Cellular adaptation to xenobiotics: Interplay between xenosensors, reactive oxygen species and FOXO transcription factors

Lars-Oliver Klotz, Holger Steinbrenner

Institute of Nutrition, Department of Nutrigenomics, Friedrich-Schiller-Universität Jena, D-07743 Jena, Germany

ARTICLE INFO

Keywords:
Xenobiotic metabolism
Biotransformation of xenobiotics
Forkhead box transcription factors
Redox regulation

ABSTRACT

Cells adapt to an exposure to xenobiotics by upregulating the biosynthesis of proteins involved in xenobiotic metabolism. This is achieved largely via activation of cellular xenosensors that modulate gene expression. Biotransformation of xenobiotics frequently comes with the generation of reactive oxygen species (ROS). ROS, in turn, are known modulators of signal transduction processes. FOXO (forkhead box class O) transcription factors are among the proteins deeply involved in the cellular response to stress, including oxidative stress elicited by the formation of ROS. On the one hand, FOXO activity is modulated by ROS, while on the other, FOXO target genes include many that encode antioxidant proteins – thereby establishing a regulatory circuit. Here, the role of ROS and of FOXOs in the regulation of xenosensor transcriptional activities will be discussed. Constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptors (PPARs), arylhydrocarbon receptor (AhR) and nuclear factor erythroid 2-related factor 2 (Nrf2) all interact with FOXOs and/or ROS. The two latter not only fine-tune the activities of xenosensors but also mediate interactions between them. As a consequence, the emerging picture of an interplay between xenosensors, ROS and FOXO transcription factors suggests a modulatory role of ROS and FOXOs in the cellular adaptive response to xenobiotics.

1. Introduction

Exposure of mammalian cells to xenobiotics – i.e., compounds that are “foreign” to the organism of interest, such as (environmental) toxins, metal ions, drugs, phytochemicals – elicits responses ranging from signaling and adaptation to cell death. Cells are equipped with enzymatic means of metabolizing xenobiotics for the purpose of eliminating these compounds. Xenobiotic metabolism and biotransformation occurs in stages; for example, in the case of hydrophobic xenobiotics, these are devoted to generating functional groups in these compounds (phase I) that serve as docking sites for hydrophilic compounds they are coupled with (phase II). These transformations then allow for transport and excretion (phase III) of xenobiotic metabolites.

Cells react to an exposure to xenobiotics by upregulating the formation of the xenobiotic metabolism machinery, i.e., of proteins involved in the above-mentioned phases of biotransformation. This adaptive cellular response is largely due to the interaction of xenobiotics with signaling cascades and transcriptional regulators, i.e. “xenosensors”. Cellular structures that are targeted by xenobiotics, triggering a cellular response, may of course be considered as xenobiotic sensors, i.e. xenosensors, in a very broad sense. However, the actual term rather refers to proteins less “accidentally” interacting with their ligands. Here, we will focus on xenosensors in the latter sense, i.e. xenobiotic-sensitive transcriptional regulators.

Biotransformation comes with the generation of reactive oxygen species (ROS) through a multitude of reactions both directly releasing ROS as part of the respective reaction and indirectly as a consequence of the products generated by transformation of a xenobiotic [1–3] (Fig. 1). The generation of ROS is a natural consequence of biotransformation using serial redox reactions and exploiting the presence
of oxygen. Electron transfer to molecular oxygen will result in the generation of superoxide and derivatives thereof, such as hydrogen peroxide. These ROS (this generic term will be used here when referring to superoxide and/or hydrogen peroxide) are known modulators of cellular signaling processes by interfering with signaling cascades at several levels, including at the level of transcriptional regulators. Moreover, cells are now known to exploit the transient formation of superoxide/hydrogen peroxide as vital components of signaling cascades, including growth factor-dependent signaling.

In summary, xenobiotics trigger xenosensor-dependent adaptation of xenobiotic metabolism, and in parallel, through xenobiotic metabolism, contribute to the generation of ROS and to ROS-dependent regulation of cellular signaling events.

Several transcription factors are now known to be redox-regulated and affected by the generation of ROS (see [4] for a comprehensive review). Here, we will focus on a group of factors known not only to be regulated by ROS but to also control cellular stress response and antioxidant defense: Forkhead Box, class O (FOXO) transcription factors.

The purpose of this article is to provide a brief overview on the interplay between xenosensors, ROS and FOXO transcription factors in regulating the cellular response to an exposure to xenobiotics. Following a discussion of the modulation of FOXO signaling by ROS, we will provide examples of xenosensors directly targeted by xenobiotics (CAR, PXR, PPARs, AhR and Nrf2) and delineate their relation with ROS and FOXOs.

2. Modulation of FOXO transcriptional activity: “ROS” and “non-ROS” routes

Metabolism of xenobiotics may cause the generation of ROS (most frequently, superoxide and hydrogen peroxide, see Fig. 1), which, in turn, were demonstrated to affect the activity of FOXO transcription factors (for a recent summary, see [5]; in short, both activation and inactivation of FOXOs may be elicited by ROS, depending on location, timing and extent of their formation). Four FOXO isoforms (FOXOs 1, 3, 4, 6) exist in humans that are ubiquitously expressed (albeit with varying expression levels in different tissues); currently, most data in the body of literature on FOXOs is on isoforms FOXO1, FOXO3 and FOXO4. FOXO transcription factors not only regulate the expression of genes encoding proteins involved in antioxidant defense [5], but also

![Fig. 1. Xenobiotics and the formation of reactive oxygen species.](image-url)
L.-O. Klotz, H. Steinbrenner

regulate the formation of enzymes involved in fuel metabolism [6] or the regulation of cellular proliferation and cell death [7], among many others.

2.1. Regulation of FOXOs by ROS

ROS-dependent FOXO modulation occurs at several stages: FOXO protein levels, for example, are regulated posttranscriptionally by redox-sensitive RNA binding proteins or redox-regulated microRNAs (for a recent review, see [8]). FOXO activity, in turn, is modulated by upstream signaling cascades that affect FOXO subcellular localization, DNA binding and transactivation activity. The most prominent of these cascades is the signaling cascade emanating from insulin receptor or insulin-like growth factor-1 (IGF-1) receptor which, via phosphoinositide 3-kinase (PI3K) and the serine/threonine kinase Akt, causes phosphorylation and inactivation as well as nuclear exclusion of FOXOs [6]. As described in Fig. 2, this cascade is affected by conditions causing the generation of ROS. Moreover, FOXOs themselves were also demonstrated to interact with coregulators, such as CREB-binding protein (CBP), via formation of intermolecular disulfides if exposed to hydrogen peroxide [9,10]. As several FOXO target genes encode proteins involved in antioxidant defense, those may in turn decrease intracellular ROS levels that would otherwise modulate the activation of FOXOs. Importantly, oxidative processes elicited by ROS may generate reaction products that affect cellular signaling cascades. One such example is 4-hydroxynonenal (HNE), which is a lipid peroxidation product and may interact with Akt [11] (see Fig. 2c).

2.2. ROS-independent regulation of FOXOs by xenobiotics

Several xenobiotic compounds may affect FOXO signaling also independently of the formation of ROS. Here, we will briefly analyze the effects of metal (Cu, Zn) ions that – albeit of physiological relevance – may also trigger undesired effects, of metalloids such as arsenic, as well as of naphthoquinones of various origins. These compounds share an affinity for susceptible thiol moieties on proteins.

Whereas oxidation of proteins by ROS may be reversible (e.g., thiol oxidation to disulfide or to sulfenic acid may be reversed intracellularly, in part through the action of enzymes of the redox family [12]), the non-oxidative interaction with xenobiotics may or may not be. For example, metal ions can interfere with enzyme activities (such as Zn ions with PTEN, see Fig. 2b), but binding is noncovalent and usually reversible; in contrast, alkylation (such as a Michael addition of the lipid peroxidation product 4-hydroxynonenal (HNE) to a susceptible cysteine residue (i.e. HNEylation of Akt), result in Akt inhibition [11]; (c) Akt oxidation, e.g. to form sulfenylated Akt [62], and reaction with electrophiles, such as a Michael addition of the lipid peroxidation product 4-hydroxynonenal (HNE) to a susceptible cysteine residue (i.e. HNEylation of Akt), result in Akt inhibition [11]; (d) FOXO transcription factors may be regulated by hydrogen peroxide-induced covalent binding to coregulators such as CBP, which can both affect acetylation status of FOXOs as well as of histones near the transcription start site [9,10].

3. Interactions between FOXOs and xenosensors

Here, we will briefly introduce xenosensors that FOXO proteins were demonstrated to interact with, discuss the nature of this

Fig. 2. “ROS” and “non-ROS” routes for modulation of FOXO activity along the insulin receptor/IGF-1 receptor dependent signaling cascade. Right panel: Insulin (or insulin-like growth factor, IGF1), via stimulation of insulin (or IGF1) receptor (InsR, or IGF1-R), via phosphoinositide 3-kinase (PI3K)-induced generation of 3'-phosphorylated phosphoinositides and via the subsequent activation of the serine/threonine kinase Akt, causes inactivation of FOXO transcription factors. This cascade is controlled by protein tyrosine phosphatases (PTPs) such as PTP1B that dephosphorylate and inactivate the insulin receptor. Moreover, the lipid phosphatase, PTEN (phosphatase and tensin homolog) attenuates PI3K signaling by catalyzing the 3'-dephosphorylation of phosphoinositides. (a) Most known PTP harbor an oxidation-sensitive (low-pKₐ) cysteine at their active site and may therefore be inactivated by oxidants such as H₂O₂, peroxynitrite or singlet oxygen [60]. This inactivation may be reversible, depending on the extent of oxidation [61]; (b) Similar to PTPs, PTEN may be inactivated by oxidation, e.g. upon exposure to H₂O₂, which yields a disulfide [62]; PTEN inactivation may also occur in a “non-ROS” fashion, e.g. through interaction with metal ions such as Zn²⁺ [63]. (c) Akt oxidation, e.g. to form sulfenylated Akt [64,65], and reaction with electrophiles, such as a Michael addition of the lipid peroxidation product 4-hydroxynonenal (HNE) to a susceptible cysteine residue (i.e. HNEylation of Akt), result in Akt inhibition [11]; (d) FOXO transcription factors may be regulated by hydrogen peroxide-induced covalent binding to coregulators such as CBP, which can both affect acetylation status of FOXOs as well as of histones near the transcription start site [9,10].
interaction and the role of ROS therein. In this regard, Fig. 3 presents an overview on the modes of activation of these xenosensors by their ligands (e.g., xenobiotics).

3.1. CAR/PXR

Constitutive androstane receptor (CAR) and pregnane xenobiotic receptor (PXR) are RXR heterodimer forming members of the nuclear receptor family (Fig. 3a/b) and are widely regarded as the major...
fox sensors as they interact with a multitude of ligands and drive the transcription of genes encoding major phase I/II enzymes, including CYP2C and CYP3A monoxygenases, which were estimated to catalyze approx. 60% of all cytochrome P450 (CYP)-mediated drug oxidations [21], as well as UDP-glucuronosyl transferases (UGTs), sulfo- transferases (SULTs) and others. As with their target genes, there is an overlap between the two receptors also with respect to their activators, although ligands selective for CAR (such as phenytoin) as well as PXR (such as rifampicin and hyperforin) were identified [22–24]. For a list of CAR and PXR activators as well as target genes, see [25].

Both CAR and PXR were demonstrated to interact with FOXO1 in HepG2 human hepatoma cells, and FOXO1 was then identified as a coactivator of CAR and PXR in these cells [26]. As expected for a FOXO-dependent effect, this activity was attenuated by insulin via PI3K and Akt [26]. Interestingly, while FOXO1 coactivates CAR and PXR, the two latter (as CAR/RXR or PXR/RXR heterodimers) were shown to act as corepressors of FOXO activity on its DNA binding elements [26]. This interaction was demonstrated to result in a CAR-induced attenuation of FOXO target gene expression, such as of genes coding for glucocorticoid enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase), or the cyclin-dependent kinase inhibitor, p21, and also FOXO1 itself [27]. CAR-mediated suppression of FOXO1 (along with hepatocyte nuclear factor 4α, HNF4α) was suggested to be responsible for the anti-glucocorticoid effects of certain alkaloids [28]. Similarly, PXR activation was shown to affect glucocorticoid expression not only through FOXO1 inhibition but also through competition for PPARγ coactivator (PGC) 1α, a known coregulator of FOXOs as well as other transcriptional regulators, such as HNF4α (for review, see [29]).

An interaction between insulin signaling and CAR was shown in (murine and human) hepatocytes as insulin not only causes the above inactivation of FOXOs (and thus a loss of coactivation of CAR) but also attenuates CAR activity [30] – likely at the level of preventing its dephosphorylation, as seen also with EGF [31]. Therefore, a direct link exists between energy and xenobiotic metabolism through FOXO/CAR interaction and through the effect of insulin on CAR activation. Based on these findings, two types of CAR activators can be distinguished – those acting as CAR ligands (e.g., CITCO) and those stimulating CAR in an indirect fashion, via control of CAR dephosphorylation, such as phenobarbital. The latter was demonstrated to interfere with binding of EGF or insulin to their respective receptors [30,31], hence antagonizing CAR inactivation.

Regarding the role of ROS in fine-tuning CAR/PXR signaling, one can reasonably assume an interaction with these nuclear receptors through the known capability of ROS to modulate FOXO signaling. A recent study suggests that the stimulation of CAR-dependent expression of the Cyp2b10 and Ugt1a1 genes in murine liver by exposure of mice to phenethyl isothiocyanate is mediated by ROS [32]. However, the notion of a ROS-mediated effect of the isothiocyanate on CAR is based solely on the use of the thiol N-acetyl cysteine, a rather unspecific and indirect modulator of ROS levels and obviously also a modulator of thiol/disulfide equilibria in proteins.

In summary, xenobiotics may affect FOXO activity through stimulation of CAR and/or PXR. Owing to their interaction with FOXOs, CAR and PXR are not only regulators of xenobiotic metabolism but also play an important role in the modulation of energy metabolism [33].

3.2. PPARs

Peroxisome proliferator-activated receptors (PPARs) represent a family of transcription factors that can be activated by diverse endogenous and exogenous ligands (such as the compounds originally categorized as “peroxisome [or microbody] proliferators”, i.e. compounds triggering peroxisome production [34]), rendering them targets susceptible to pharmacological and environmental xenobiotics and/or their cellular metabolites. The three PPAR proteins PPARα, PPARβ/δ and PPARγ exert isoform- and cell type-specific functions in the regulation of nutrient homeostasis and energy balance with emphasis on lipid and carbohydrate metabolism. PPARα and PPARγ show tissue-specific expression patterns: PPARα is enriched in metabolically active tissues such as liver, heart, kidney and intestine, while PPARγ is most highly expressed in mature adipocytes of the white adipose tissue. PPARβ/δ is ubiquitously expressed. Similar to CAR and PXR, active (ligand-bound) PPARs form heterodimers with retinoid X receptor (RXR) and bind to defined consensus sequences, PPAR response elements (PPREs), in the promoters of their target genes [35]. It is not fully established yet to what extent ligand binding supports nuclear localization of PPARs [36] see Fig. 3e).

FOXO1, the most abundant FOXO isoform in adipose tissue, has been found to interfere with PPARγ-controlled gene expression in mature adipocytes in two ways (Fig. 4a): (i) FOXO1 may repress the promoters, and thus impair transcription of, the PPARγ1 and PPARγ2 genes [37]; and (ii) FOXO1 may directly bind to PPREs in promoters of PPARγ target genes, thus acting as a trans-repressor of PPARγ [38]. Binding of FOXO1 to PPREs is not mediated by its N-terminal DNA binding domain [38] that is otherwise required for interaction of FOXO1 with FOXO-responsive (DBE) sites within promoters of its proper target genes [5]. Instead, FOXO1 binds to PPREs through a centrally located and evolutionarily conserved 31 amino acid-domain containing an LXXLL motif [38]. The FOXO1-PPARγ interaction has been shown to be influenced by factors that control differentiation of preadipocytes into adipocytes as well as metabolic and endocrine functions of mature adipocytes (Fig. 4a): by inducing phosphorylation and subsequent nuclear exclusion of FOXO1, insulin counteracted FOXO1-mediated repression of PPARγ1 and PPARγ2 promoters as well as FOXO1 occupancy at PPRE sites in the promotors of PPARγ target genes, which in turn enhanced PPARγ transcriptional activity [37,38]. This has been considered as a feed-forward mechanism contributing to maintaining insulin responsiveness of adipocytes [37,38]. Insulin also rescued the induction of
PPARγ target genes in FOXO1-overexpressing adipocytes exposed to rosiglitazone [38]. The anti-diabetic thiazolidinedione derivative rosiglitazone is a pharmacological PPARγ agonist that promotes adipocyte differentiation and lipid accumulation [35]. FOXO1 overexpression was capable of dampening but not of completely suppressing the rosiglitazone-induced increase in PPARγ transcriptional activity in adipocytes [38]. In this regard, it would be of interest to explore the impact of environmental PPARγ-targeting obesogens (adipocyte differentiation and lipid accumulation-promoting xenobiotics) such as tributyltin or phthalates [39] on the FOXO1-PPARγ interaction. Additionally, other substances derived from environment or nutrition may affect binding of FOXO1 to PPRE sites in PPARγ target genes, as demonstrated for the essential trace element iron: exposure of adipocytes to iron ions inhibited transcription and secretion of the PPARγ target gene adiponectin, an insulin-sensitizing adipokine, through induction of FOXO1 binding to a PPRE site in the adiponectin promoter [40].

The above-discussed actions of FOXO1 as a transcriptional repressor appear to be restricted to PPARγ, as FOXO1 overexpression neither inhibited PPARα nor PPARβ/δ transcriptional activity [38]. However, a more indirect link was reported for FOXOs (FOXO1 and FOXO3) and PPARs in kidney (Fig. 4b). Two groups found that up-regulation/activation of PPARα was accompanied by enhanced FOXO1 transcriptional activity due to inhibition of Akt-mediated FOXO phosphorylation [41,42]. Treatment with the pharmacological PPARα agonist, fenofibrate, protected spontaneously hypertensive rats from renal lipod accumulation and apoptotic cell death induced by feeding a high-fat diet [41]. Concomitantly, Akt and FOXO3 phosphorylation was suppressed in the kidneys of fenofibrate-treated animals, resulting in increased gene expression of the FOXO target genes SOD2, an antioxidant enzyme, and Bcl-2, an anti-apoptotic protein [41]. A recent study shed light on the molecular mechanism underlying the closely linked PPARα and FOXO activation by applying another pharmacological PPARα agonist, 2-(4-(5-chlorobenzothiazol-2-yl)phenoxyl)-2-methyl-propionic acid (MHY908). Similar to fenofibrate, MHY908 suppressed basal as well as insulin-induced Akt and FOXO1 phosphorylation in the kidneys of aged rats and in HEK293T human embryonic kidney cells, respectively; FOXO3 activation resulted in up-regulation of two FOXO-dependent antioxidant enzymes, SOD2 and catalase [42]. Use of PPARα-specific siRNA revealed that MHY908, through activation of PPARα, suppressed the insulin-induced and NADPH oxidase (Nox) 4-mediated increase in intracellular hydrogen peroxide levels in HEK293T cells [42]. Downregulation of Nox4 expression is likely to explain the activation of FOXO signaling by PPARα agonists, as the insulin signaling cascade that promotes phosphorylation (inactivation) of FOXOs is known to be enhanced by Nox4-mediated generation of H2O2 [5,43].

3.3. Arylhydrocarbon receptor (AhR)

The arylhydrocarbon receptor (AhR, Fig. 3d) is a member of the bHLH-PAS family [with a basic helix-loop-helix DNA binding domain and Per-ARNT-Sim (PAS) domains] that is relatively ubiquitously expressed in human tissues (albeit to varying extents). Stimulation of AhR, which is kept in the cytosol by binding partners (including Hsp90 as well as AhR interacting protein, AIP), is achieved through binding of a ligand and the subsequent release of AhR binding partners, followed by nuclear translocation. AhR then heterodimerizes with ARNT (AhR nuclear translocator) and binds to DNA at specific sites, the xenobiotic response elements (XRE). Classical ligands promoting AhR activation include TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) and other halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH, such as 3-methylcholanthrene or benzo(a)pyrene), as well as indole derivatives and certain flavonoids. Target genes include CYPs, predominantly CYPs 1A1, 1A2, 1B1, as well as genes coding for phase II enzymes (e.g., UGTs) [25,44].

AhR interacts with a major regulator of the cellular antioxidant response, Nrf2 (see 3.4.); as summarized in a recent review [45], this occurs (i) via stimulation of CYP1A1-dependent metabolism of xenobiotics that results in the generation of ROS, which in turn stimulate Nrf2 (see Fig. 5), (ii) via transcriptional stimulation of Nrf2 synthesis by AhR, and (iii) by cooperation of AhR and Nrf2 in regulating antioxidant proteins such as NAD(P)H:quione oxidoreductase-1 (NQO1; see Fig. 1c). A link between AhR and FOXO-dependent signaling was suggested in preliminary studies on FOXO-responsive promoter constructs that were activated by AhR agonists and then demonstrated to contain an artificially inserted XRE; deletion of this XRE rendered these FOXO-responsive luciferase constructs refractory to FOXO1-induced stimulation, suggesting that XRE and FOXO-responsive elements might interact [46]. Moreover, AhR was demonstrated to drive transcription of the CAR gene [47], enhancing CAR synthesis. As CAR is a corepressor of FOXO activity (see above), this would establish a link between AhR and FOXOs. PXR, which interacts with FOXO1 (see above), also interacts with AhR, yet it suppresses AhR transcriptional activity, apparently preventing AhR binding to XRE [48].

In summary, CAR/PXR, PPARs and AhR are closely linked in regulating xenobiotic metabolism, and redox regulation may occur at the
level of their interaction with redox-regulated coregulators or transcription factors, such as FOXOs or nuclear factor erythroid 2-related factor 2 (Nrf2).

3.4. Nrf2

Nrf2 is a transcription factor of the cap’n’collar basic region leucine zipper (CNC-bZIP) family [49] stimulated by xenobiotics through suppression of its negative regulator, Keap-1 (Kelch-like ECH-associated protein 1). The latter constitutively mediates ubiquitination (and degradation) of Nrf2, thus keeping Nrf2 transcriptional activity under tight control (Fig. 5). Xenobiotics that affect Keap-1/Nrf2 interaction, leading to Nrf2 activation, are usually compounds interacting with thiols, such as (i) electrophiles capable of modifying one of the Keap-1 cysteine residues, (ii) compounds metabolized to such electrophiles, (iii) certain metal(loid) ions or (iv) compounds that cause the cellular generation of reactive oxygen or nitrogen species. Such reactive species will then directly oxidize Keap-1 cysteines or contribute to the cellular generation of electrophiles, such as 4-hydroxynonenal (HNE), a lipid peroxidation product (Fig. 5). Exposure to HNE causes stimulation of Nrf2 transcriptional activity in PC12 cells, followed by an enhanced generation of electrophiles, such as 4-hydroxynonenal (HNE) [72]; different electrophiles may react with different Keap-1 cysteines, as was demonstrated for compounds such as diethyl maleate and HNE [73,74].

It was demonstrated in several human tumor cell lines cells that FOXO3 (but interestingly neither FOXO1 nor FOXO4) stimulates the transcription of the Keap-1 gene [52], regulating Keap-1 protein levels (Fig. 6); of note, this FOXO3/Keap axis appears to exist in human but not murine cells [52]. FOXO3 would, therefore, attenuate Nrf2 action by elevating Keap-1 levels, whereas active Akt would tend to stimulate Nrf2 by blocking said FOXO activity. This is in line with the positive action of Akt on Nrf2 activity described above. An additional link between the two transcription factor systems was hypothesized: xenobiotoxin-induced FOXO3 formation may require Nrf2, as shown in murine ovaries exposed to 4-vinylcyclohexene diepoxide [53].

A similar link was seen in Caenorhabditis elegans exposed to an electrophilic thiol depleting compound, diethyl maleate (DEM). Whereas DEM lowered C. elegans lifespan at higher concentrations, exposure of the nematodes to 10–100-fold lower concentrations enhanced their stress resistance and lifespan. This extension of lifespan was then demonstrated to require both DAF-16 (the FOXO ortholog in C. elegans) and SKN-1 (the C. elegans ortholog of Nrf2) [54]. The nature of their cooperation remains to be explored.

4. Conclusions

Exposure of cells to xenobiotics comes with an activation of xenosensors, such as nuclear receptor xenosensors (e.g., CAR, PXR, PPARs), AhR or Nrf2. It is also frequently accompanied by the generation of ROS, which may, in turn, affect xenosensor activities. Here, we have summarized literature data that lead us to conclude that FOXO transcription factors interact with all of the described xenosensors (Fig. 7). In some cases, this is a direct physical interaction with functional consequences, as in the case of CAR and PXR (Fig. 7b). In others (Fig. 7c–e), the interaction is indirect, either established through ROS (see PPARα, which may downregulate cellular H₂O₂ generation and thus modulate FOXOs, Fig. 4b; or see Nrf2, Fig. 6), through a competition for DNA binding sites (e.g., Fig. 4a) or through the mutual influence established by proteins whose biosynthesis is under the transcriptional control of either FOXOs or xenosensors (e.g., Fig. 6).

Altogether, these findings establish a picture that suggests a regulatory or modulating role of FOXOs and ROS regarding xenosensor activities. FOXOs, as redox-regulated transcription factors [5], may bridge xenobiotoxin-induced generation of ROS and the modulation of xenosensor activities. Finally, there are some consequences that point
The role of ROS in the interaction maps emerging from the sum-chapters is briefly summarized (Fig. 7).

### References

1. J.P. Kehrer, Free radicals as mediators of tissue injury and disease, Crit. Rev. Toxicol. 23 (1993) 21–48.
2. J.P. Kehrer, L.O. Klotz, Free radicals and related reactive species as mediators of tissue injury and disease: implications for Health, Crit. Rev. Toxicol. 45 (2015) 765–798.
3. F.P. Guengerich, Cytochrome P450 and other enzymes in drug metabolism and toxicity, AAPS J. 8 (2006) E101–E111.
4. R. Brigelius-Flohe, L. Flohe, Basic principles and emerging concepts in the redox control of transcription factors, Antioxid. Redox Signal 15 (2011) 2335–2381.
5. L.O. Klotz, C. Sanchez-Ramos, I. Prieto-Arroyo, P. Urbanek, H. Steinbrenner.
6. M. Monsalve, Redox regulation of FoxO transcription factors, Redox Biol. 6 (2015) 51–72.
7. A. Barthel, D. Schmoll, T.G. Unterman, FoxO proteins in insulin action and metabolism, Trends Endocrinol. Metab. 16 (2005) 183–189.
8. A. Eijkelenboom, B.M. Burgersing, FOXO signalling integrators for homeostasis maintenance, Nat. Rev. Mol. Cell Biol. 14 (2013) 83–97.
9. P. Urbanek, L.O. Klotz, Posttranscriptional regulation of FOXO expression: microRNAs and beyond, Br. J. Pharmacol. 174 (2017) 1514–1532.
10. T.B. Dansen, L.M. Smith, M.H. van Triest, P.L. de Keijzer, D. van Leenen, M.G. Koeckamp, A. Szypowska, A. Meppelder, A.B. Brekenkam, J. Yodoi, F.C. Holstege, B.M. Burgersing, Redox-sensitive cysteine bridges p38/ERK-mediated acetylation and FoxO4 activity, Nat. Chem. Biol. 5 (2009) 664–672.
11. M. Putker, H.R. Vos, K. van Doremalen, H. de Ruiter, A.G. Duran, B. Snel, B.M. Burgersing, M. Vermeulen, T.B. Dansen, Evolutionary acquisition of cysteines determines FOXO paralog-specific redox signalling, Antioxid. Redox Signal 22 (2015) 15–28.
12. M.J. Long, S. Parvez, Y. Zhao, S.L. Surya, Y. Wang, S. Zhang, Y. Aye, Akt3 is a privileged first responder in isozyme-specific electrophilic response, Nat. Chem. Biol. 13 (2017) 333–338.
13. E.M. Hahsenschmitt, J.R. Godoy, C. Berndt, C. Hudemann, C.H. Lillig, Thioderioxins, glutaredoxins, and peroxiredoxins—molecular mechanisms and health significance: from cofactors to antioxidants to redox signalling, Antioxid. Redox Signal 19 (2013) 1539–1605.
14. A. Eckers, K. Reimann, L.O. Klotz, Nickel and copper ion-induced stress signaling in human hepatoma cells: analysis of phosphoisoforms 3′-kinase/Akt signaling, Biochim. Biophys. Acta 22 (2009) 307–316.
15. J.P. Kehrer, T. Yasujima, K. Saito, R. Moore, M. Negishi, Phenobarbital and insulin reciprocate effects of arsenite in HepG2 cells: modulation of insulin signaling, Biochim. Biophys. Acta 496 (2010) 93–100.
16. I. Hamann, L.O. Klotz, Arsenite-induced stress signaling: modulation of the phosphoisoforms 3′-kinase/Akt signaling cascade, Redox Biol. 1 (2013) 104–109.
17. I. Hamann, K. Petroll, X. Hou, A. Anwar-Mohamed, A.O. El-Kadi, L.O. Klotz, Acute and long-term effects of arsenite in HepG2 cells: modulation of insulin signaling, Biochim. Biophys. Acta 27 (2014) 317–332.
18. I. Hamann, K. Petroll, L. Grimm, A. Hartwig, L.O. Klotz, Insulin-like modulation of Akt/FoxO signaling by copper ions is independent of insulin receptor, Arch. Biochem. Biophys. 558 (2014) 42–50.
19. E.A. Ostrakovich, M.R. Lordjevod, F. Sliets, H. Sies, L.O. Klotz, Copper ions strongly activate the phosphoisoform 3′-kinase/Akt pathway independent of the generation of reactive oxygen species, Arch. Biochem. Biophys. 397 (2002) 232–239.
20. L.O. Klotz, X. Hou, C. Jacob, L.-O. Klotz, Nuclear receptors as mediators of cellular and inter-cellular signaling, Molecules 19 (2014) 14902–14918.
21. V. Klau, T. Hartmann, J. Gambini, P. Graf, W. Stahl, A. Hartwig, L.O. Klotz, 1,4-Naphthoquinones as inducers of oxidative damage and stress signaling in HaCaT human keratinocytes, Arch. Biochem. Biophys. 496 (2010) 93–100.
22. L.C. Winterkess, T.G. Heath, Predicting in vivo drug interactions from in vitro drug discovery data, Nat. Rev. Drug Discov. 4 (2005) 825–833.
23. H. Wang, S. Faucette, R. Moore, T. Sueyoshi, M. Negishi, E. LeCluyse, Human constitutive androstane receptor mediates induction of CYP2B6 gene expression by phenoxytins, J. Biol. Chem. 279 (2004) 29295–29301.
24. L.B. Moore, B. Goodwin, S.A. Jones, G.B. Wisely, J.C. Serabi-Singh, T.M. Willson, J.L. Collins, S.A. Klierer, St. John’s wort induces hepatic drug metabolism through activation of the pregnane X receptor, Proc. Natl. Acad. Sci. USA 97 (2000) 7500–7502.
25. L.B. Moore, D.J. Parks, S.A. Jones, R.K. Beddoes, T.G. Consler, J.B. Stimmel, B. Goodwin, C. Liddle, S.G. Blanchard, T.M. Willson, J.L. Collins, S.A. Klierer, Orphan nuclear receptor constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands, J. Biol. Chem. 275 (2000) 15122–15127.
26. A. Parkinson, B.W. Ogilvie, D.B. Buckley, F. Kazmi, M. Czerwinski, O. Parkinson, Biotransformation of xenobiotics, in: C. Klaessien (Ed.), Caserat & Doull’s Toxicology, The Basic Science of Poisons, McGraw-Hill Education, New York, NY, USA, 2013, pp. 185–366.
27. S. Kodama, C. Koike, M. Negishi, Y. Yamasato, Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and glutathione conjugative enzymes, Mol. Cell Biol. 24 (2004) 7931–7940.
28. Y.A. Kazantseva, A.A. Yarushkin, V.P. Vostynkin, CAR-mediated repression of FoxO1 transcriptional activity regulates the cell cycle inhibitor p21 in mouse livers, Toxicology 321 (2012) 74–79.
29. L. Xu, Z. Wang, M. Huang, Y. Li, K. Zeng, J. Lei, H. Hu, B. Chen, J. Lu, W. Xie, S. Zeng, Evodia alkaloids suppress gluconeogenesis and lipogenesis by activating the constitutive androstane receptor, Biochim. Biophys. Acta 1859 (2016) 1100–1111.
30. J. Hakola, J. Rysa, J. Hukkanen, Regulation of hepatic energy metabolism by the nuclear receptor PXR, Biochim. Biophys. Acta 1859 (2016) 1072–1082.
31. T. Yasumaga, K. Saito, R. Moore, M. Negishi, Phenobarbital and insulin reciprocate activation of the nuclear receptor constitutive androstane receptor through the insulin receptor, J. Pharmacol. Exp. Ther. 357 (2016) 366–374.
32. S. Mutoh, M. Sohbany, R. Moore, L. Perea, L. Pederseen, T. Sueyoshi, M. Negishi, Phenobarbital indirectly activates the constitutive active androstane receptor (CAR) by inhibition of epidermal growth factor receptor signaling, Sci. Signal. 6 (2013) ra11.
33. E. Yoda, M. Pastek, C. Konopnicki, R. Fujiwara, S. Chen, R.H. Tukey, Isothiocyanates induce UGT1A1 in humanized UGT1A1 mice in a CAR-dependent fashion that is highly dependent upon oxidative stress, Sci. Rep. 7 (2017) 46499.
34. Y. Konno, M. Negishi, S. Kodama, The roles of nuclear receptors CAR and PXR in...
hepatic energy metabolism, Drug Metab. Pharmacokinet. 23 (2008) 8–13.
[34] R. Hess, W. Stübibi, W. Riess, Nature of the hepatopancreatic effect produced by ethyl-
chlorophenoxy-isobutyrate in the rat, Nature 208 (1965) 856–858.
[35] B. Grgièl-Gorniak, Peroxoxime proliferator-activated receptors and their ligands: nutritional and clinical implications – a review, Nutr. J. 13 (2014) 17.
[36] T. Umemoto, Y. Fujiki, Ligand-dependent nucleo-cytoplasmic shuttling of peroxi-
some proliferator-activated receptors, PPARalpha and PPARgamma, Genes Cells 17 (2012) 576–596.
[37] M. Armoni, C. Harel, S. Karmi, H. Chen, F. Bar-Yoseph, M.R. Ver, M.J. Quon, E. Karnieli, FOXO1 represses peroxoxime proliferator-activated receptor-gamma1 and –gamma2 gene promoters in primary adipocytes. A novel paradigm to increase insulin sensitivity, J. Biol. Chem. 281 (2006) 19881–19891.
[38] W. Fan, T. Inamura, N. Sonoda, D.D. Sears, D. Patounis, J.J. Kim, J.M. Olefsky, FOX01 transrepresses peroxoxime proliferator-activated receptor gamma transac-
summation, coordinating an insulin-induced feed-forward response in adipocytes, J. Biol. Chem. 284 (2009) 12188–12197.
[39] A. Jänisiek, B. Blumberg, Minireview: PPARalpha as a target of obesogens, J. Steroid Biochem. Mol. Biol. 127 (2011) 4–8.
[40] J.S. Gabrielsen, Y. Gao, J.A. Simcox, J. Huang, D. Thorup, D. Jones, R.C. Cooksey, J. S. Lambeth, B.J. Goldstein, The NAD(P)H oxidase homolog Nox4 modulates in-
flammation through modulation of the ROS/Akt/FoxO3 pathway, Exp. Gerontol. 92 (2017) 87–95.
[41] K. Mahadev, H. Motoshima, X. Wu, J.M. Ruddy, R.S. Arnold, G. Cheng, J.D. Lambeth, B.J. Goldstein, The NAD(P)H oxidase homolog Nox4 modulates in-
flammation through regulation of cell cycle progression, PLoS One 4 (2009) e7523.
[42] N. Urban, D. Tuitsipats, F. Hausig, K. Kreuzer, K. Erler, V. Stein, M. Ristow, H. Steinbrenner, L.O. Klotz, Non-linear impact of glutathione depletion on C. ele-
taste produced by ethyl-
[43] B. Grygiel-Gorniak, Peroxisome proliferator-activated receptors and their ligands:
nutritional and clinical implications – a review, Nutr. J. 13 (2014) 17.
[44] L. Guan, L. Zhang, Z. Gong, X. Hou, Y. Xu, X. Feng, H. Wang, H. You, FoxO3 in-
activation promotes human cholangiocarcinoma tumorigenesis and chemoresis-
tance through Keap1-Nrf2 signaling, Hepatology 63 (2016) 1914–1927.
[45] X. Hu, J.R. Roberts, P.L. Apopo, Y.W. Kan, Q. Ma, Accelerated ovarian failure in-
duced by 4-vinyl cyclohexene diepoxide in Nrf2 null mice, Mol. Cell. Biol. 26 (2006) 940–954.
[46] A. Krasnianski, S.M. Kuusmanen, H. Leinonen, A.L. Levonen, The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer, Redox Biol. 1 (2013) 45–49.
[47] J.D. Hayes, T. Sueyoshi, M. Negishi, Dephosphorylation of threonine 38 is required for nuclear trans-
lation of the tumor suppressor PTEN by H2O2, J. Biol. Chem. 277 (2002) 20336–20342.
[48] E. Karnieli, FOXO1 represses peroxoxime proliferator-activated receptor-gamma1 and –gamma2 gene promoters in primary adipocytes. A novel paradigm to increase insulin sensitivity, J. Biol. Chem. 281 (2006) 19881–19891.
[49] E. Burgermeister, R. Seger, MAPK kinases as nucleo-cytoplasmic shuttles for

Redox Biol. 13 (2017) 646–654