MA, Timmermans L, Godschalk RW, *et al.* Diminished carcinogen detoxification is a novel mechanism for hypoxia-inducible factor 1-mediated genetic instability. *J Biol Chem* 2010; 285: 14558-64.
- [265] Terzuoli E, Puppo M, Rapisarda A, *et al.* Aminoflavone, a ligand of the aryl hydrocarbon receptor, inhibits HIF-1 α expression in an AhR-independent fashion. *Cancer Res* 2010; 70: 6837-48.
- [266] Rivera SP, Wang F, Saarikoski ST, *et al.* A novel promoter element containing multiple overlapping xenobiotic and hypoxia response elements mediates induction of cytochrome P4502S1 by both dioxin and hypoxia. *J Biol Chem* 2007; 282: 10881-93.
- [267] Shimada T, Hiramatsu N, Hayakawa K, *et al.* Dual suppression of adipogenesis by cigarette smoke through activation of the aryl hydrocarbon receptor and induction of endoplasmic reticulum stress. *Am J Physiol Endocrinol Metab* 2009; 296: E721-30.
- [268] Surh Y.J. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 2003; 3: 768-80.
- [269] Bode AM, Dong Z. Cancer prevention research - then and now. *Nat Rev Cancer* 2009; 9: 508-16.
- [270] Lee KW, Bode AM, Dong Z. Molecular targets of phytochemicals for cancer prevention. *Nat Rev Cancer* 2011; 11: 211-8.
- [271] Mandlekar S, Hong JL, Kong AN. Modulation of metabolic enzymes by dietary phytochemicals: a review of mechanisms underlying beneficial versus unfavorable effects. *Curr Drug Metab* 2006; 7: 661-75.
- [272] Hayes JD, Kelleher MO, Eggleston IM. The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *Eur J Nutr* 2008; 47(Suppl 2): 73-88
- [273] Shu L, Cheung KL, Khor TO, Chen C, Kong AN. Phytochemicals: cancer chemoprevention and suppression of tumor onset and metastasis. *Cancer Metastasis Rev* 2010; 29: 483-502.
- [274] Kohle C, Bock KW. Activation of coupled Ah receptor and Nrf2 gene batteries by dietary phytochemicals in relation to chemoprevention. *Biochem Pharmacol* 2006; 72: 795-805.
- [275] Surh YJ, Kundu JK, Na HK. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med* 2008; 74: 1526-39.
- [276] Lee JS, Surh YJ. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett* 2005; 224: 171-84.
- [277] Yu X, Kensler T. Nrf2 as a target for cancer chemoprevention. *Mutat Res* 2005; 591: 93-102

- [278] Kwak MK, Kensler TW. Targeting NRF2 signaling for cancer chemoprevention. *Toxicol Appl Pharmacol* 2011; 244: 66-76.
- [279] Hayes JD, McMahon M. Molecular basis for the contribution of the antioxidant responsive element to cancer chemoprevention. *Cancer Lett* 2001; 174: 103-13.
- [280] Haddad JJ. Antioxidant and prooxidant mechanisms in the regulation of redox(y)-sensitive transcription factors. *Cell Signal* 2002; 14: 879-97.
- [281] Kamata H, Hirata H. Redox regulation of cellular signalling. *Cell Signal* 1999; 11: 1-14.
- [282] Jones DP. Redefining oxidative stress. *Antioxid Redox Signal* 2006; 8: 1865-79.
- [283] Itoh K, Mimura J, Yamamoto M. Discovery of the negative regulator of Nrf2, Keap1: a historical overview. *Antioxid Redox Signal* 2010; 13: 1665-78.
- [284] Leung L, Kwong M, Hou S, Lee C, Chan JY. Deficiency of the Nrf1 and Nrf2 transcription factors results in early embryonic lethality and severe oxidative stress. *J Biol Chem* 2003; 278: 48021-9.
- [285] Kwong M, Kan YW, Chan JY. The CNC basic leucine zipper factor, Nrf1, is essential for cell survival in response to oxidative stress-inducing agents. Role for Nrf1 in γ -gcs(l) and gss expression in mouse fibroblasts. *J Biol Chem* 1999; 274: 37491-8.
- [286] Zhang Y, Lucocq JM, Yamamoto M, Hayes JD. The NHB1 (N-terminal homology box 1) sequence in transcription factor Nrf1 is required to anchor it to the endoplasmic reticulum and also to enable its asparagine-glycosylation. *Biochem J* 2007; 408: 161-72.
- [287] Zhang Y, Hayes JD. Identification of topological determinants in the N-terminal domain of transcription factor Nrf1 that control its orientation in the endoplasmic reticulum membrane. *Biochem J* 2010; 430: 497-510.
- [288] Zhang Y, Lucocq JM, Hayes JD. The Nrf1 CNC/bZIP protein is a nuclear envelope-bound transcription factor that is activated by t-butyl hydroquinone but not by endoplasmic reticulum stressors. *Biochem J* 2009; 418: 293-310.
- [289] Miyamoto, N., Izumi, H., Miyamoto, R., Kondo, H., Tawara, A., Sasaguri, Y. and Kohno, K. Quercetin induces the expression of peroxiredoxins 3 and 5 via the Nrf2/NRF1 transcription pathway. *Invest Ophthalmol Vis Sci* 2011; 52: 1055-1063.
- [290] Hernandez-Montes, E., Pollard, S. E., Vauzour, D., Jofre-Montseny, L., Rota, C., Rimbach, G., Weinberg, P. D. and Spencer, J. P. Activation of glutathione peroxidase via Nrf1 mediates genistein's protection against oxidative endothelial cell injury. *Biochem Biophys Res Commun* 2006; 346: 851-859.

Received: 00 00, 2013

Revised: 00 00, 2013

Accepted: 00 00, 2013