Formation and Signaling Actions of Electrophilic Lipids

ARTICLE *in* CHEMICAL REVIEWS · SEPTEMBER 2011

Impact Factor: 46.57 · DOI: 10.1021/cr200131e · Source: PubMed

3 AUTHORS:

Francisco J Schopfer
University of Pittsburgh
75 PUBLICATIONS **3,079** CITATIONS
SEE PROFILE

Chiara Cipollina
Fondazione Ri.MED
16 PUBLICATIONS **416** CITATIONS
SEE PROFILE

Bruce A Freeman
University of Pittsburgh
350 PUBLICATIONS **36,770** CITATIONS
SEE PROFILE

Published in final edited form as:
Chem Rev. 2011 October 12; 111(10): 5997–6021. doi:10.1021/cr200131e.

Formation and Signaling Actions of Electrophilic Lipids

Francisco J. Schopfer[†], **Chiara Cipollina**[‡,§], and **Bruce A. Freeman**[†,*]

[†]Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, United States

[‡]Fondazione Ri.MED, Piazza Sett'Angeli 10, 90134 Palermo, Italy

[§]Institute of Biomedicine and Molecular Immunology, Italian National Research Council, Via U. La Malfa 153, 90146 Palermo, Italy

1. INTRODUCTION

The post-translational modification (PTM) of proteins (e.g., phosphorylation, acetylation, ubiquitinylation, SUMOylation) is a fundamental mechanism of cell signaling that modulates protein structure and function, leading to the multiplication of proteome diversity. Current data reveal that oxidative inflammatory conditions and dietary constituents result in the PTM of proteins by electrophilic species. Electrophiles contain an electron-poor moiety, conferring attraction to electron-rich nucleophiles. In turn, nucleophiles donate electrons to electrophilic species to form a covalent bond via Michael addition.[1,2] A better understanding of the chemistry of electrophilic species will permit prediction of downstream reactions and subsequent biological responses that also include tissue-protective and anti-inflammatory actions.

Because of the susceptibility of unsaturated fatty acids to diverse oxidation and radical addition reactions, the formation of electrophilic byproducts is extensive during both basal metabolism and inflammatory responses and is the focus of this article. The PTM of proteins by electrophilic fatty acid oxidation and nitration products predominantly occurs by S-alkylation of protein thiols, with other nucleophilic amino acids serving as less favorable targets. Recently, high-performance liquid chromatography–mass spectrometry (HPLC-MS) and affinity chemistry-based strategies have revealed that there is both extensive protein PTM by electrophiles and a high degree of selectivity for electrophilic PTM reactions. Signal selectivity and reversibility of fatty acid electrophile-mediated PTM also occurs, indicating that essential prerequisites are fulfilled for this signaling mechanism as a feasible cell regulatory process that links metabolic and inflammatory status with redox-dependent information transfer. Because of the broad anti-inflammatory signaling actions observed for lipid-derived electrophiles, these species also hold promise as potential drug candidates that can shift inflammatory signaling reactions from propagation and injury mechanisms to resolution. For some classes of electrophiles, less favorable genotoxic and cytotoxic responses can also occur.[3–7]

This review addresses lipid-derived electrophilic species by first focusing on biochemical characteristics of electrophiles and the mechanisms underlying the endogenous formation of electrophilic species. This information is placed in the context of the cell signaling pathways modulated by electrophilic fatty acid derivatives and how these reactions can impact

[*]**Corresponding Author**, Bruce A. Freeman, Ph.D., E1340 Biomedical Science Tower, 200 Lothrop Street, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, United States. Phone: 412-648-9319. Fax: 412-648-2229 freerad@pitt.edu.

physiological and pathophysiological processes. In aggregate, we advance the concept that organisms have evolved a population of redox-sensing proteins, in particular transcriptional regulatory proteins, containing electrophile-reactive amino acids that respond to electrophilic species by regulating metabolic and inflammatory-related gene expression. This facet of transcriptional regulation presents abundant pharmacological opportunities.

1.1. Chemical Properties of Electrophiles

Several approaches have been used to explain the molecular determinants underlying the electrophilicity and nucleophilicity of molecules.[8,9] The most accepted concepts stem from early studies that establish a classification model for electrophiles and nucleophiles based on the hard/soft acid–base model (HSAB).[10] The initial terms were defined by Pearson,[11] who classified molecules based on their characteristics as soft bases, hard bases, soft acids, and hard acids. The main differences between soft and hard species rely on the ease of ionization and polarization of the outer electron shell.[12] Whereas hard electrophiles (acids) have a high positive charge density or a formal positive charge at the electrophilic center, soft electrophiles have a more diffuse charge density. In general, hard electrophiles have a small size and electrons in the outer shell are not easily excited, making these molecules difficult to polarize. In contrast, soft electrophiles are formed by the electron-withdrawing substituents (e.g., nitro and keto groups) reacting with the carbon atoms of unsaturated fatty acid acyl chains. These soft electrophiles are characterized by more diffuse distribution of electron deficiency or partial positive charge. This can be exemplified by α,β-unsaturated carbonyls (keto-fatty acids), which have no net charge but rather manifest polarization of electron density across a multiatom structure.

The major determinant for the selectivity of electrophile reaction with nucleophiles is determined by the relative ability of the outer shell electrons to polarize. The bases (nucleophiles) can also be characterized in these terms, with hard bases being more difficult to polarize and highly electronegative. In contrast, soft bases are more readily polarized and oxidized, being associated with empty, low-lying electron orbitals. This classification is important, because it gives a framework for understanding and predicting the biological reactions of electrophilic species. In this regard, soft electrophiles will preferentially react with soft nucleophiles and hard electrophiles will react with hard nucleophiles. The energy transition states between electrophiles and nucleophiles determine the preferential reactivities of these groups.

Most of the characteristics of these chemical reactions can be rationalized through the electrophilicity and/or nucleophilicity of the different species involved in reactions, highlighting the importance of a rigorous definition of electrophilicity.[1] The HSAB theory establishes that the selectivity and reaction rates between nucleophiles and electrophiles will depend on the hardness state of the reactants, with maximal rates of reaction expected between molecules with similar hardness values. In this regard, for reactions to occur between hard/soft combinations of electrophiles and nucleophiles, high energy transitions are needed.[13,14] The concept of electrophilicity can also be conceptualized as the lowering of energy that occurs with a maximum amount of electron flow between the nucleophile and the electrophile. These concepts were pioneered by Pearson and Songstad[14] and then further refined by experimental findings from Parr et al. in their “electrophilicity index” studies.[15]

In a biological context, the concept of electrophilicity has been applied to understand the reactions of soft electrophile constituents of proteins such as those found in the HIV nucleocapsid.[16] The mechanism of soft electrophile neurotoxicity has been evaluated in terms of quantum mechanical parameters, with different parameters of electrophilicity also correlated with experimental kinetic values and synaptosome inhibition.[17] The chemical rules that govern the reactions between lipid-derived electrophiles and proteins are relevant,

because they allow for a prediction of cellular responses to change in the generation of electrophilic species. To better understand biologically relevant electrophiles, the hardness (η), softness (σ), and chemical potential (μ) can be calculated and used as quantum mechanical predictors of their reactivity. For example, softness (σ) was the most relevant predictor of reactivity between electrophiles and thiols.[17] From a growing body of work, it is now appreciated that cells and tissues have developed complex sensors and signaling mechanisms that react with and respond to different types of electrophiles. These systems are able to detect electrophiles in different hardness categories and respond accordingly. Moreover, these determinants will define the patterns of gene expression that will occur in response to various electrophilic stimuli.

A common type of reaction of soft electrophiles with nucleophiles is termed Michael addition (Figure 1).[18] Most of the biological Michael addition reactions addressed herein pertain to soft–soft reactions. The softest biological nucleophiles are cysteine thiols, constituents of proteins and the tripeptide antioxidant glutathione (GSH), followed by the harder primary and secondary amines of lysine and histidine.[18] Despite a weaker Michael donor reactivity than cysteine, lysine and histidine also undergo covalent reaction with aldehydes. In the case of 4-hydroxy-2-nonenal (HNE), this yields Schiff's base adducts that are not readily reversible (Figure 1). Most proteomic analyses of lipid-derived electrophile reactions thus reveal cysteine, histidine, and lysine as principal targets.

The degree of lability of electrophile-protein reactions is in part dictated by the nature of the bond energy between the electrophile and its target. Notably, protein stability (e.g., renal clearance, proteolysis), further metabolism of the electrophilic lipid (e.g., double-bond saturation, β-oxidation, additional oxidation or nitration) and the reversibility of the Michael addition reaction via chemical or enzymatic reactions are all important but largely unexplored factors also influencing adduct lability. The cellular turnover of biotinylated iodoacetamide and maleimide-based electrophile adducts with proteins has been studied in cells using proteomic approaches and occurred largely a result of metabolism.[19] The authors concluded that the lower toxicity responses to the maleimide-based electrophilic species studied was a consequence of more transient protein adduction characteristics. Recent murine studies, where electrophilic fatty acid (FA) nitroalkene derivatives were injected intravenously, show that electrophilic FA nitroalkene derivatives acquire an extended half-life in vivo by undergoing reversible and exchangeable electrophilic reactions with nucleophilic plasma protein targets.[20] Loss of nitro-fatty acids in blood and tissues was via saturation of the alkenyl group, and the formation of coenzyme A derivatives and subsequent β-oxidation reactions that terminate at the site of acyl-chain nitration. Radioisotope tracing studies also suggest that esterification into complex lipids and other not yet identified metabolic routes were also occurring.

The metabolic fate of electrophilic lipids in vivo is an important issue from both pharmacological and toxicological perspectives and has not been well-defined kinetically or mechanistically. Current data supports the precept that the alkylation of proteins by lipid-derived electrophiles is a dynamic process. Now investigators are faced with the challenge of determining the reversibility of lipid electrophile–protein reactions, specifically, whether adducted electrophiles can undergo exchange reactions between different proteins. Also, the kinetics of a) exchange reactions between an electrophile and different proteins and between a protein adduct and GSH and b) GSH adduction and ultimate excretion are unknown. and the kinetics of both these reactions and GSH adduction and clearance from the cell via multidrug resistance protein transport mechanisms[21] and ultimate excretion.

The constraints of proteomic approaches for detecting posttranslational modifications can sometimes introduce bias into the determination of target residues. This is largely due to

differences in the ionization and detection of peptides from individual proteins. Thus, allied approaches such as introducing point mutations in susceptible amino acids of a target protein can assist in addressing the importance of particular electrophile target residues in the overall modification of protein function. A proviso for this approach is that one or more point mutations may influence overall protein structure, function, and susceptibility of other protein substituents to electrophile reaction. Finally, despite the usefulness of the softness parameter in prediciting electrophile reactivities, other properties will also strongly influence the reactivity of electrophiles toward nucleophilic targets. The microenvironment of target nucleophiles, such as hydrogen bonding reactions with neighboring amino acids, will modulate nucleophile ionization. In particular, the electrophile reactivity of a thiolate anion is orders of magnitude greater than for its protonated and less nucleophilic thiol counterpart. In other words, protein conformation-induced decreases in cysteine pK_a will increase reactivity for Michael adduct formation.[22,23]

2. BIOLOGICAL FORMATION OF ELECTROPHILIC FATTY ACID DERIVATIVES

Four principal substituents confer electrophilic properties to unsaturated fatty acids: aldehyde, α-β-unsaturated carbonyl, epoxide, and nitroalkene. The most common electrophilic substituents of fatty acids are type 2 alkenes. Molecules in this group are defined by an electron-withdrawing group linked to an alkenyl carbon and include α,β-unsaturated carbonyls and nitroalkenes (Figure 2).[24] The electron-withdrawing oxygen(s) of keto and nitro groups renders the β-carbon electron poor and thus susceptible to Michael addition. A fundamental characteristic of α,β-unsaturated carbonyl and nitroalkene derivatives is a soft electrophile nature, thus restricting the breadth of molecular targets in cell and tissue compartments.

2.1. Nonenzymatic Oxidation Reactions

The nonenzymatic formation of electrophilic fatty acid derivatives stems from oxidant-induced autocatalytic lipid peroxidation. Early studies of food rancidification showed that the decay in food quality included changes in color, loss of flavor, development of off-flavors, unpleasant odor, and an overall loss of nutrient value caused by lipid oxidation.[25] The free radical chemistry governing these reactions is well established, and the biochemical properties of major oxidation products have been extensively studied. Lipid peroxidation occurs with both free and esterified fatty acids, leading to the formation of hydrophobic and hydrophilic electrophilic products. In particular, the S_n2 position of phospholipids is commonly occupied by mono- or polyunsaturated fatty acids, providing the most relevant pool for lipid peroxidation reactions. Molecular oxygen, a key modulator of lipid peroxidation, is hydrophobic and concentrates in membranes.

The electrophilic products derived from nonenzymatic oxidation of polyunsaturated fatty acids result from free radical-induced oxidation reactions that abstract a hydrogen. Specifically, bisallylic hydrogens have an acidity that makes abstraction favorable, and their radical products are resonance-stabilized. After this initiation phase, a propagation phase takes place that includes reaction of carbon-centered radicals with oxygen to form a peroxyl radical. This radical can reduce itself to a lipid hydroper-oxide by abstracting a hydrogen atom from adjacent polyunsaturated fatty acids or lipophilic reductants such as α-tocopherol, generating secondary radical species. During propagation reactions, alkoxyl and epoxyallylic radicals are also formed. These radicals are responsible for chain breakdown pathways that yield short-chain aldehydes typically having α,β-unsaturated aldehyde substituents [i.e., acrolein, 4-hydroxy 2-nonenal (HNE), 4-hydroperoxy 2-nonenal (HpNE), 4-oxo 2-nonenal (ONE), 4-hydroxyhexenal (HHE), 4-hydroperoxyhexenal (HpHE), and 4-

oxohexenal (OHE)] (Figure 3). Notably, ONE, OHE, and HNE have an α,β-unsaturated ketone as an additional functional group, yielding a class of bifunctional species that contain both a conjugated aldehyde and a second functional group that can be a conjugated ketone, epoxy, hydroxy, or hydroperoxy group.[26] These bifunctional molecules can react with proteins by either Michael addition or irreversible Schiff's base reactions, or via both mechanisms (Figure 1).[27,28] The formation of a Schiff's base adduct typically requires harder electrophiles such as aldehydes that preferentially react with primary and secondary amines, such as those present in proteins and DNA bases. In contrast, the α,β-unsaturated ketone moieties would be expected to preferentially form Michael adducts with cysteine thiols. Alkenals are also generated and are harder electrophiles that can react with the exocyclic amino groups of deoxypurines (deoxyadenine and deoxyguanosine) and deoxypyrimidines (deoxycytosine) to form etheno adducts.[29] We will not address the nucleophilic oxygen atoms of the purine and pyrimidine bases of DNA and RNA, which generally react irreversibly with hard electrophiles such as nitrosamines to induce mutagenic responses.[30]

The nonenzymatic formation of isoprostanes shares the initial steps of free radical-induced lipid peroxidation. Upon reaction of a carbon-centered radical with oxygen, instead of a further hydrogen abstraction, the peroxyl radical can also undergo a 5-exo cyclization with a second addition of oxygen, resulting in the formation of prostaglandin-like compounds. Like prostaglandins, isoprostanes can also undergo metabolic oxidation to form hydroxyl substituents that can in turn be metabolized to electrophilic α,β-unsaturated ketone derivatives.[31–35] The endogenous formation of A_4/J_4-neuroprostanes (A_4/J_4-NPs) was originally viewed as a marker of oxidative stress;[36] however, it has also become evident that electrophilic A_4/J_4-NPs can mediate anti-inflammatory signaling actions that have the potential to attenuate inflammation and oxidative stress.

2.2. Nonenzymatic Nitration Reactions

The formation of lipid electrophiles containing electron-withdrawing groups other than oxygen has not been studied until recently. Halogenated unsaturated fatty acids, predominantly found as vinyl ether-linked complex lipids, would be expected to have electrophilic reactivity.[37–39] Unfortunately, the cell signaling actions of these inflammatory oxidant [hypochlorous acid (HOCl), hypobromous acid (HOBr)]-derived species have not been addressed. The appreciation of the free radical species nitric oxide (NO) as a diffusible and lipophilic mediator of endothelial function, neurotransmission, and inflammation has also expanded our perspective about endogenous mechanisms of membrane and lipoprotein oxidation.[40–42] In particular, previously unappreciated endogenous reactive species can potently impact lipid oxidation and downstream cell signaling responses. Reactions that result in the generation of electrophilic fatty acids are mediated by oxides of nitrogen, including the product of the reaction of NO with superoxide ($O_2^{\cdot -}$), peroxynitrite ($ONOO^-$). Peroxynitrite also readily undergoes protonation to peroxynitrous acid (ONOOH) or reacts with carbon dioxide (CO_2) to form nitrosoperoxocarbonate ($ONOOCO_2$), both of which can undergo homolytic scission to form $\bullet NO_2$.[43] Also, both the protonation and oxidation of nitrite (NO_2^-) yield nitrogen dioxide ($\bullet NO_2$).[44–46] Nitrite is ultimately converted to $\bullet NO_2$ following its protonation to nitrous acid during acidic conditions or after its direct oxidation by heme peroxidases.[46] Notably, many of these reactive inflammatory mediators stem from enzymatic origins including nitric oxide synthases and NAD(P)H oxidases.

Nitrogen dioxide readily abstracts bisallylic hydrogens, which in turn initiates lipid peroxidation in the presence of oxygen. Because both $\bullet NO_2$ and NO readily react with carbon-centered fatty acid radicals,[42] these species can also inhibit inhibit propagation reactions during lipid peroxidation. These reactions will give rise to nitrosylated, nitrated, or nitrito-containing fatty acids, with only nitro derivatives stable enough to be detectable in

biological milieu.[47] The most striking characteristic of nitro-fatty acids is an electrophilic character. Specifically, $\bullet NO_2$ reacts with the π-electron of alkenes via an addition reaction (Figure 4). Then, the reaction of a second $\bullet NO_2$ results in the reformation of the double bond and yields a nitroalkene, with no apparent hydrogen abstraction occurring during $\bullet NO_2$-dependent oxidation of unsaturated fatty acids.[47–49] This is in strong contrast with the view of lipid peroxidation reactions wherein hydrogen abstraction of bisallylic carbons forms a carbon-centered radical first, followed by double-bond rearrangement to a conjugated diene. Subsequent reactions of oxygen then form peroxy radicals and hydroxy radicals to finally give hydroxy- and peroxy-containing fatty acids.[50] Notably, (i) polyunsaturated fatty acids with conjugated dienes are particularly susceptible to nitration in vitro and in vivo, as opposed to methylene-interrupted species and (ii) a significant product detected during fatty acid nitration in vivo includes the formation of individual polyunsaturated fatty acids having both α,β-unsaturated-keto and nitroalkenyl groups. This electrophilic species is favored in the presence of oxygen and involves a double-bond rearrangement and reaction with oxygen instead of reaction with a second $\bullet NO_2$ molecule (Figure 4).

2.3. Enzymatic Formation of α,β-Unsaturated Keto Fatty Acids

Several enzymatic oxidation mechanisms lead to the biological formation of electrophilic species. Although the levels of these electrophilic species are low under normal physiological conditions, stimuli such as the enhanced expression and activation of specific enzymatic sources of partially reduced oxygen and NO-derived species during inflammation can significantly increase extents of formation of electrophilic fatty acid derivatives. Three heme- and nonheme-containing metallo-enzyme families are predominantly responsible for the enzymatic oxygenation of fatty acids: lipoxygenases (LOX), cycloxygenases (COX),[51] and cytochromes P450.[52] These enzymes display a myriad of biological effects that depend on the context and patterns of product formation. For example, depending on biological conditions, pro-inflammatory, anti-inflammatory, vasodilatory, or vasoconstrictor responses can be observed. Net cell signaling and physiological responses will depend on factors such as the patterns of oxygenase distribution in the different cell types in an inflammatory locus. This in turn will impact patterns of lipid signaling mediator formation. In some cases, a concerted transcellular oxygenation of fatty acids may be induced by multiple oxygenases residing in different cell types, a scenario suggested by the detection of trihydroxy derivatives of polyunsaturated fatty acids.[53] Notably, there are multiple NAD^+ and $NADP^+$-dependent dehydrogenases capable of readily oxidizing α,β-unsaturated mono-, di-, and trihydroxy fatty acids into electrophilic α,β-unsaturated ketones.[54–58] This mechanism supports the production of electrophilic α,β-unsaturated ketones from omega-3 fatty acids, which reside in an equilibrium between free and protein-adducted species reactions. The further oxidation of hydroxy fatty acids will also strongly influence mechanisms of downstream signaling, by shifting from the putative G-protein-coupled receptor signaling actions proposed for trihydroxy fatty acid derivatives[59] to the broader array of electrophile-regulated anti-inflammatory signaling processes described herein.[60]

Electrophilic α,β-unsaturated ketones are produced by activated macrophages and other cell types via a two-step enzymatic process involving (at least) COX-2-dependent hydroxylation, followed by oxidation of the hydroxyl group to a ketone by multiple dehydrogenases.[57] Mono-oxygenation occurred with 20 and 22 carbon fatty acids having 4–6 double bonds, with 22:5 and 22:6 yielding predominantly 13-oxo and 17-oxo derivatives, respectively. During the metabolic stress often associated with the activation of inflammatory cells, changes in the redox state of NAD^+ and NADH typically promote higher NAD^+ levels. For example, this occurs in activated inflammatory cells during the neutrophil respiratory burst and upon macrophage, microglial, and eosinophil activation. These conditions will support lipid oxidation and thus provide hydroxy derivatives for subsequent dehydrogenase

reactions, favoring the formation of α,β-unsaturated ketone derivatives.[56,58,61] Unsaturated fatty acid constituents of complex lipids are also enzymatically oxygenated. In neutrophils, LOX-catalyzed oxidation of membrane-bound arachidonic acid yields multiple hydroxylated phosphatidylethanolamine and phosphatidylcholine derivatives, which are then oxidized by dehydrogenases to yield electrophilic glycerophospholipids.[62–64]

2.4. Conditions That Promote Endogenous Electrophilic Fatty Acid Generation

Inflammatory conditions create a microenvironment that promotes the enzymatic and nonenzymatic oxidation of unsaturated fatty acids. This milieu is characterized by an increased rate of production of partially reduced oxygen species, oxides of nitrogen (NO, $ONOO^-$, $\bullet NO_2$, NO_2^-), and the increased expression of oxidases and oxygenases. Concomitantly, alterations in lipase activation promote the release of free fatty acids from complex membrane lipids that may already be oxidized or nitrated, or serve to provide free fatty acid substrates for subsequent generation of electrophilic lipid derivatives. The innate immune system (e.g., macrophages, eosinophils, and neutrophils) contributes significantly to this milieu. Depending on specific inflammatory conditions and tissue compartments, different gene expression patterns and inflammatory cell types can predominate, in turn impacting on oxidized and nitrated fatty acid profiles. Examples of biological conditions where electrophilic fatty acids are generated at increased levels abound; herein we focus on cardiovascular and cerebrovascular generation of these species.[18,60,65–67]

Myocardial ischemia and reperfusion injury (I/R) is characterized by elevated concentrations of activated neutrophils (a source of 5-LOX) and reactive species such as O_2^-, hydrogen peroxide (H_2O_2), HOCl, and NO and secondary nitrosating and nitrating species.[68] The rates of production of these reactive species are elevated in both I/R events and ischemic preconditioning (IPC). Reperfusion injury significantly contributes to tissue damage during myocardial infarction, due to the sudden increase of oxygen concentration in previously highly reduced ischemic myocardial cells, leading to inflammatory cell infiltration and extensive oxidative damage.[68,69] IPC is induced by short episodes of I/R that protect the heart from more extended periods of ischemia, followed by reperfusion.[70] In both conditions, the reperfusion phase is characterized by a transient increase in rates of reactive oxygen species generation, elevated rates of production of NO and its metabolites, an acidic pH, and the activation of both LOXs and mitochondrial phospholipase A_2.[70] This milieu promotes the generation of nitrating species and the formation of electrophilic fatty acid nitroalkene derivatives that are generated by free radical-mediated nitration of fatty acids through mechanisms enhanced by acidic pH (Figure 3). This scenario for how free radical-mediated fatty acid nitration might occur is further reinforced by the findings that (a) nitroalkene formation requires the reintroduction of oxygen during reperfusion and (b) nitric oxide synthase inhibition limits fatty acid nitration.[68]

Activated microglia are the primary cell type in the brain responsible for the inflammatory phenotype in Alzheimer's disease (AD), contributing to accelerated rates of oxygen reduction and increased expression of COX-2 activity.[71] Docosahexaenoic acid (DHA), rich in the brain, serves as the substrate for A_4/J_4-NP production by free radical mechanisms. Formation of A_4/J_4-NPs involves the generation of a DHA radical followed by addition of molecular oxygen, resulting in a peroxyl radical that mediates cyclic endoperoxide intermediate formation. This in turn isomerizes to E_4/D_4-NPs that, upon dehydration, yield electrophilic cyclopentenone A_4/J_4-NPs. A_4/J_4-NPs found in the brain either as free acids or phospholipid-esterified species, where they remain available for further release by phospholipases.[36]

Other electrophilic species that are formed during inflammatory reactions are HNE and 15d-PGJ_2. HNE is generated upon oxidation of omega-6 fatty acids such as arachidonic acid

(AA) and linoleic acid. The electrophilic 15d-PGJ_2 is generated via a semienzymatic pathway, wherein COX-2 catalyzes the first step by converting AA into prostaglandin H_2 (PGH_2), which is further oxidized by PGD_2 synthetase into an unstable PGD_2 intermediate that undergoes dehydration to 15d-PGJ_2. Endogenous generation of 15d-PGJ_2 has been observed in inflammatory conditions in tissues characterized by high concentrations of COX-2 expressing cells such as activated macrophages.[72]

2.5. Concentrations of Endogenously Generated Electrophilic Fatty Acids

The low concentration of endogenously generated electrophilic lipids measured in plasma or tissues has raised concerns regarding their biological significance.[73] Importantly, the facile Michael addition reactions that occur in biological milieu support protein and GSH adduction reactions can abrogate one's ability to directly detect net extents of electrophilic lipid generation. Because of their innate reactivities, electrophiles will also be expected to react and signal proximally to sites of generation where they would be at higher concentrations than species that have diffused from sites of generation in a specific tissue compartment to remote anatomic locations (e.g., plasma). For this reason, as observed for in vitro studies of cellular responses to various eicosanoids, it is not unusual to have to use bolus addition of μM electrophilic mediator concentrations to elicit responses that might be induced by nM concentrations generated over time in vivo. In support of this precept, the time-dependent accumulation of covalent target protein adducts has been observed to occur with the electrophilic lipid 15d-PGJ_2. Thus, functionally significant adduction of susceptible proteins can occur and result in signaling responses in spite of very low concentrations of an electrophilic species being detectable in vivo.[74]

The quantification of endogenous electrophilic fatty acid derivatives will thus also pose multiple challenges. Electrophilic lipids are susceptible to β-oxidation, reduction of functionally critical olefins, transport from the site of generation by binding to albumin or other carriers, or covalent adduction with glutathione or other low molecular weight thiols to be further degraded to mercapturic acids and excreted through urine and bile. Because electrophilic lipids can potentially exist in both adducted and free forms, the equilibrium depends on the rate constant of nucleophile reactions, the reversibility of Michael addition, and the above factors. Because of their reactivity and amphipathic nature, the detection of adducted electrophiles has to consider both their binding to large proteins (which can be precipitated) and the challenges lent by binding to smaller peptides that can escape precipitation (e.g., GSH).[57] Thus, extraction efficiency and how robust one's detection approaches are will add complexity to electrophilic lipid quantification. The capture of free electrophiles and the exchange of adducted species to high concentrations of an added low molecular weight nucleophile such as β-mercaptoethanol can provide a quantitative method for the measurement of total electrophile levels in biological systems. This also limits the variability inherent in determining and detecting the distribution of species between free and bound forms.[75] This method has been successfully applied to the measurement of both free and adducted α,β-unsaturated keto- and nitro-fatty acid levels in experimental models of inflammation, I/R, and IPC.[57,75,76] A remaining challenge is to chemically or enzymatically de-esterify electrophilic fatty acids from complex lipids without modification of the substituents conferring electrophilic reactivity.

Formation of nitro-oleic acid (OA-NO_2) and nitro-linoleic acid (LNO_2) has been observed in murine cardiac tissue after I/R injury (Table 1). No such nitro-FAs were detectable under basal conditions following sham surgery.[69] Similarly, a 50-fold increase of LNO_2 levels were observed following I/R injury in a rat model, going from basal levels of 0.011 pmol/mg mitochondrial protein to 0.57 pmol/mg mitochondrial protein.[75] Endogenous levels of LNO_2, primarily stemming from conjugated linoleic acid precursors, showed a significant increase in a rat IPC model, becoming elevated to 0.619 pmol/mg mitochondrial protein

while the levels of OA-NO_2 remained stable at 0.230 pmol/mg before and after IPC. This contrasts with I/R data, where OA-NO_2 concentrations in infarcted regions of the heart significantly increased. Such discrepancies may be due to differences in the experimental model. In fact, the occurrence of necrosis and apoptosis, typical of I/R but not of IPC, may lead to a different profile of hydrolyzed fatty acids and nitration reactions.[68] It is again noted that the quantification of esterified nitroalkenes is hindered by their instability under enzymatic and base- and acid-catalyzed hydrolysis strategies.

The formation of A_4/J_4-NPs has been measured in brains of healthy humans and patients affected by Alzheimer's disease (AD). Basal levels of ~0.3 pmol/mg of brain were measured in healthy humans,[31,36] probably due to the basal generation of reactive species by the high mitochondrial activity of the brain. In AD, A_4/J_4-NPs significantly increased to ~0.8 pmol/mg[31] due to the oxidative stress typical of this condition. These levels of A_4/J_4-NPs correspond to a concentration of ~950 nM, which falls within the range of concentrations observed to induce cell-signaling responses (0.5–5 μM).[31]

The formation of 15d-PGJ_2 in a carrageenin-induced pleurisy murine model showed levels as high as 2.5 nM in inflammatory exudates during the resolution phase.[72] A recent study also reported 15d-PGJ_2 in plasma samples from both patients affected by multiple sclerosis (MS) and healthy controls. Plasma 15d-PGJ_2 levels were ~47 nM, and levels did not change in the presence of this pathological condition (Table 1).

In experimental models where concentrations of electrophiles were measured and normalized based on protein content, concentrations were calculated to fall within the range of 0.01–0.8 pmol/mg of protein. An exception is the much higher levels measured for HNE in healthy and diseased states.[77,78] Concentrations of HNE in human brain have been reported to range from 0.18 nmol/mg of protein to ~1.28 nmol/mg of protein in brains of Alzheimer's disease patients.[77,78] Assuming that these reported concentrations can be compared, this represents more than a 1000-fold difference in concentration as compared to the neuroprotective A_4/J_4-NPs measured in brains of Alzheimer's disease patients or the nitroalkene generation measured in IPC.[31,70] This difference, combined with differences in structure and reactivity, may explain why HNE is frequently viewed as a toxic rather than cytoprotective electrophile.[79–81] As for other lipid electrophiles, however, challenges remain for incisive determination of HNE levels in different tissue compartments; thus, the net actions of especially lower concentrations of HNE in biological milieu remain to be established.

2.6. Toxic Actions of Electrophilic Fatty Acid-Derived Species

The toxicity of electrophilic species observed in cell and in vivo models is not just a function of the rate of electrophile—target molecule reactions. Other factors such as the downstream metabolism of electrophilic species, the actions of their metabolic byproducts, and the reversibility of Michael addition also play an important role.[65] The initial reactivity of an electrophile towards a nucleophile is influenced by the pK_a of both thiol and amino-containing residues and the tertiary structure of the protein. The metabolic inactivation of the electrophilic group by enzymes such as aldehyde dehydrogenases, aldo-keto reductases, and nitroalkene reductases can also impact the reaction and downstream signaling events mediated by electrophile—target molecule complexes.

As opposed to the beneficial effects reported for other classes of electrophilic lipids, the literature predominantly supports that HNE manifests detrimental actions. This is likely due to the high concentrations it can reach, a potent reactivity, and its capacity for dual participation in irreversible Schiff's base formation and poorly reversible Michael addition. In autopsy brain tissue samples from Alzheimer's disease patients, for example, HNE

adducts were detected in the amyloid β-peptide (Aβ) and were related to pathogenic aggregation responses. Furthermore, in AD brain, HNE covalently adducted the electrophile detoxifying enzymes glutathione S-transferase (GST) and multidrug resistance protein-1 (MRP-1). This contributed to HNE-dependent impairment of cellular detoxification systems and overall toxic responses to HNE.[79] Formation of HNE has also been observed in systemic lupus erythematosus (SLE), a disorder characterized by the presence of autoantibodies where oxidative damage contributes to the development of the pathology. HNE was found in SLE patient red blood cells, forming covalent adducts with the detoxifying enzyme catalase, suggesting a possible pathophysiological mechanism in SLE.[82]

3. ELECTROPHILE SENSING MECHANISMS AND METABOLIC RESPONSES

A broad array of electrophilic species are detectable under basal physiological conditions. When steady-state concentrations rise in response to dietary intake of electrophile-containing foods, metabolic stress, and inflammation, alterations in cell and tissue homeostasis can occur as a consequence of electrophile adduction of functionally significant nucleophilic amino acids of proteins. The post-translational modifications induced by electrophiles lead to changes in tertiary and quaternary structures, catalytic acitivities that depend on reactive nucleophilic amino acids, alterations in charge and hydrophobicity, subcellular relocalization, and, in the case of bifunctional electrophiles, protein cross-linking. These target proteins can serve a variety of metabolic functions including enzyme catalysis, transcriptional regulation, ion and macromolecule transport, cytoskeletal function, and host defense, to name a few.[83–87] Notably, many cytoprotective and anti-inflammatory responses are induced by electrophilic species, suggesting that equilibrium can be established between inciting events, extents of electrophile generation, protein adduction, and adaptive cellular responses. We discuss in the next section functionally significant electrophile-responsive targets that allow organisms to respond and adapt to alterations in metabolic and inflammatory status.

3.1. Kelch-like ECH-Associated Protein 1 (Keap1) and Nuclear Factor (Erythroid-Derived-2)-like 2 (Nrf2)

Studies of combustion-related carcinogenic compounds in 1918 led to the discovery that exposure of rabbits and mice to a coal tar extract induced skin tumors.[88] In 1930, these carcinogenic effects were ascribed to a purified constituent of coal tar, 3,4-benzopyrene.[89,90] This discovery of a genotoxic agent was followed by studies showing that organisms responded to benzopyrene exposure by induction of the expression of phase II detoxifying enzymes.[91–93] In the late 1970s it was appreciated that exposure of cell and animal models to low doses of electrophiles inhibited carcinogen-induced tumorigenesis[94] via a phase II detoxifying enzyme-mediated mechanism that was under the control of cis elements associated with target genes termed both antioxidant response elements (ARE)[95] and electrophile response elements (EpRE).[96] Using a Nrf2-deficient murine model, it was proven that Nrf2 modulated the induction of phase II genes[97] and that Keap1 is a negative regulator of Nrf2 that binds to the Neh2 region of Nrf2. It was hypothesized that electrophile adduction of Keap1 would induce dissociation from Nrf2, permitting Nrf2 translocation to the nucleus and its binding to specific cis elements, thus promoting the activation of ARE-regulated genes.[98] This pioneering work has revealed that Nrf2 plays an essential role in modulating cell differentiation and protecting against oxidative and inflammatory injury by regulating the expression of phase II genes and antioxidant enzymes.[99–101]

Under basal conditions, Nrf2 is retained in the cytoplasm through binding with its inhibitor, the ubiquitin ligase adaptor Keap1. Keap1 contains five functional domains: the N-terminal region; the Bric-a-Brac, tramtrack, broad complex (BTB) domain; the intervening region (IVR); the Kelch domain; and the C-terminal region (Figure 5A). Keap1 binds Nrf2 via the

Kelch domain and triggers a signal for cullin-3 (Cul3)-dependent ubiquitination and proteasomal degradation.[102] Elevated levels of reactive species such as electrophiles disrupt the interaction between Keap1 and Nrf2 and facilitate newly synthesized Nrf2 to escape Keap1 association and ultimate proteasomal degradation (Figure 5B). Nrf2 then is competent to accumulate in the nucleus where it forms heterodimers with small Maf proteins and recruits other factors required for the transcriptional activation of ARE transcriptional elements. This induces the activation of the ARE-dependent genes heme oxygenase-1 (HO-1), Mn superoxide dismutase, glutathione S-transferases (GSTs), NAD(P)H oxidoreductase, and other key defensive or adaptive enzymes.[103–106]

The redox-dependent regulation of Nrf2 activity depends on the oxidation or alkylation of several susceptible reactive cysteines on Keap1, which thus act as a sensor for the redox status of cells.[107] In particular, C-273, C-288, and C-151 have been identified as critical cysteines responsible for electrophile-dependent regulation of Keap1.[102,104,105,108,109] C-273 and C-288 are located in the Keap1 intervening region (IVR) and are required for maintaining Nrf2 turnover under basal conditions.[102,105,109–111] C-151 is located in the BTB domain and is critical for Nrf2 accumulation in response to electrophiles such as sulphoraphane.[102] The mitigating role of C-273 as an electrophile target may be species-dependent, as the murine Keap1 C-273 shows a higher reactivity to electrophiles than C-273 of human Keap1.[112] Several biologically relevant electrophiles, such as 15deoxy-PGJ_2, AA-derived cyclopentenone isoprostanes, nitroalkenes, sulphoraphane and HNE, covalently react with Keap1 (Table 2).[99,109,113] The activation of Nrf2-dependent gene expression responses accounts for a major component of the cytoprotective actions of electrophilic fatty acids. Table 2 summarizes the “cysteine code” that different electrophiles can display, wherein specific patterns of Keap1 cysteine reactions will occur depending on the physical nature of the electrophile and its interactions with Keap1.[109]

Multiple alterations of Nrf2-regulated signaling modify disease processes. For example, alveolar macrophages from the lungs of COPD patients express much lower levels of Nrf2 compared to macrophages from healthy subjects, suggesting an impairment of phase II gene expression.[114] In a murine model of asthma, disruption of Nrf2 leads to enhanced severity of the asthmatic response due to an impairment of the antioxidant system.[115] Besides chronic inflammatory disorders, impairment of Nrf2 is linked to metabolic syndrome. For example, a high-fat diet represses Nrf2 expression in mice and leads to increased hepatic and serum cholesterol and free fatty acids.[116] From a different perspective, although the impairment of Nrf2-regulated gene expression leads to pathological conditions, upregulation of this pathway can manifest protective effects. This is the case for atherosclerosis where it has been shown that Nrf2 is induced in regions of arteries exposed to high shear stress, providing protection from plaque development and anti-inflammatory effects.[117]

In contrast to the above, recent studies have also revealed that persistent stabilization of Nrf2 by genetic and epigenetic mechanisms, such as mutating or gene silencing the Keap1 gene or oncogene-directed increased expression of Nrf2, can be associated with development of cancers.[101,118,119] Thus, under some circumstances there can be either beneficial or detrimental effects of electrophiles that target Nrf2 activation for chemoprevention. For this reason, it is proposed that future clinical electrophile-based chemopreventive studies be designed so that Nrf2 activators are administered within a dose range that induces activation of the Nrf2 pathway in a manner that promotes protective rather than undesired carcinogenic side-effects.[118]

3.2. Heat Shock Factor 1 (HSF1)

Heat shock proteins (HSPs) are molecular chaperones whose expressions are induced in response to stresses including heat, metabolic dysregulation, electrophiles, and exposure to

inflammatory-derived reactive species. There are several families of HSPs that contribute to cellular homeostasis by reducing protein denaturation, preventing aggregation of denatured or oxidized proteins and assisting in the translocation of proteins to their sites of intracellular localization[120] A broader involvement of HSPs in cell physiology is now evident, including the modulation of signaling events such as NF-κB-regulated inflammatory responses, the Keap1/Nrf2-dependent antioxidant responses, and apoptotic signaling.[121–124]

The anti-inflammatory actions of HSPs are mediated to a significant extent by HSP70, which associates with TRAF6, prevents the degradation of IκBα, and inhibits p65 nuclear translocation. These actions are manifested by the inhibition of LPS-induced NFκB inflammatory responses.[123,124] The heat shock protein HSP90 is also involved in the regulation of Nrf2 function by binding with Keap1 and weakening its interaction with Nrf2.[125] The antiapoptotic actions of the heat shock response rely on HSF1-dependent induction of the protein BAG3, which contributes to the stabilization of the antiapoptotic protein Bcl-X_L. This in turn limits HNE-induced toxicity[121,122] and further underscores the importance of HSPs in maintaining cellular homeostasis.

HSPs act via noncovalent interactions with exposed hydrophobic surfaces of unfolded proteins. The expression of HSPs is controlled predominantly at the transcriptional level by the transcription factor HSF1. Under physiological conditions, HSF1 is localized in the cytoplasm and associates with the heat shock proteins HSP90 and HSP70 (Figure 6).[126,127] When protein folding is perturbed by electrophile adduction or oxidative reactions, HSF1 translocation to the nucleus is promoted, resulting in the transcriptional activation of HSP genes. Notably, electrophilic lipid derivatives promote the activation of the heat shock response by interacting with HSP90 and HSP72.[128] Both of these proteins share a thiol-specific mechanism of inactivation that renders them sensitive to electrophile adduction.[84] For example, HNE binds to the C-572 of HSP90 and other susceptible Cys residues of the same protein.[129] Similarly, HNE binds to Cys-267 in the ATPase domain of HSP72, impairing its chaperone function.[130]

Although the specific mechanism for electrophile-dependent activation of HSF1 has not been established, one tenable hypothesis is that electrophile adduction of Hsp90 and Hsp72 induces dissociation from HSF1, leading to nuclear accumulation of HSF1, its trimerization and phosphorylation, to finally the induction of heat shock responsive genes by binding to and activating heat shock response elements (HSEs)[128] (Figure 6). In addition, protein unfolding or increased hydrophobicity induced by electrophile adduction can facilitate protein association with HSPs, thus releasing HSF1 and activating the heat shock response. Similar to what has been observed for HNE, HSF1 is activated by other electrophiles such as sulforaphane,[131] nitroalkenes,[100] and 15d-PGJ_2.[132] Considering the broad involvement of the heat shock response in protecting cells and tissues from external insults and in suppressing the inflammatory response, the sensitivity of HSF1-regulated gene expression to activation by electrophilic species further reinforces that a broad and highly conserved pattern of salutary signaling actions are responsive to this class of signaling mediators.[122,128,132]

3.3. Peroxisome Proliferator-Activator Receptor γ

Peroxisome proliferator-activator receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily that is highly expressed in multiple tissue compartments including adipose, cardiovascular, and pulmonary tissues, as well as differentiated macrophages.[133] PPARγ commonly forms a heterodimer with the retinoid X receptor (RXR) and regulates the expression of genes involved in adipogenesis, glucose and lipid metabolism, macrophage differentiation, and immune responses.[76,133,134] Transcriptional activation requires the binding of the PPARγ DNA-binding domain to PPARγ response elements (PPREs), a

process strongly modulated by the association and dissociation of coregulatory proteins.[135,136] The regulatory domain of PPARγ resides in the C-terminal region, within the ligand-binding domain (LBD). This region contains a large, generally hydrophobic ligand-binding domain that accommodates a broad array of lipophilic ligands including long-chain fatty acids.[137,138] The presence of a cysteine at position 285 within the LBD confers a special sensitivity for electrophilic lipids, specifically α,β-unsaturated ketone and nitroalkene derivatives.[137,139,140] Several endogenously generated electrophilic fatty acids activate PPARγ by undergoing covalent adduction of C-285 via Michael addition. Covalent binding of PPARγ has been reported for 15d-PGJ2, 4-oxo-DHA, 5-oxo-EPA, 6-oxo-OTE, arachidonic acid oxo-derivatives, and nitroalkenes.[76,141–144] Despite variability in chain length, molecular structure, and electrophilic moiety, all of these electrophiles react with the same C-285 (Table 2), with the presence of an electrophilic group on a long-chain polyunsaturated fatty acid the only requirement for covalent binding. The PPARγ ligand pocket is so promiscuous that it even allows the docking of up to two long-chain fatty acids. This characteristic may provide different electrophilic lipids the opportunity to be accommodated simultaneously in the pocket and react with C-285. Upon ligand binding, the PPARγ LBD undergoes conformational changes that both stabilize and recruit specific coregulatory proteins, specifically activators and repressors.[145] The specificity of this regulatory mechanism is a consequence of each ligand inducing a different conformational change in helix 12 of the LBD, leading to the recruitment of a specific set of coregulators.[143,146] The potency of the inducing ligand correlates with the degree of stabilization of the LBD.

Importantly, the covalent binding of electrophilic lipids to the LBD of PPARγ provides a unique form of receptor activation, because the saturation kinetics that typically governs ligand binding to receptors do not apply to a covalent interaction.[141] Thus, very low concentrations of an electrophilic PPARγ ligand can accumulate over time and result in significantly greater extents of receptor occupancy and activation than a nonelectrophilic counterpart. This precept is reinforced by the observation that nonelectrophilic PUFAs are much less potent PPARγ activators than electrophilic derivatives.[57] The activation of PPARγ by electrophilic lipids, which are best described as partial ligands, results in a cascade of downstream events that remain to be identified in detail. Depending on the cell type and condition, electrophile-dependent PPARγ activation displays a range of beneficial actions. For example, nitroalkene-dependent activation of PPARγ restores insulin sensitivity and blood glucose levels in a murine model of diabetes.[76] Also, angiotensin II-induced hypertension, the restenosis of injured vessels, atherosclerotic plaque accumulation, inflammatory bowel injury, and ischemic myocardial damage is limited by in vivo nitro-fatty acid administration at nM levels in preclinical disease models.[69,147–150] Furthermore, these PPARγ agonists display PPARγ-independent anti-inflammatory actions, repressing the expression of several pro-inflammatory enzymes and cytokines, such as iNOS, IL-10, and IFNγ.[134] Because of the pluripotent signaling actions of electrophilic fatty acids, it remains a challenge to ascribe a specific signaling pathway, such as PPARγ activation, as the mitigating mechanism responsible for the broad tissue-protective and anti-inflammatory actions that these species exert.

3.4. Nuclear Factor κB

The electrophile-dependent modulation of nuclear factor κB (NF-κB) function represents one of the best-defined mechanisms accounting for the anti-inflammatory actions of lipid-derived electrophiles. NF-κB is a transcriptional regulatory protein complex that plays a major role in control of inflammation and immune responses.[151] NF-κB is a heterodimer composed of two subunits, p50 and p65. Under basal conditions, NF-κB is retained in the cytoplasm by interaction with its inhibitor IκB. Pro-inflammatory stimuli activate IκB kinase

(IKK), which in turn phosphorylates IκB, leading to its ubiquitinylation and proteasomal degradation. Upon IκB degradation, NF-κB is free to translocate to the nucleus and activate the transcription of pro-inflammatory genes including cytokines (TNFα, IL-1, IL-6), chemokines (IL-8, MCP-1), inflammatory enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), adhesion molecules (ICAM-1, VCAM-1), and transmembrane receptors.[152,153] Electrophilic lipids potently inhibit the activation of NF-κB and downstream pro-inflammatory events[154] (Figure 7).

A complex mechanism of action for electrophile-dependent modulation of NF-κB signaling has emerged, wherein electrophiles act at multiple levels. Upstream, 15d-PGJ_2, A_4/J_4-NPs, and HNE all covalently adduct the highly conserved C-179 in the activation loop of IKKβ, resulting in IKK inhibition, IκBα stabilization, and NF-κB inhibition.[31,155,156] A more direct mechanism of NF-κB inhibition involves Michael addition of electrophiles at the C-38 residue of p65, located in the DNA-binding domain.[157,158] This modification is induced by nitroalkenes and 15d-PGJ_2 and results in the loss of DNA binding and transcriptional activity by p65. Similarly, p50 is susceptible to electrophile inhibition upon covalent modification with the reactive cysteine at position C-61 in the DNA binding domain.[159] Through these mechanisms of NF-κB signaling inhibition, electrophilic lipids act as redox-derived anti-inflammatory mediators that downregulate the inflammatory response to resolve inflammation. This precept has been supported by several in vivo models of inflammation.[69,72,147,149] For example, in an in vivo model of carrageenin-induced pleurisy where endogenous 15-PGJ_2 generation has been measured, further supplementation with 15d-PGJ_2 mediates anti-inflammatory actions by reducing inflammatory cell infiltration and exudate volume.[72] To add further complexity to this scenario, NF-κB and Nrf2 share target genes, as in the case of IL-6. In fact, Nrf2 may promote specific inflammatory processes through activation of IL-6.[160] On the other hand, an antioxidant role has been proposed for IL-6. Although it appears a treatment that would at the same time repress NF-κB and induce Nrf2-dependent transcription may be a successful strategy to develop new therapies against inflammatory disorders, attention must be paid to the undesired side-effects due to excessive induction of Nrf2.

Abnormal activation of NF-κB is a hallmark of virtually all chronic inflammatory disorders, as well as acute events such as I/R injury. In a rat model of cerebral ischemia, activation of NF-κB expression and downstream signaling was observed after reperfusion. Consistent with this, disruption of the NF-κB subunit p50 resulted in reduced infarct size.[161] Similarly, NF-κB was induced in a mouse model of cardiac I/R with consequent upregulation of pro-inflammatory cytokine and adhesion molecule expression.[68] Persistent and dysregulated activation of NF-κB is a typical feature of chronic lung diseases, such as asthma and COPD, and neurodegenerative disorders. In the case of Alzheimer's disease, activation of NF-κB in neurons and astroglia occurs in the proximity of early plaques of brains from Alzheimer disease patients. The pro-inflammatory transcriptional remodeling observed in AD patients was thus related to the activation of NF-κB. Interestingly, the neurotoxic Aβ peptide induced NF-κB transcriptional activation.[162] NF-κB-dependent transcriptional reprogramming is also observed in COPD, where increased expression of the p65 subunit has been observed in bronchial biopsies of smokers with or without COPD, compared to nonsmokers.[163] This suggests a possible cause—effect relationship in which persistent smoke-induced activation of pro-inflammatory NF-κB leads to a chronic pathological state. Abnormal activation of NF-κB has also been observed in asthmatic patients that display increased levels of NF-κB p65, IκB phosphorylation, and IKKβ protein levels compared to normal individuals.[164]

Persistent activation of NF-κB also leads to chronic inflammation, which can favor the development of neoplastic malignancies.[165] Inflammation may favor cancer development through the production of enzymes such as matrix metalloproteinases (MMPs) that promote

the metastatic evolution of tumors. Notably, fatty acid nitroalkene derivatives also stimulate activation of proMMP-7 and proMMP-9 proteolytic activity via adduction of the conserved cysteine switch domain thiolate, confirming a role for electrophile-mediated thiol alkylation in MMP activation.[166] In addition, MMP expression is suppressed by nitroalkenes via activation of PPARγ to an extent similar to that induced by the PPARγ agonist Rosiglitazone, subsequently limiting the further progression of inflammatory processes. Another mechanism by which chronic inflammation contributes to tumorigenesis is the production of reactive species during inflammatory reactions. In this respect, the role of Nrf2 in chemoprevention is relevant.[165,167] There is a strong interplay between Nrf2 and NF-κB signaling responses, where activation of Nrf2 blunts NF-κB-dependent inflammatory responses, while NF-κB represses Nrf2 transcriptional activity via binding to its promoter elements.[168] Therefore, it appears that interrupting pro-inflammatory loops with an approach that would concomitantly repress NF-κB and induce Nrf2-dependent transcription simplify and state may be a successful therapeutic strategy. In this regard, electrophilic lipids offer great potential.

3.5. Peroxiredoxins

Peroxiredoxins (Prx) are thiol-dependent antioxidant proteins that exert tissue-protective actions through their peroxidase activity. Prx play a crucial role in redox regulation, defense against oxidative stress, regulation of redox-sensitive transcription factors, and the refolding of disulfide-containing proteins. Prx are maintained in their reduced state by the thioredoxin system, composed of thioredoxins (Trx) and thioredoxin reductase (TrxR). The activity of Prx, Trx, and TrxR depends on both catalytic and structural cysteine residues. Electrophiles impact redox homeostasis of the cell by direct reactions with both cytosolic and mitochondrial Prx, Trx, and TrxR.[169] Cyclopentenone prostaglandins, HNE, and isothiocyanates adduct TrxR and Trx, inhibiting their catalytic activity[170–172] and enhancing the oxidation of Prx.[173] Alternatively, HNE can also adduct Prx6 at noncatalytic cysteines, resulting in conformational changes that will impair catalytic activity.[174] By interacting with the Trx—Prx system, electrophiles can activate redox-sensitive signaling pathways. In this regard, the mitochondrial Prx—Trx system is more susceptible to electrophile-dependent regulation than its cytosolic counterpart.[169,173] This is highly relevant, because mitochondria are important sources of reactive species and electrophilic lipid oxidation products.[75,175,176]

3.6. Histone Deacetylases

Histone deacetylases (HDACs) mediate transcriptional regulation by modulating chromatin structure, which in turn determines the accessibility of DNA to transcription factor binding. HDACs remove acetyl groups from histone lysine residues, promoting chromatin condensation and inhibiting transcription. In particular, class I HDACs play a major role in gene silencing by cooperating with corepressor complexes.[177,178] The activity of HDACs is modulated by the redox state of the cells because of the presence of several reactive cysteines that can be readily oxidized by reactive oxygen species. 15d-PGJ_2 and HNE both covalently adduct class I HDACs by binding with two highly conserved noncatalytic cysteine residues present in HDAC-1, -3, and -4, but not HDAC-8.[177,179] Alkylation by reactive carbonyls also blocks the interaction of HDACs with their histone substrates, causing an increase in histone acetylation and transcriptional activation of HDAC-repressed genes such as HO-1 and HSP70. The regulation of HDAC activity by electrophilic lipids thus operates in concert with the modulation of redox-dependent transcription factors such as Nrf2, PPARγ, and HSF1 to promote transcriptional activation. These parallel mechanisms contribute to the salutary and synergistic transcriptional reprogramming induced by electrophiles.

3.7. Mitogen-Activated Protein Kinases (MAPKs)

Phosphorylation has historically been viewed as the principal post-translational modification that expands the scope of the functional proteome. Tyrosine and serine phosphorylation provide signaling pathways with the capability of operating via fast “on/off switches” when acting in concert with phosphatases. Notably, it is now evident that both kinases and phosphatases are critical targets of electrophile reactions.

The MAPK pathway is a signaling cascade involved in the regulation of physiological events including cell proliferation, differentiation, and apoptosis. The MAPK pathway includes several subfamilies, among which are the extracellular signal-regulated protein kinase (ERK) pathway, the JNK pathway, and the p38 MAPK pathway. Each of these signaling pathways consists of three levels of kinases that are regulated upstream by the Ras protein and Rac/Cdc42. Although it is traditionally recognized that the regulation of these kinases occurs via phosphorylation, it has recently emerged that MAP kinases are sensitive to electrophile-dependent regulation. For example, 15-PGJ_2 adduction activates H-Ras to induce downstream activation of the MEK/ERK and p38 MAPK pathway.[180–183] This covalent modification appears to be specific for the isoform H-Ras, because 15d-PGJ_2 fails to alkylate N-Ras or K-Ras.[180] Evidence also supports that HNE covalently binds Erk2 at H-178 within the kinase phosphorylation lip and inhibits Erk enzymatic activity and signaling.[184]

An alternative mechanism through which electrophiles may contribute to the activation of MAPK signaling pathway is via the apoptosis signal-regulating kinase 1 (ASK 1), a MAPKKK upstream of JNK and p38. Under basal conditions, ASK1 is bound to Trx, forming an inactive complex. Oxidation of Trx reactive cysteines causes the release of ASK1, leading in turn to activation of p38 and JNK signaling. Depending on the persistance of this activation and the extent of cellular damage, ASK1 may modulate inflammation by either regulating cytokine production or leading cells to apoptosis.[185] Electrophile-dependent modulation of Trx may also participate in this signaling mechanism. Overall, although current data strongly support an involvement of electrophiles in the modulation of MAPK pathways, controversies remain because of the high degree of complexity, interconnectivity, and redundancy of MAPK signaling responses.

3.8. Protein Tyrosine Phosphatases

Protein tyrosine phosphatases (PTPs) are a large family of enzymes that hydrolyze the phosphate group from phosphotyrosine residues. Their actions counterbalance the effect of the protein tyrosine kinases (PTKs), which promote the activation of signaling reactions involved in cell proliferation, differentiation, and metabolic regulation.[186,187] The mechanism of action of PTPs involves the formation of a phospho-cysteine intermediate, in which the highly conserved catalytic cysteine binds the phosphate group, followed by hydrolysis. Because of its low pK_a (~5.4), the PTP catalytic cysteine exists as thiolate anion within physiological pH ranges and confers redox-dependent regulation of PTP activity.[187,188] Electrophiles are thus strong candidates for Michael addition to this catalytic cysteine, which is conserved in >95% of the ~100 PTPs described to date. Therefore, it is not suprising that acrolein and 1,2-naphthoquinone (1,2-NQ) covalently adduct the C-215 of PTP1B, inhibiting enzymatic activity.[186,189] Similarly, less-direct proteomic evidence reveals that the electrophile HNE inhibits PTP1B via similar mechanisms.[190] In addition to adducting the catalytic cysteine, 1,2-NQ alkylates C-121 and H-25.[186] It also appears that acrolein-mediated inhibition of PTP1B is dependent on the modification of a noncatalytic cysteine. We have also observed nitro-fatty acid modification of the catalytic thiol of PTP1B, leading to enzyme inactivation and alterations in downstream insulin signaling (unpublished) (Figure 8). In aggregate, these data support an additional linkage between

electrophilic fatty acid actions and the regulation of signaling events critical for intermediary metabolism, cell differentiation, and inflammatory responses.

3.9. Energy Metabolism

Mitochondria generate significant amounts of $O_2^{\cdot -}$ and H_2O_2 and are rich in unsaturated fatty acids that can serve as substrates for the generation of electrophilic byproducts. During inflammation, elevated rates of O_2^{-} and H_2O_2 generation and exposure of mitochondria to increased levels of NO and NO_2^{-} create conditions ideal for fatty acid oxidation and nitration. Mitochondria are thus a rich source of electrophilic signaling mediators, including nitro- and keto-fatty acids, as revealed by an ex vivo ischemic preconditioning model using rodent hearts.[70] Mitochondria are also highly susceptible to reaction with fatty acid-derived electrophiles because (i) many mitochondrial proteins contain functionally significant electrophile reactive cysteines;[191] (ii) there is an increase in matrix pH during coupled respiration, promoting thiol reactivity by increasing thiolate anion concentrations available for alkylation;[191] (iii) fatty acid-derived electrophiles impact respiratory chain function, alter oxidative phosphorylation, and modulate rates of $O_2^{\cdot -}$ production;[192–195] (iv) NO_2-FA can promote tissue-protective mitochondrial uncoupling reactions;[70] (v) electrophilic lipids covalently adduct and inhibit cytochrome c oxidase activity, thus imparing respiratory chain function;[196] and (vi) electrophiles react with the sulfhydryl group(s) of the lipoic acid present in the catalytic pocket of the two NADH-generating enzymes α-ketoglutarate dehydrogenase (KGDH) and pyruvate dehydrogenase, thus inhibiting their activity.[193,196,197] Additional mechanisms through which electrophiles modulate mitochondrial activity are via interaction with the uncoupling proteinsUCP1, UCP2, and UCP3 and with the adenine nucleotide translocase (ANT).[198] The mitochondrial ATP-sensitive potassium channel (mK(ATP)) is also activated by fatty acid nitroalkene derivatives.[199] This redox modulation of mK(ATP) may be an underlying mechanism for the regulation of this ion channel in the context of ischemic preconditioning.

Although under physiological conditions the inhibition of mitochondrial activity by electrophilic lipids may negatively impact energy metabolism, under many circumstances this also manifests clearly tissue-protective responses. For example, the partial uncoupling of mitochondria by electrophiles such as HNE and nitroalkenes reduce reactive oxygen species (ROS) production and protect cells and myocardial tissue from oxidative stress.[70,198,200] This is reinforced by a recent report describing a nutrient-sensitized screening strategy that differentiated changes in cellular respiratory versus glycolytic acivity, which showed that several already FDA-approved drugs shifted cellular energy metabolism from mitochondrial respiration to glycolysis. The impressive aspect of this study was that there was a very strong correlation between the extent of respiratory inhibition by small-molecule drugs and the limitation of ischemia-reperfusion injury to the heart and brain of cell and animal models.[201] One can expect sometimes divergent effects of electrophiles to be observed in animal model systems and intact cell studies versus investigations based on isolated mitochondria;[194,202] thus, more study is warranted to translate the above insights into more clinically relevant perspectives.

3.10. Glycolytic Enzymes

During glycolysis, energy is generated in the form of ATP and reducing equivalents such as NADH. Tissue glycolytic rates are modulated in response to energy requirements and the redox state of the cell. This modulation occurs at all levels of cell regulation—transcriptional, translational, and post-translational. In particular, post-translational modifications of critical glycolytic enzymes confer the possibility of rapid activity switches that are responsive to metabolic demand and environmental conditions. In addition to being regulated by phosphorylation-dependent mechanisms, several glycolytic enzymes are

sensitive to additional modes of regulation by virtue of functionally significant nucleophilic histidine and cysteine residues that in turn serve as electrophile sensors and readily undergo modification via Michael addition. For example, the cyclopentenone prostaglandin 15d-PGJ_2 adducts with and inhibits the enzymes enolase and lactate dehydrogenase.[203] Similarly, HNE covalently adducts enolase and fructose bisphosphate aldolase at H-246,[204] with the functional consequences of these modifications remaining to be elucidated. There is a strong functional linkage between adduction of the neuronal glucose transporter GLUT3 by HNE that inhibits glucose influx.[205] Similarly, modification of glyceraldehyde-3-P dehydrogenase (GAPDH) by HNE at multiple, noncatalytic residues leads to inhibition of enzymatic activity (Table 2).[206] Notably, fatty acid nitroalkene adduction of GAPDH, besides impairing enzymatic activity, promotes translocation of GAPDH to the membrane. This shifts the subcellular distribution of the enzyme and confers lipophilic properties in a manner that can affect metabolic complex formation.[207] Overall, electrophile-dependent modulation of glycolysis provides a means to coordinate energy metabolism with the redox state of the cell, in a manner akin to that observed for mitochondrial respiration.

4. Electrophile-Based Pharmacologic Strategies

The potent anti-inflammatory and tissue-protective signaling actions of soft electrophiles observed in diverse cell and rodent models have encouraged investigation of the potential for these species as drug candidates for treating acute and chronic inflammatory conditions, cancer, and metabolic disorders. To date, these studies have been conducted in animal models and earlyphase clinical trials.[118,208,209] There are additional issues that have also motivated this area of drug development with respect to fatty acid-derived electrophiles. First, many electrophilic fatty acids, their precursors, and other soft electrophiles are found endogenously or are present in food. Beneficial nutritionally related actions and a lack of toxicity have been linked with these dietary constituents that are sometimes sold as over-the-counter supplements (e.g., conjugated linoleic acid, omega-3 fatty acids, polyphenols). Second, electrophilic fatty acids may be particularly effective as drugs because they modulate at a defined molecular level specific metabolic and inflammatory signaling processes involved in disease processes.[115,117,168,210] For example, the induction of Nrf2-dependent antioxidant responses, the activation of HSF-regulated heat shock responses, the suppression of NF-κB-dependent pro-inflammatory gene expression, and ligand activity that activates the PPARγ-dependent transcriptional program represent the best-characterized actions of electrophiles. Consequently, the diseases presented are impacted by one or more of these signaling pathways and thus may be eligible for an electrophile-based therapeutic strategy. The following are the most promising areas for electrophile-based interventions: chemoprevention and chronic inflammatory pathologies, including respiratory, cardiovascular, and neurodegenerative disorders and metabolic diseases.

4.1. Chemoprevention

Carcinogenesis involves multiple stages during which genetic and environmental factors contribute to the malignant transformation of the cell. In particular, chemical modification of DNA due to irreversible reactions of hard electrophiles, oxidative stress, or inheritance of critical mutations in growth-regulatory mediators all can contribute to cell transformation. In addition, a link between chronic inflammation and cancer development has been established, where in some cases chronic inflammation predisposes one to cancer development.[165,211,212] Alternatively, the tumor may cause inflammation, which in turn would promote cancer development and metastatic evolution through production of ROS and tissue-damaging factors such as MMP expression and activation. In this respect electrophiles have shown potential in the prevention of cancer by acting on the major causes of cancer: DNA damage induced by chemicals and inflammation. The induction of Nrf2 has been extensively investigated in this regard as an explanation for electrophile-dependent

chemopreventive effects. The involvement of HSFs and inhibition of NF-κB along with other less well characterized actions of electrophiles may also contribute to reduce tumor development.

The induction of phase II enzymes is a central mechanism by which electrophiles prevent neoplastic transformation of cells in vitro. These encouraging in vitro results have led to translation of these strategies to clinical applications. For example, the chemopreventive potential of electrophiles present in food, such as sulforaphane, is being investigated in early-stage clinical trials focusing on cancer.[118,213] This has encouraged the progression of clinical trials to assess the efficacy of curcumin and sulforaphane for treating colon cancer in humans.[214] In the case of breast cancer, encouraging results stem from studies where it has been shown that dietary intake of sulforaphane results in overexpression of phase II enzymes in rat mammary epithelium and in humans.[215–219] In aggregate, electrophile-stimulated signaling events regulate several cell growth and differentiation-related mechanisms including expression of conjugating and antioxidant genes, anti-inflammatory responses, and molecular chaperone/stress response systems, all of which can alter susceptibility to carcinogenesis. The challenge for the field at this stage is to advance key drug candidates into later-stage efficacy studies in humans.

4.2. Neuroprotection

While ischemia-reperfusion injury is more of an acute event, neurodegenerative pathologies such as Parkinson's disease (PD) and Alzheimer's disease (AD) are chronic disorders with a significant inflammatory component that take years, if not decades, to evolve. AD and PD manifest late in life and present a complex pathological situation wherein oxidative stress and persistent inflammation contribute in part to protein aggregation and tissue degeneration. The involvement of oxidative stress, and consequently Nrf2-regulated signaling, in the evolution of AD and PD is supported by several lines of evidence. Besides coordinating the response to oxidative stress, the Keap1–Nrf2 pathway promotes a virtuous cycle in which the antioxidant effect sustains anti-inflammatory actions. Conversely, impairment of this cycle is often related to the development of neurodegenerative disorders.[115,117,168,210] This is the case in a murine model of AD, where Nrf2 protein levels and Nrf2 target genes such as NQO1 and those involved in GSH biosynthesis are downregulated in age-matched AD mice, as contrasted with healthy mice.[220] Moreover, several mutations of the PARK7 gene, coding for the Nrf2 stabilizer DJ-1, have been identified as sufficient for inducing a monogenic form of PD. Loss of DJ-1 causes instability of Nrf2, with a consequent reduction in detoxification enzyme expression, including NQO1.[221] This genetic alteration is characterized by enhanced oxidative stress, which constitutes a threat for nigral neuron stability. The auto-oxidation of the endogenous neurotransmitter dopamine will also induce the generation of locally high levels of reactive quinones and partially reduced oxygen species. This renders neurons particularly sensitive to oxidative stress and emphasizes the importance of the antioxidant response for protection from neurodegenerative disorders.[222]

The mitigating actions of electrophilic species have been studied in several in vivo models of central nervous system disorders, including PD and cerebral ischemic injury.[223–225] In all cases, the focus of the study was to test responses to the activation of Nrf2. Oral administration of the Nrf2 inducer 3*H*-1,2-dithiole-3-thione (D3T) in a murine model of PD protected neurons from death, with this effect abolished in Nrf2 null mice.[223] Similarly, intracerebroventricular or intraperitoneal pretreatment of mice with *tert*-butylhydroquinone (tBHQ) reduced motor deficit and cortical damage in a murine model of cerebral ischemia.[225] This effect was related to the tBHQ-induced increases in Nrf2 target gene expression, including NAD(P)H/quinone oxidoreductase (NQO1) and glutathione S-transferase (GST). While D3T and tBHQ represent chemical compounds not structurally

related to endogenous electrophiles, a new class of neuroprotective electrophiles has been recently synthesized that are related to cyclopentenone prostaglandins, termed neurite outgrowth-promoting prostaglandins (NEPPs).[224] NEPPs preferentially localize in neurons, resulting in neuronal cell protection in a murine model of ischemia-reperfusion injury by reducing infarct volume. This effect was significant when NEPPs were administered as a preventive strategy rather than postreperfusion, due to the requirement for Nrf2-dependent transcriptional activation.[224] Because of their neuron-specific localization, NEPPs may also be suitable for treatment of PD, which is characterized by the loss of dopaminergic neurons in the substantia nigra.[223]

An alternative approach to the administration of electrophiles would be the administration of precursors of electrophilic fatty acids, such as by supplementing the diet with the omega-3 fatty acids DHA and EPA, which can then be converted into anti-inflammatory and cytoprotective electrophilic derivatives such as resolvins, protectins, and electrophilic α,β-unsaturated ketones.[57] DHA and EPA can be consumed in relatively large doses and are suitable for performing clinical trials in humans. In this respect, a diet rich in DHA decreases the risk of AD and protects against neuronal damage.[226] In contrast, lower plasma concentrations of DHA esterified to complex lipids are correlated with the development of AD.[227] The identification of the active metabolite responsible for the beneficial effects of DHA will be crucial for therapies based on omega-3 FAs and would facilitate therapeutic optimization.

4.3. Cardiovascular Protection

The in vivo administration of electrophilic fatty acids shows protective cardiovascular effects when administered either before or after an episode of cardiac ischemia-reperfusion.[69] The transient phase of oxygen depletion, combined with the sudden increase of oxygen following reperfusion, leads to cell death and tissue damage that is in large part mediated by reactive inflammatory mediators and activation of NF-κB.[68] It is widely recognized that increased NO generation and mitochondrial preservation both play key roles in modulating ischemic injury. The finding that there is an increase in cytoprotective mitochondrial nitro-fatty acid generation following IPC and I/R injury also reveals new hypotheses that can reconcile previous findings.[69,228] Assuming a mitochondrial volume of 0.65 μL/mg of protein, endogenous intramitochondrial LNO_2 levels have been estimated at ~1.0 μM, close to the concentrations required for LNO_2 to induce mitochondrial uncoupling and cardioprotection. [70] In an I/R model, externally supplemented OA-NO_2 resulted in significant reduction of myocardial infarct size and preserved left ventricular function. These beneficial effects were observed even when administered after reperfusion.[68] OA-NO_2 suppressed NF-κB signaling by covalently binding NF-κB p65 and inhibiting its transcriptional activity.[69,229] This downregulated the expression of downstream inflammatory effectors such as the chemokine MCP-1 and the adhesion proteins VCAM-1 and ICAM-1, with a consequent reduction of neutrophil infiltration in the infarct zone.[68] An additional mechanism has also been proposed to explain the cardioprotective role of LNO_2 in IPC, wherein adduction of LNO_2 to the mitochondrial adenine nucleotide transporter (ANT) and uncoupling protein-2 (UCP-2) induced mild uncoupling, which likely contributed to tissue protection.[70] Also, the mitochondrial ATP-sensitive potassium channel was activated by LNO_2.[199]

After vessel wall mechanical injury, the systemic administration of OA-NO_2 also inhibited neointimal hyperplasia in the femoral artery, in a murine model of restenosis after angioplasty.[147] Both the progression of vessel injury and drug administration (given immediately after wire injury) went on for 21 days. Increased HO-1 expression, presumably under Nrf2 regulation, accounted for much of the vascular protection induced by OA-NO_2 in both cultured aortic smooth muscle cells and in vivo, because inhibition of HO by Sn(IV)-

protoporphyrin, small interfering RNA for HO-1, and genetic ablation of HO-1 expression and activity reversed all induced protective effects.

Systemic administration of OA-NO_2 also causes a sustained, dose-dependent reduction of Ang II-induced hypertension in mice and exerts a significant blood pressure-lowering effect on pre-existing hypertension established by Ang II infusion.[150] The improved vasodilation was specific for Ang II type 1 receptor (AT1R)-mediated signaling, because vascular constriction by other G-protein-coupled receptors was not altered in response to OA-NO_2 treatment. OA-NO_2, but not native oleic acid, specifically adducted the AT1R, reduced heterotrimeric G-protein coupling, and inhibited inositol-1,4,5-trisphosphate and calcium mobilization without inhibiting Ang II binding to the receptor.

Finally, the systemic administration of OA-NO_2 to apolipoprotein E-deficient mice fed a high-fat pro-atherogenic diet for 12 weeks induced extensive reduction in atherosclerotic lesion formation.[149] Immunochemical, histological, and gene expression analyses revealed that OA-NO_2 attenuated atherosclerotic lesion formation by suppressing tissue oxidant generation, inhibiting adhesion molecule expression, and decreasing vessel wall infiltration of inflammatory cells. In addition, OA-NO_2 reduced foam cell formation by attenuating oxidized low-density lipoprotein- induced phosphorylation of STAT-1, a transcription factor linked to foam cell formation in atherosclerotic plaques. Notably, atherosclerotic lesions of OA-NO_2-treated animals showed increased collagen content and α-smooth muscle actin levels, indicating greater plaque stability. In aggregate, these findings strongly suggest an involvement of fatty acid nitroalkene derivatives in conferring multiple cardiovascular protective mechanisms.

Additional electrophile-based pharmacologic studies have shown promise for the treatment of atherosclerosis. The use of sulforaphane derivatives, present in cruciferous vegetables, induces protective effects in a murine model of atherosclerosis.[117] The intraperitoneal injection of sulforaphane activates Nrf2 in susceptible regions of aorta, inducing anti-inflammatory and antiatherogenic gene expression. This suggests that eating cruciferous vegetables may lead to similar effects and links these beneficial actions to the activation of Nrf2. An alternative mechanism through which electrophiles may protect from the development of atherosclerosis is via the capablitiy of nitroalkene and α,β-unsaturated ketone fatty acid derivatives to serve as partial agonists of PPARγ-dependent gene expression.[57,140,143,230] Although this has not been proven in an electrophile-specific context, it has been reported that targeting PPARγ with specific agonists reduces the expression of inflammatory genes in atheroscleorotic lesions and the formation of foam cells.[231]

In the context of omega-3 fatty acids, there are strong associations with cardiovascular benefit and the dietary ingestion of DHA/EPA, fish oil supplements, and diets rich in seafood. For example, the GISSI clinical trial shows a very significant reduction in adverse myocardial events and mortality when dietary supplementation of omega-3 polyunsaturated fatty acids is provided after an initial myocardial infarction.[232] Omega-3 fatty acid supplementation, using both natural marine oil supplements and prescription preparations of DHA and EPA, is now widespread in clinical practice, after positive benefits have been shown in the reduction of blood pressure, arrhythmias, and the progression of heart failure.[233–235] One can expect continuing debate regarding the molecular and signaling events responsible for these clinically beneficial effects. In the context of the present discussion, however, the discovery that omega-3 fatty acids are readily converted to potent electrophilic signaling mediators and receptor ligands suggests that an even more targeted and effective therapeutic approaches for multiple inflammatory-related cardiovascular disorders can be anticipated.

4.4. Antidiabetic Strategies

Two precepts provide strong motivation for testing the impact of electrophilic fatty acids in treating type 2 diabetes. First, extensive data reveal that the hyperglycemic and hyperlipidemic conditions that lead to type 2 diabetes also induce a proinflammatory state in the organism.[236–238] Second, the PPARγ receptor is a primary drug target for restoring insulin sensitivity and lowering blood glucose in type 2 diabetics. In the context of diabetes and vascular diseases, PPARγ is expressed throughout the vasculature, including monocytes/macrophages, endothelial cells, adipocytes, and vascular smooth muscle cells.[239] The actions of the thiazolidinedione (TZD) class of synthetic PPARγ ligands, which includes Rosiglitazone and Pioglitazone, exemplify the broad role that this receptor class plays in regulating tissue homeostasis. In various animal models and clinical studies, TZDs (a) increase insulin sensitivity, (b) suppress chronic inflammatory processes, (c) reduce circulating free fatty acid levels, (d) correct endothelial dysfunction, (e) reduce fatty streak formation, (f) delay plaque evolution, (g) limit vessel wall thickening, and (h) enhance plaque stabilization and regression.[240] These benefits have been offset by recent meta-analyses of treatment trials of TZDs (compared with other therapies for type 2 diabetes or placebo). Recent data convincingly show that there is an increased risk of bladder cancer, myocardial infarction, and death from cardiovascular causes.[241–243] The net clinical benefits of TZDs are thus presently a source of intense debate and motivate further drug discovery wherein electrophilic fatty acids may present new options.

Two recent studies support antidiabetic potential for electrophilic fatty acids. Multiple α,β-unsaturated ketone derivatives of EPA and DHA have been shown to covalently adduct the C-285 of PPARγ and activate its gene expression program.[139,143] Translating this molecular insight into experimental models of obesity-induced diabetes, the α,β-unsaturated ketone derivative of DHA lowered blood glucose without inducing adverse changes in weight gain and serum lipids.[244] Also, leptin-deficient hyperglycemic mice supplemented with OA-NO_2 had lowered insulin and glucose levels. Compared to treatment with other PPARγ activators such as the TZD Rosiglitazone, OA-NO_2 displayed no adverse side effects such as increased adipogenesis and accelerated body weight gain.[76] Additional work has also shown that inhibition of the electrophile-sensitive protein tyrosine phosphatase 1B (PTP1B) will increase insulin sensitivity and resistance to obesity. This suggests that PTP inhibition may also be an electrophile-mediated mechanism of action in the treatment of diabetes and underscores the potential benefits of the multiple “off-target” pharmacologic actions expected for electrophilic species in vivo.[245] Overall, these data are promising and strongly support further studies of the efficacy of electrophilic fatty acids as partial PPARγ agonists and new drug strategies for treating diabetes.

4.5. Pharmacodynamics of the In Vivo Administration of Electrophiles

A promising aspect of electrophilic fatty acids as drugs is their favorable pharmacokinetics profile. Because of a covalent protein and GSH adduction character, a long binary complex residence time is expected between electrophilic fatty acid derivatives and molecular targets. This issue has been recognized to be even a more significant aspect of drug development than the apparent affinity of a drug for its target.[246] An additional hallmark of electrophiles as drug candidates rests in their pluripotent signaling actions, with the extensive supporting data discussed herein revealing that these species regulate multiple signaling pathways and protein effectors to transduce tissue-protective and anti-inflammatory actions.

The covalent reaction of electrophiles with nucleophiles can potentially pose bioavailability challenges upon oral or intravenous administration. For example, intravenously administered nitro-fatty acids readily undergo covalent reaction with blood proteins, with the reversibility of this reaction essential for downstream signaling actions. From another perspective, the

reversible adduction of an electrophilic species with gastric, intestinal, or plasma proteins may even be viewed as a mechanism to extend effective half-life and facilitate tissue delivery.[247] The use of liposomes or other nanoparticle formulations as carriers to encapsulate the electrophilic drug may also be useful.[248] This approach may improve absorption and bioavailability and allow a more targeted approach upon oral administration.

The distribution of systemically administered electrophiles has been investigated by measuring the parent compound and its metabolites in several target organs. In the case of OA-NO_2, the plasma half-life of both free and adducted parent compound is ~10 h when administered either by gavage or intravenously. Oral bioavailability is ~20% when calculated against plasma levels (thus an underestimate), and parent OA-NO_2 was distributed to all organs and tissues, with maximal distribution in liver and muscles.[247] Similarly, oral administration of the electrophile cucurmin showed systemic bioavailability and led orally bioavailable and led to increased levels of Nrf2 and the Nrf2 target gene NQO1 in lung and liver.[249] Similar results were obtained when sulforaphane (SFN) was orally administered to rats. The SFN metabolite dithiocarbamate (DTC) was measured in plasma and reached peak concentrations 1 h after oral administration. Tissue (mammary gland) expression of Nrf2-dependent NQO1 and HO-1 was observed in mammary glands 12 h after oral administration.[215] This work was translated to a clinical study in which women were given a filtered broccoli sprout drink containing sulforaphane about 1 h before undergoing breast surgery. The SFN metabolite DTC was found in breast epithelial cells and its concentration significantly increased in urine and plasma. Notably, the increased expression of NQO1 and HO-1 was observed in women's breast tissue after SFN administration. [215] Finally, in a short-term clinical study using a sulforaphane- rich beverage made from broccoli sprouts, there was ~70% oral bioavailability of sulforaphane, as indicated by analysis of urinary parent compound and metabolites.[250]

These data encourage further development of enriched or purified natural electrophilic products and synthetic homologues of biological electrophiles that replicate or improve bioavailability and pharmacologic potential. This is exemplified by the synthetic triterpenoid derivative 2-cyano-3,12-dioxooleana-1,9-dien-28-oic imidazolide (CDDO-Im), which, after oral administration at doses as low as 0.3 μmol/kg in rats, induced an increase in Nrf2 levels and its target gene NQO1 in several organs and tissues 6–9 h after treatment.[251] CDDO-Im thus represents an example of a naturally occurring compound that has been chemically modified to enhance its electrophilic character by altering the electron-withdrawing group and altering patterns of tissue distribution by the masking of charged moieties.

Although electrophilic fatty acid derivatives may provide a multilayered defense against inflammatory and oxidative disorders because of their multiple targets and modes of action, this broad reactivity may also present a risk for undesired side-effects. For example, although the efficacy of curcumin for chemoprevention may give specific promising results, the inhibition of COX-2 by curcumin may lead to increased cardiovascular risk and altered patterns of resolution of inflammation.[214] Also, the use of electrophile-based strategies for chemoprevention may result in “excessive” activation of Nrf2-regulated gene expression, conferring resistance to chemotherapy and possibly promoting carcinogenesis.[252] The fact that overexpression of the Nrf2 stabilizer DJ-1 is found in biopsies from lung and prostate cancers and that elevated expression of DJ-1 inversely correlates with clinical outcomes of nonsmall-cell lung carcinoma patients highlights this risk.[221] It has been proposed that, for a chemopreventive strategy to be effective, Nrf2 activators must be administered within a dose-range that induces minimal activation of Nrf2 signaling, to ensure protective responses without causing undesired pro-carcinogenic side-effects. Another potential negative outcome when using electrophile-based cancer therapeutic strategies may be the inactivation of the tumor suppressor PTEN (phosphatase and tensin homologue deleted on chromosome

10).[211] Although under normal physiological or inflammatory conditions this event may contribute to the resolution of inflammation by promoting tissue regeneration, this may also constitute a risk of malignant degeneration associated with inflammation. Although current data encourage the study of electrophilic species as chemotherapeutic agents, one must be open to unexpected signaling actions and net responses.

5. Future Perspectives

The data presented herein support the precept that metabolic and inflammatory events of organisms are under strong regulation by a population of redox-sensing transcriptional regulatory proteins and enzymes that respond to electrophilic lipid derivatives. This rapidly unfolding area of basic and clinical investigation motivates the search for additional endogenous electrophilic signaling mediators and reveals new pharmacological opportunities. If these objectives are to be pursued critically, one must always address evolving issues in the context of the broad reactivities that electrophilic lipids will display. When searching for novel electrophilic species in biological systems, new advances can be made in affinity capture and unique chemical trapping reactions that can assist in improving the sensitivity and specificity of electrophile detection—either in the free or macromolecule-adducted form.[18,75] As the field evolves, benefit also stems from sharing standardized procedures and reagents between laboratories, so that inevitable debates about concentrations, reactivities, and chemical structures can be minimized. In the context of drug discovery, exciting opportunities still abound for defining new signaling pathways and additional pathogenic events that can be modulated with electrophilic lipid derivatives — using both natural products and synthetic homologues. Detailed toxicological studies of potential adverse side-effects of both parent molecules and their metabolites will always be crucial in therapeutics development. This area would also benefit from being addressed in the context of drug delivery strategies that (i) provide greater extents of local delivery, (ii) employ prolonged pharmacokinetics (e.g., slow-release electrophile derivatives or encapsulation strategies), (iii) utilize pro-drugs that can be metabolized to electrophilic species (e.g., omega-3 fatty acids), or (iv) devise second-generation nonbiologicals that better target specific signaling events.

Biographies

Francisco J. Schopfer received his B.S. in Biology in 1999 and Ph.D. in Biochemistry in 2001 from the University of Buenos Aires, where he worked on mitochondrial redox regulation with Dr. Juan Jose Poderoso. He then worked as a postdoctoral fellow with Dr. Bruce Freeman studying the formation and signaling actions of endogenously generated redox species, including nitro- and keto-fatty acids. In 2006, Dr. Schopfer moved to University of Pittsburgh as an Instructor and then became Research Assistant Professor (2006) in the Department of Pharmacology & Chemical Biology. His work entails the formation, detection, quantification, signaling mechanisms, and physiological actions of electrophilic fatty acid derivatives.

Chiara Cipollina obtained her Ph.D. in Industrial Biotechnology at the University of Milan-Bicocca, Milan, Italy, in 2006. During her graduate training, she collaborated with Delft University of Technology, The Netherlands, studying cellular and molecular mechanisms that regulate cell growth and metabolism. She then conducted postdoctoral training at Delft University of Technology, where she developed strategies for the quantitative analysis of cell metabolism based on mass spectrometry techniques. In 2008, she moved to the Department of Pharmacology & Chemical Biology at University of Pittsburgh where she joined the Freeman group and described new endogenous lipid-derived electrophiles involved in anti-inflammatory signaling processes. Since April 2010, she has been at the Institute of Biomedicine and Molecular Immunology, Italian National Research Council, in Palermo, Italy. Her research investigates the anti-inflammatory actions of endogenous electrophilic lipids in chronic inflammatory disorders.

Bruce A. Freeman, Ph.D., is the Irwin Fridovich Professor and Chair of Pharmacology & Chemical Biology at the University of Pittsburgh School of Medicine. He received his B.S. and Ph.D. degrees from the University of California, Riverside, in biochemistry, was a postdoctoral fellow at Duke University, and then joined the faculty at Duke as an Assistant Professor in the Department of Medicine. From 1985 to 2005 he was a Professor of Anesthesiology, Biochemistry and Molecular Genetics at the University of Alabama at Birmingham. His research interests are focused on the production, reactions, and signal transduction properties of oxidizing and free-radical inflammatory mediators. Currently, Dr. Freeman’s lab is investigating the biochemical links between partially reduced oxygen species, oxides of nitrogen, and cell signaling events that impact on cell and organ function. His research on redox-derived signaling mediators has revealed new therapeutic approaches to the treatment of acute inflammation, respiratory disorders, and metabolic and cardiovascular diseases.

Acknowledgments

The research of the authors is supported by the American Heart Association, American Diabetes Association (F.J.S.), the Fondazione Ri.MED (C.C.), and NIH grants R01-HL058115, R01-HL64937, P30-DK072506, and P01-HL103455 (B.A.F.). B.A.F. acknowledges financial interest in Complexa, Inc. and Nitromega, Inc.

ABBREVIATIONS

1,2-NQ	1,2-naphthoquinone
15d-PGJ$_2$	15-deoxy-delta-12,14-prostaglandin J_2
4-HpNE	4-hydroperoxy 2-Nonenal
A$_4$/J$_4$-NPs	A_4/J_4 cyclopentenone neuroprostanes

AA	arachidonic acid
AD	Alzheimer's disease
ANT	adenine nucleotide translocase (ANT)
ARE	antioxidant response elements
Aβ	amyloid β-peptide
BTB	Bric-a-Brac, tramtrack, broad complex
CDDO-Im	1[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole
COPD	chronic obstructive pulmonary disease
COX	cycloxygenase
Cul-3	cullin 3
D3T	3H-1, 2-dithiole-3-thione
DHA	docosa-4,7,10,13,16,19-hexaenoic acid
DPA	docosa-7,10,13,16,19-pentaenoic acid
DTC	dithiocarbamate
EPA	eicosa- 5,8,11,14,17-pentenoic acid
EpRE	electrophile response elements
ERK	extracellular signal-regulated protein kinase
ETE	eicosa-5,8,11,13-tetraenoic acid
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GCLM	glutamate-cysteine ligase, modifier subunit
GSH	glutathione
GST	glutathione S-transferases
HDAC	histone deacetylase
HHE	4-hydroxyhexenal
HNE	4-hydroxy-2-nonenal
H_2O_2	hydrogen peroxide
HO-1	heme oxygenase 1
HOCl	hypochlorous acid
HpHE	4-hydroperoxyhexenal
HpNE	4-hydroperoxynonenal
HSAB	hard/soft acid base
HSE	heat shock response elements
HSF1	heat shock factor 1
HSP	heat shock protein
I/R	ischemia and reperfusion
ICAM-1	intercellular adhesion molecule 1

IFNγ	interferon γ
IKK	IκB kinase
iNOS	inducible nitric oxide synthase
IP_3	inositol-1,4,5-trisphosphate
IPC	ischemic preconditioning
IVR	intervening region
Keap1	Kelch-like ECH-associated protein 1
KGDH	α-ketoglutarate dehydrogenase
LBD	ligand-binding domain
LNO_2	nitro-linoleic acid
LOX	lypoxygenase
MCP-1	monocyte chemotactic protein
MMP	matrix metalloproteinase
MRP-1	multidrug resistance protein-1
NEPPs	neurite outgrowth-promoting prostaglandins
NO_2-FAs	nitro-fatty acids
NF-κB	Nuclear Factor-kappa B
NQO1	NAD(P)H quinone oxidoreductase 1
Nrf2	nuclear factor E2-related factor-2
$O_2^{\cdot -}$	superoxide anion
OA-NO_2	nitro-oleic acid
OHE	4-oxohexenal
ONE	4-oxo 2-nonenal
OTE	octadecatrienoic acid
PD	Parkinson’s disease
PDH	pyruvate dehydrogenase
PGH_2	prostaglandin H2
PPARγ	peroxisome proliferator-activator receptor γ
PPRE	PPARγ response elements
Prx	peroxiredoxins
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
PUFA	polyunsaturated fatty acid
RXR	retinoid X receptor
SFN	sulforaphane
SLE	systemic lupus erythematosus

tBHQ	*tert*-butylhydroquinone
TNFα	tumor necrosis factor-alpha
Trx	thioredoxins
TrxR	thioredoxin reductase
TZD	thiazolidinediones
UCP	uncoupling protein
VCAM-1	vascular cell adhesion molecule 1

REFERENCES

1. Chattaraj PK, Sarkar U, Roy DR. Chem Rev. 2006; 106:2065. [PubMed: 16771443]
2. Makosza M, Wojciechowski K. Chem. Rev. 2004; 104:2631. [PubMed: 15137803]
3. Schultz TW, Carlson RE, Cronin MTD, Hermens JLM, Johnson R, O'Brien PJ, Roberts DW, Siraki A, Wallace KB, Veith GD. SAR QSAR Environ. Res. 2006; 17:413. [PubMed: 16920662]
4. LoPachin RM, Barber DS, Gavin T. Toxicol. Sci. 2008; 104:235. [PubMed: 18083715]
5. Enoch SJ, Cronin MTD. Crit. Rev. Toxicol. 40:728. [PubMed: 20722585]
6. Gamboa da Costa G, Churchwell MI, Hamilton LP, Von Tungeln LS, Beland FA, Marques MM, Doerge DR. Chem. Res. Toxicol. 2003; 16:1328. [PubMed: 14565774]
7. Hinson JA, Roberts DW. Annu. Rev. Pharmacol. Toxicol. 1992; 32:471. [PubMed: 1605575]
8. Chermette H. J. Comput. Chem. 1999; 20:129.
9. Parthasarathi R, Subramanian V, Roy DR, Chattaraj PK. Bioorg. Med. Chem. 2004; 12:5533. [PubMed: 15465330]
10. Swain CG, Scott CB. J. Am. Chem. Soc. 1953; 75:141.
11. Pearson RG. J. Am. Chem. Soc. 1963; 85:3533.
12. Pearson RG. J. Chem. Educ. 1987; 64:561.
13. Coles B. Drug Metab. Rev. 1984; 15:1307. [PubMed: 6398776]
14. Pearson RG, Songstad J. J. Am. Chem. Soc. 1967; 89:1827.
15. Parr RG, Szentpály LV, Liu S. J. Am. Chem. Soc. 1999; 121:1922.
16. Maynard AT, Huang M, Rice WG, Covell DG. Proc. Natl. Acad. Sci. U. S. A. 1998; 95:11578. [PubMed: 9751708]
17. LoPachin RM, Gavin T, Geohagen BC, Das S. Toxicol. Sci. 2007; 98:561. [PubMed: 17519395]
18. Rudolph TK, Freeman BA. Sci. Signaling. 2009; 2:re7.
19. Lin D, Saleh S, Liebler DC. Chem. Res. Toxicol. 2008; 21:2361. [PubMed: 19548357]
20. Rudolph V, Schopfer FJ, Khoo NK, Rudolph TK, Cole MP, Woodcock SR, Bonacci G, Groeger AL, Golin-Bisello F, Chen CS, Baker PR, Freeman BA. J. Biol. Chem. 2009; 284:1461. [PubMed: 19015269]
21. Alexander RL, Bates DJ, Wright MW, King SB, Morrow CS. Biochemistry. 2006; 45:7889. [PubMed: 16784241]
22. Nagahara N, Matsumura T, Okamoto R, Kajihara Y. Curr. Med. Chem. 2009; 16:4490. [PubMed: 19903155]
23. Vogel EW, Nivard MJ. Mutat. Res. 1994; 305:13. [PubMed: 7508544]
24. Kemp DS, VF. Carbonyl condensation reactions. New York: Worth Publishers; 1980.
25. Addis PB. Food Chem. Toxicol. 1986; 24:1021. [PubMed: 3542756]
26. Gueraud F, Atalay M, Bresgen N, Cipak A, Eckl PM, Huc L, Jouanin I, Siems W, Uchida K. Free Radical Res. 44:1098. [PubMed: 20836659]
27. Parthasarathy S, Steinberg D, Witztum JL. Annu. Rev. Med. 1992; 43:219. [PubMed: 1580586]
28. Marnett LJ. IARC Sci Publ. 1999:17. [PubMed: 10626205]

29. Marnett LJ, Riggins JN, West JD. J. Clin. Invest. 2003; 111:583. [PubMed: 12618510]
30. Saparbaev M, Laval J. IARC Sci. Publ. 1999:249. [PubMed: 10626225]
31. Musiek ES, Brooks JD, Joo M, Brunoldi E, Porta A, Zanoni G, Vidari G, Blackwell TS, Montine TJ, Milne GL, McLaughlin B, Morrow JD. J. Biol. Chem. 2008; 283:19927. [PubMed: 18490445]
32. Brooks JD, Milne GL, Yin H, Sanchez SC, Porter NA, Morrow JD. J. Biol. Chem. 2008; 283:12043. [PubMed: 18263929]
33. Musiek ES, Breeding RS, Milne GL, Zanoni G, Morrow JD, McLaughlin B. J.Neurochem. 2006; 97:1301. [PubMed: 16638022]
34. Milne GL, Musiek ES, Morrow JD. Antioxid. Redox Signaling. 2005; 7:210.
35. Milne GL, Gao L, Porta A, Zanoni G, Vidari G, Morrow JD. J. Biol. Chem. 2005; 280:25178. [PubMed: 15878849]
36. Fam SS, Murphey LJ, Terry ES, Zackert WE, Chen Y, Gao L, Pandalai S, Milne GL, Roberts LJ, Porter NA, Montine TJ, Morrow JD. J. Biol. Chem. 2002; 277:36076. [PubMed: 12133837]
37. Albert CJ, Thukkani AK, Heuertz RM, Slungaard A, Hazen SL, Ford DA. J. Biol. Chem. 2003; 278:8942. [PubMed: 12643282]
38. Thukkani AK, Hsu FF, Crowley JR, Wysolmerski RB, Albert CJ, Ford DA. J. Biol. Chem. 2002; 277:3842. [PubMed: 11724792]
39. Albert CJ, Crowley JR, Hsu FF, Thukkani AK, Ford DA. J. Biol. Chem. 2002; 277:4694. [PubMed: 11836259]
40. Baker PR, Schopfer FJ, O'Donnell VB, Freeman BA. Free Radical Biol. Med. 2009; 46:989. [PubMed: 19200454]
41. Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA. J. Biol. Chem. 1994; 269:26066. [PubMed: 7929318]
42. O'Donnell VB, Chumley PH, Hogg N, Bloodsworth A, Darley-Usmar VM, Freeman BA. Biochemistry. 1997; 36:15216. [PubMed: 9398249]
43. Radi R, Denicola A, Freeman BA. Methods Enzymol. 1999; 301:353. [PubMed: 9919584]
44. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Proc. Natl. Acad. Sci. U. S. A. 1990; 87:1620. [PubMed: 2154753]
45. O'Donnell VB, Eiserich JP, Chumley PH, Jablonsky MJ, Krishna NR, Kirk M, Barnes S, rley-Usmar VM, Freeman BA. Chem. Res. Toxicol. 1999; 12:83. [PubMed: 9894022]
46. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, Van der Vliet A. Nature. 1998; 391:393. [PubMed: 9450756]
47. Freeman BA, Baker PR, Schopfer FJ, Woodcock SR, Napolitano A, d'Ischia M. J. Biol. Chem. 2008; 283:15515. [PubMed: 18285326]
48. Napolitano A, Camera E, Picardo M, d'Ischia M. J. Org. Chem. 2000; 65:4853. [PubMed: 10956463]
49. Napolitano A, Crescenzi O, Camera E, Giudicianni I, Picardo M, d'Ischia M. Tetrahedron. 2004; 58:5061.
50. Gutteridge JM, Halliwell B. Trends Biochem. Sci. 1990; 15:129. [PubMed: 2187293]
51. Lee SH, Rangiah K, Williams MV, Wehr AY, DuBois RN, Blair IA. Chem. Res. Toxicol. 2007; 20:1665. [PubMed: 17910482]
52. Murphy RC, Zarini S. Prostaglandins Other Lipid Mediators. 2002; 68–69:471.
53. Serhan CN, Yacoubian S, Yang R. Annu. Rev. Pathol. 2008; 3:279. [PubMed: 18233953]
54. Patel P, Cossette C, Anumolu JR, Erlemann KR, Grant GE, Rokach J, Powell WS. J. Pharmacol. Exp. Ther. 2009; 329:335. [PubMed: 19164464]
55. Wei C, Zhu P, Shah SJ, Blair IA. Mol. Pharmacol. 2009; 76:516. [PubMed: 19535459]
56. Powell WS, Rokach J. Prog. Lipid Res. 2005; 44:154. [PubMed: 15893379]
57. Groeger AL, Cipollina C, Cole MP, Woodcock SR, Bonacci G, Rudolph TK, Rudolph V, Freeman BA, Schopfer FJ. Nat. Chem. Biol. 2010; 6:433. [PubMed: 20436486]
58. Grant GE, Rokach J, Powell WS. Prostaglandins Other Lipid Mediators. 2009; 89:98. [PubMed: 19450703]

59. Chiang N, Serhan CN, Dahlen SE, Drazen JM, Hay DWP, Rovati GE, Shimizu T, Yokomizo T, Brink C. Pharmacol. Rev. 2006; 58:463. [PubMed: 16968948]
60. Khoo NK, Freeman BA. Curr. Opin. Pharmacol. 2010; 10:179. [PubMed: 20080062]
61. Cossette C, Patel P, Anumolu JR, Sivendran S, Lee GJ, Gravel S, Graham FD, Lesimple A, Mamer OA, Rokach J, Powell WS. J. Biol. Chem. 2008; 283:11234. [PubMed: 18287092]
62. Thomas CP, Morgan LT, Maskrey BH, Murphy RC, Kuhn H, Hazen SL, Goodall AH, Hamali HA, Collins PW, O'Donnell VB. J. Biol. Chem. 2010; 285:6891. [PubMed: 20061396]
63. Morgan LT, Thomas CP, Kuhn H, O'Donnell VB. Biochem. J. 2010; 431:141. [PubMed: 20653566]
64. Clark SR, Guy CJ, Scurr MJ, Taylor PR, Kift-Morgan AP, Hammond VJ, Thomas CP, Coles B, Roberts GW, Eberl M, Jones SA, Topley N, Kotecha S, O'Donnell VB. Blood. 2011; 117:2033. [PubMed: 21177434]
65. Lin D, Saleh S, Liebler DC. Chem. Res. Toxicol. 2008; 21:2361. [PubMed: 19548357]
66. Farmer EE, Davoine C. Curr. Opin. Plant Biol. 2007; 10:380. [PubMed: 17646124]
67. Ceaser EK, Moellering DR, Shiva S, Ramachandran A, Landar A, Venkartraman A, Crawford J, Patel R, Dickinson DA, Ulasova E, Ji S, rley-Usmar VM. Biochem. Soc. Trans. 2004; 32:151. [PubMed: 14748737]
68. Rudolph V, Rudolph TK, Schopfer FJ, Bonacci G, Woodcock SR, Cole MP, Baker PR, Ramani R, Freeman BA. Cardiovasc. Res. 2010; 85:155. [PubMed: 19666678]
69. Rudolph V, Rudolph TK, Schopfer FJ, Bonacci G, Woodcock SR, Cole MP, Baker PR, Ramani R, Freeman BA. Cardiovasc. Res. 2010; 85:155. [PubMed: 19666678]
70. Nadtochiy SM, Baker PR, Freeman BA, Brookes PS. Cardiovasc. Res. 2009; 82:333. [PubMed: 19050010]
71. Wyss-Coray T. Nat. Med. 2006; 12:1005. [PubMed: 16960575]
72. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Nat. Med. 1999; 5:698. [PubMed: 10371510]
73. Bell-Parikh LC, Ide T, Lawson JA, McNamara P, Reilly M, FitzGerald GA. J. Clin. Invest. 2003; 112:945. [PubMed: 12975479]
74. Oh JY, Giles N, Landar A, Darley-Usmar V. Biochem. J. 2008; 411:297. [PubMed: 18237271]
75. Schopfer FJ, Batthyany C, Baker PR, Bonacci G, Cole MP, Rudolph V, Groeger AL, Rudolph TK, Nadtochiy S, Brookes PS, Freeman BA. Free Radical Biol. Med. 2009; 46:1250. [PubMed: 19353781]
76. Schopfer FJ, Cole MP, Groeger AL, Chen CS, Khoo NK, Woodcock SR, Golin-Bisello F, Motanya UN, Li Y, Zhang J, Garcia-Barrio MT, Rudolph TK, Rudolph V, Bonacci G, Baker PR, Xu HE, Batthyany CI, Chen YE, Hallis TM, Freeman BA. J. Biol. Chem. 2010; 285:12321. [PubMed: 20097754]
77. Markesbery WR, Lovell MA. Neurobiol. Aging. 1998; 19:33. [PubMed: 9562500]
78. Williams TI, Lynn BC, Markesbery WR, Lovell MA. Neurobiol. Aging. 2006; 27:1094. [PubMed: 15993986]
79. Butterfield DA, Bader Lange ML, Sultana R. Biochim. Biophys. Acta. 2010; 1801:924. [PubMed: 20176130]
80. Codreanu SG, Zhang B, Sobecki SM, Billheimer DD, Liebler DC. Mol. Cell. Proteomics. 2008
81. Lee JY, Je JH, Kim DH, Chung SW, Zou Y, Kim ND, Ae YM, Suck BH, Yu BP, Chung HY. Eur. J. Biochem. 2004; 271:1339. [PubMed: 15030484]
82. D'Souza A, Kurien BT, Rodgers R, Shenoi J, Kurono S, Matsumoto H, Hensley K, Nath SK, Scofield RH. BMC Med. Genet. 2008; 9:62. [PubMed: 18606005]
83. Wong HL, Liebler DC. Chem. Res. Toxicol. 2008
84. Vila A, Tallman KA, Jacobs AT, Liebler DC, Porter NA, Marnett LJ. Chem. Res. Toxicol. 2008; 21:432. [PubMed: 18232660]
85. Szapacs ME, Kim HY, Porter NA, Liebler DC. J. Proteome Res. 2008; 7:4237. [PubMed: 18778096]
86. Shin NY, Liu Q, Stamer SL, Liebler DC. Chem. Res. Toxicol. 2007; 20:859. [PubMed: 17480101]

87. Dennehy MK, Richards KA, Wernke GR, Shyr Y, Liebler DC. Chem. Res. Toxicol. 2006; 19:20. [PubMed: 16411652]
88. Yamagiwa K, Ichikawa K. J. Cancer Res. 1918; 3:1.
89. Kennaway EL, Hieger I. Br. Med. J. 1930; 1:1044. [PubMed: 20775497]
90. Cook JW, Hewett CL, Hieger I. J. Chem. Soc. 1933:395.
91. Primiano T, Sutter TR, Kensler TW. Adv. Pharmacol. 1997; 38:293. [PubMed: 8895814]
92. Buetler TM, Gallagher EP, Wang C, Stahl DL, Hayes JD, Eaton DL. Toxicol. Appl. Pharmacol. 1995; 135:45. [PubMed: 7482539]
93. Hayes JD, Pulford DJ. Crit. Rev. Biochem. Mol. Biol. 1995; 30:445. [PubMed: 8770536]
94. Wattenberg LW. Adv. Cancer Res. 1978; 26:197. [PubMed: 204165]
95. Rushmore TH, Morton MR, Pickett CB. J. Biol. Chem. 1991; 266:11632. [PubMed: 1646813]
96. Friling RS, Bensimon A, Tichauer Y, Daniel V. Proc. Natl. Acad. Sci. U. S. A. 1990; 87:6258. [PubMed: 2166952]
97. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. Biochem. Biophys. Res. Commun. 1997; 236:313. [PubMed: 9240432]
98. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. Genes Dev. 1999; 13:76. [PubMed: 9887101]
99. Itoh K, Mochizuki M, Ishii Y, Ishii T, Shibata T, Kawamoto Y, Kelly V, Sekizawa K, Uchida K, Yamamoto M. Mol. Cell. Biol. 2004; 24:36. [PubMed: 14673141]
100. Kansanen E, Jyrkkanen HK, Volger OL, Leinonen H, Kivela AM, Hakkinen SK, Woodcock SR, Schopfer FJ, Horrevoets AJ, Yla-Herttuala S, Freeman BA, Levonen AL. J. Biol. Chem. 2009; 284:33233. [PubMed: 19808663]
101. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, Scrimieri F, Winter JM, Hruban RH, Iacobuzio-Donahue C, Kern SE, Blair IA, Tuveson DA. Nature. 2011; 475:106. [PubMed: 21734707]
102. Zhang DD, Hannink M. Mol. Cell. Biol. 2003; 23:8137. [PubMed: 14585973]
103. Hong F, Sekhar KR, Freeman ML, Liebler DC. J. Biol. Chem. 2005; 280:31768. [PubMed: 15985429]
104. Eggler AL, Liu G, Pezzuto JM, van Breemen RB, Mesecar AD. Proc. Natl. Acad. Sci. U. S. A. 2005; 102:10070. [PubMed: 16006525]
105. McMahon M, Lamont DJ, Beattie KA, Hayes JD. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:18838. [PubMed: 20956331]
106. Lee TS, Chau LY. Nat. Med. 2002; 8:240. [PubMed: 11875494]
107. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P. Proc. Natl. Acad. Sci. U. S. A. 2002; 99:11908. [PubMed: 12193649]
108. Gao L, Wang J, Sekhar KR, Yin H, Yared NF, Schneider SN, Sasi S, Dalton TP, Anderson ME, Chan JY, Morrow JD, Freeman ML. J. Biol. Chem. 2007; 282:2529. [PubMed: 17127771]
109. Levonen AL, Landar A, Ramachandran A, Ceaser EK, Dickinson DA, Zanoni G, Morrow JD, Darley-Usmar VM. Biochem. J. 2004; 378:373. [PubMed: 14616092]
110. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, Talalay P. Proc. Natl. Acad. Sci. U. S. A. 2004; 101:2040. [PubMed: 14764894]
111. Kansanen E, Bonacci G, Schopfer FJ, Linna S, Tong KI, Leinonen H, Woodcock SR, Yamamoto M, Carlberg C, Yla-Herttuala S, Freeman BA, Levonen AL. J. Biol. Chem. 2011
112. Sekhar KR, Rachakonda G, Freeman ML. Toxicol. Appl. Pharmacol. 244:21. [PubMed: 19560482]
113. Kobayashi M, Li L, Iwamoto N, Nakajima-Takagi Y, Kaneko H, Nakayama Y, Eguchi M, Wada Y, Kumagai Y, Yamamoto M. Mol. Cell. Biol. 2009; 29:493. [PubMed: 19001094]
114. Suzuki M, Betsuyaku T, Ito Y, Nagai K, Nasuhara Y, Kaga K, Kondo S, Nishimura M. Am. J. Respir. CellMol. Biol. 2008; 39:673.
115. Rangasamy T, Guo J, Mitzner WA, Roman J, Singh A, Fryer AD, Yamamoto M, Kensler TW, Tuder RM, Georas SN, Biswal S. J. Exp. Med. 2005; 202:47. [PubMed: 15998787]

116. Tanaka Y, Aleksunes LM, Yeager RL, Gyamfi MA, Esterly N, Guo GL, Klaassen CD. J. Pharmacol. Exp. Ther. 2008; 325:655. [PubMed: 18281592]
117. Zakkar M, Van der Heiden K, Luong le A, Chaudhury H, Cuhlmann S, Hamdulay SS, Krams R, Edirisinghe I, Rahman I, Carlsen H, Haskard DO, Mason JC, Evans PC. Arterioscler. Thromb. Vasc. Biol. 2009; 29:1851. [PubMed: 19729611]
118. Kensler TW, Wakabayashi N. Carcinogenesis. 2010; 31:90. [PubMed: 19793802]
119. Taguchi K, Motohashi H, Yamamoto M. Genes Cells. 16:123. [PubMed: 21251164]
120. Benjamin IJ, McMillan DR. Circ. Res. 1998; 83:117. [PubMed: 9686751]
121. Jacobs AT, Marnett LJ. J. Biol. Chem. 2007; 282:33412. [PubMed: 17873279]
122. Jacobs AT, Marnett LJ. J. Biol. Chem. 2009; 284:9176. [PubMed: 19179333]
123. Chen H, Wu Y, Zhang Y, Jin L, Luo L, Xue B, Lu C, Zhang X, Yin Z. FEBS Lett. 2006; 580:3145. [PubMed: 16697380]
124. Shi Y, Tu Z, Tang D, Zhang H, Liu M, Wang K, Calderwood SK, Xiao X. Shock. 2006; 26:277. [PubMed: 16912653]
125. Niture SK, Jaiswal AK. J. Biol. Chem. 2010; 285:36865. [PubMed: 20864537]
126. Abravaya K, Myers MP, Murphy SP, Morimoto RI. Genes Dev. 1992; 6:1153. [PubMed: 1628823]
127. Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R. Cell. 1998; 94:471. [PubMed: 9727490]
128. Jacobs AT, Marnett LJ. Acc. Chem. Res. 2010; 43:673. [PubMed: 20218676]
129. Carbone DL, Doorn JA, Kiebler Z, Ickes BR, Petersen DR. J. Pharmacol. Exp. Ther. 2005; 315:8. [PubMed: 15951401]
130. Carbone DL, Doorn JA, Kiebler Z, Sampey BP, Petersen DR. Chem. Res. Toxicol. 2004; 17:1459. [PubMed: 15540944]
131. Gan N, Wu YC, Brunet M, Garrido C, Chung FL, Dai C, Mi L. J. Biol. Chem. 2010; 285:35528. [PubMed: 20833711]
132. Zingarelli B, Hake PW, Mangeshkar P, O'Connor M, Burroughs TJ, Piraino G, Denenberg A, Wong HR. Shock. 2007; 28:554. [PubMed: 17589386]
133. Ricote M, Huang JT, Welch JS, Glass CK. J. Leukocyte Biol. 1999; 66:733. [PubMed: 10577502]
134. Welch JS, Ricote M, Akiyama TE, Gonzalez FJ, Glass CK. Proc. Natl. Acad. Sci. U. S. A. 2003; 100:6712. [PubMed: 12740443]
135. Tontonoz P, Spiegelman BM. Annu. Rev. Biochem. 2008; 77:289. [PubMed: 18518822]
136. Ricote M, Glass CK. Biochim. Biophys. Acta. 2007; 1771:926. [PubMed: 17433773]
137. Li Y, Zhang J, Schopfer FJ, Martynowski D, Garcia-Barrio MT, Kovach A, Suino-Powell K, Baker PR, Freeman BA, Chen YE, Xu HE. Nat. Struct. Mol. Biol. 2008; 15:865. [PubMed: 18604218]
138. Villacorta L, Schopfer FJ, Zhang J, Freeman BA, Chen YE. Clin. Sci. (London). 2009; 116:205. [PubMed: 19118492]
139. Itoh T, Fairall L, Amin K, Inaba Y, Szanto A, Balint BL, Nagy L, Yamamoto K, Schwabe JW. Nat. Struct. Mol. Biol. 2008
140. Nettles KW. Nat. Struct. Mol. Biol. 2008; 15:893. [PubMed: 18769464]
141. Itoh T, Fairall L, Amin K, Inaba Y, Szanto A, Balint BL, Nagy L, Yamamoto K, Schwabe JW. Nat. Struct. Mol. Biol. 2008; 15:924. [PubMed: 19172745]
142. Shiraki T, Kamiya N, Shiki S, Kodama TS, Kakizuka A, Jingami H. J. Biol. Chem. 2005; 280:14145. [PubMed: 15695504]
143. Waku T, Shiraki T, Oyama T, Fujimoto Y, Maebara K, Kamiya N, Jingami H, Morikawa K. J. Mol. Biol. 2009; 385:188. [PubMed: 18977231]
144. Schopfer FJ, Lin Y, Baker PR, Cui T, Garcia-Barrio M, Zhang J, Chen K, Chen YE, Freeman BA. Proc. Natl. Acad. Sci. U. S. A. 2005; 102:2340. [PubMed: 15701701]
145. Rosen ED, Spiegelman BM. J. Biol. Chem. 2001; 276:37731. [PubMed: 11459852]
146. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. Cell. 1995; 83:803. [PubMed: 8521497]

147. Cole MP, Rudolph TK, Khoo NK, Motanya UN, Golin-Bisello F, Wertz JW, Schopfer FJ, Rudolph V, Woodcock SR, Bolisetty S, Ali MS, Zhang J, Chen YE, Agarwal A, Freeman BA, Bauer PM. Circ. Res. 2009; 105:965. [PubMed: 19797175]

148. Borniquel S, Jansson EA, Cole MP, Freeman BA, Lundberg JO. Free Radical Biol. Med. 2010; 48:499. [PubMed: 19932165]

149. Rudolph TK, Rudolph V, Edreira MM, Cole MP, Bonacci G, Schopfer FJ, Woodcock SR, Franek A, Pekarova M, Khoo NK, Hasty AH, Baldus S, Freeman BA. Arterioscler. Thromb. Vasc. Biol. 2010; 30:938. [PubMed: 20167658]

150. Zhang J, Villacorta L, Chang L, Fan Z, Hamblin M, Zhu T, Chen CS, Cole MP, Schopfer FJ, Deng CX, Garcia-Barrio MT, Feng YH, Freeman BA, Chen YE. Circ. Res. 2010; 107:540. [PubMed: 20558825]

151. Barnes PJ, Karin M. N. Engl. J. Med. 1997; 336:1066. [PubMed: 9091804]

152. Bhatt, BA.; O'Doherty, RM. Advances in molecular and cellular endocrinology. Vol. Vol. 5. London: Reed Elsevier; 2006.

153. Pantano C, Reynaert NL, van dV, Janssen-Heininger YM. Antioxid. Redox Signaling. 2006; 8:1791.

154. Kim EH, Surh YJ. Biochem. Pharmacol. 2006; 72:1516. [PubMed: 16987499]

155. Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, Santoro MG. Nature. 2000; 403:103. [PubMed: 10638762]

156. Ji C, Kozak KR, Marnett LJ. J. Biol. Chem. 2001; 276:18223. [PubMed: 11359792]

157. Cui T, Schopfer FJ, Zhang J, Chen K, Ichikawa T, Baker PR, Batthyany C, Chacko BK, Feng X, Patel RP, Agarwal A, Freeman BA, Chen YE. J. Biol. Chem. 2006; 281:35686. [PubMed: 16887803]

158. Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, Sengchanthalangsy LL, Ghosh G, Glass CK. Proc. Natl. Acad. Sci. U. S. A. 2000; 97:4844. [PubMed: 10781090]

159. Lambert C, Li J, Jonscher K, Yang TC, Reigan P, Quintana M, Harvey J, Freed BM. J. Biol. Chem. 2007; 282:19666. [PubMed: 17491020]

160. Wruck CJ, Streetz K, Pavic G, Gotz ME, Tohidnezhad M, Brandenburg LO, Varoga D, Eickelberg O, Herdegen T, Trautwein C, Cha K, Kan YW, Pufe T. J. Biol. Chem. 2011; 286:4493. [PubMed: 21127061]

161. Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M. Nat. Med. 1999; 5:554. [PubMed: 10229233]

162. Kaltschmidt B, Uherek M, Volk B, Baeuerle PA, Kaltschmidt C. Proc. Natl. Acad. Sci.U. S. A. 1997; 94:2642. [PubMed: 9122249]

163. Di Stefano A, Caramori G, Oates T, Capelli A, Lusuardi M, Gnemmi I, Ioli F, Chung KF, Donner CF, Barnes PJ, Adcock IM. Eur. Respir. J. 2002; 20:556. [PubMed: 12358328]

164. Edwards MR, Bartlett NW, Clarke D, Birrell M, Belvisi M, Johnston SL. Pharmacol. Ther. 2009; 121:1. [PubMed: 18950657]

165. Osburn WO, Karim B, Dolan PM, Liu G, Yamamoto M, Huso DL, Kensler TW. Int. J. Cancer. 2007; 121:1883. [PubMed: 17631644]

166. Bonacci G, Schopfer FJ, Batthyany CI, Rudolph TK, Rudolph V, Khoo NK, Kelley EE, Freeman BA. J. Biol. Chem. 2011; 286:16074. [PubMed: 21454668]

167. Weitzman SA, Gordon LI. Blood. 1990; 76:655. [PubMed: 2200535]

168. Li W, Khor TO, Xu C, Shen G, Jeong WS, Yu S, Kong AN. Biochem. Pharmacol. 2008; 76:1485. [PubMed: 18694732]

169. Wong HL, Liebler DC. Chem. Res. Toxicol. 2008; 21:796. [PubMed: 18324786]

170. Go YM, Halvey PJ, Hansen JM, Reed M, Pohl J, Jones DP. Am. J. Pathol. 2007; 171:1670. [PubMed: 17982132]

171. Shibata T, Yamada T, Ishii T, Kumazawa S, Nakamura H, Masutani H, Yodoi J, Uchida K. J. Biol. Chem. 2003; 278:26046. [PubMed: 12709421]

172. Moos PJ, Edes K, Cassidy P, Massuda E, Fitzpatrick FA. J. Biol. Chem. 2003; 278:745. [PubMed: 12424231]

173. Brown KK, Eriksson SE, Arner ES, Hampton MB. Free Radical Biol. Med. 2008; 45:494. [PubMed: 18501718]
174. Roede JR, Carbone DL, Doorn JA, Kirichenko OV, Reigan P, Petersen DR. Chem. Res. Toxicol. 2008; 21:2289. [PubMed: 19548352]
175. Landar A, Zmijewski JW, Dickinson DA, Le Goffe C, Johnson MS, Milne GL, Zanoni G, Vidari G, Morrow JD, Darley-Usmar VM. Am. J. Physiol. Heart Circ. Physiol. 2006; 290:H1777. [PubMed: 16387790]
176. Higdon AN, Dranka BP, Hill BG, Oh JY, Johnson MS, Landar A, Darley-Usmar VM. Free Radical Biol. Med. 2009; 47:201. [PubMed: 19446632]
177. Doyle K, Fitzpatrick FA. J. Biol. Chem. 2010; 285:17417. [PubMed: 20385560]
178. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Nat. Rev. Cancer. 2001; 1:194. [PubMed: 11902574]
179. Codreanu SG, Zhang B, Sobecki SM, Billheimer DD, Liebler DC. Mol. Cell. Proteomics. 2009; 8:670. [PubMed: 19054759]
180. Oliva JL, Perez-Sala D, Castrillo A, Martinez N, Canada FJ, Bosca L, Rojas JM. Proc. Natl. Acad. Sci. U. S. A. 2003; 100:4772. [PubMed: 12684535]
181. Fu Y, Luo N, Lopes-Virella MF. Atherosclerosis. 2002; 160:11. [PubMed: 11755918]
182. Takeda K, Ichiki T, Tokunou T, Iino N, Takeshita A. J. Biol. Chem. 2001; 276:48950. [PubMed: 11687581]
183. Wilmer WA, Dixon C, Lu L, Hilbelink T, Rovin BH. Biochem. Biophys. Res. Commun. 2001; 281:57. [PubMed: 11178960]
184. Sampey BP, Carbone DL, Doorn JA, Drechsel DA, Petersen DR. Mol. Pharmacol. 2007; 71:871. [PubMed: 17164404]
185. Matsuzawa A, Ichijo H. Biochim. Biophys. Acta. 2008; 1780:1325. [PubMed: 18206122]
186. Iwamoto N, Sumi D, Ishii T, Uchida K, Cho AK, Froines JR, Kumagai Y. J. Biol. Chem. 2007; 282:33396. [PubMed: 17878162]
187. Samet JM, Tal TL. Annu. Rev. Pharmacol. Toxicol. 2010; 50:215. [PubMed: 20055703]
188. Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, Barford D. Nature. 2003; 423:769. [PubMed: 12802338]
189. Seiner DR, LaButti JN, Gates KS. Chem. Res. Toxicol. 2007; 20:1315. [PubMed: 17655273]
190. Lu C, Chan SL, Fu W, Mattson MP. J. Biol. Chem. 2002; 277:24368. [PubMed: 12006588]
191. Hurd TR, Costa NJ, Dahm CC, Beer SM, Brown SE, Filipovska A, Murphy MP. Antioxid. Redox Signaling. 2005; 7:999.
192. Picklo MJ, Amarnath V, McIntyre JO, Graham DG, Montine TJ. J. Neurochem. 1999; 72:1617. [PubMed: 10098869]
193. Humphries KM, Yoo Y, Szweda LI. Biochemistry. 1998; 37:552. [PubMed: 9425076]
194. Martinez B, Perez-Castillo A, Santos A. J. Lipid Res. 2005; 46:736. [PubMed: 15654126]
195. Nakamura K, Miura D, Kusano KF, Fujimoto Y, Sumita-Yoshikawa W, Fuke S, Nishii N, Nagase S, Hata Y, Morita H, Matsubara H, Ohe T, Ito H. J Card. Failure. 2009; 15:709.
196. Chen J, Schenker S, Frosto TA, Henderson GI. Biochim. Biophys. Acta. 1998; 1380:336. [PubMed: 9555085]
197. Humphries KM, Szweda LI. Biochemistry. 1998; 37:15835. [PubMed: 9843389]
198. Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otin M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ, Brand MD. EMBO J. 2003; 22:4103. [PubMed: 12912909]
199. Queliconi BB, Wojtovich AP, Nadtochiy SM, Kowaltowski AJ, Brookes PS. Biochim. Biophys. Acta. 2011; 1813:1309. [PubMed: 21094666]
200. Koenitzer JR, Freeman BA. Ann.N. Y. Acad. Sci. 2010; 1203:45. [PubMed: 20716282]
201. Gohil VM, Sheth SA, Nilsson R, Wojtovich AP, Lee JH, Perocchi F, Chen W, Clish CB, Ayata C, Brookes PS, Mootha VK. Nat. Biotechnol. 2010; 28:249. [PubMed: 20160716]
202. Ceaser EK, Ramachandran A, Levonen AL, Darley-Usmar VM. Am. J. Physiol. Heart Circ. Physiol. 2003; 285:H2298. [PubMed: 12881207]

203. Aldini G, Carini M, Vistoli G, Shibata T, Kusano Y, Gamberoni L, Dalle-Donne I, Milzani A, Uchida K. Biochemistry. 2007; 46:2707. [PubMed: 17297918]
204. Chavez J, Chung WG, Miranda CL, Singhal M, Stevens JF, Maier CS. Chem. Res. Toxicol. 2010; 23:37. [PubMed: 20043646]
205. Reagan LP, Magarinos AM, Yee DK, Swzeda LI, Van Bueren A, McCall AL, McEwen BS. Brain Res. 2000; 862:292. [PubMed: 10799703]
206. Ishii T, Tatsuda E, Kumazawa S, Nakayama T, Uchida K. Biochemistry. 2003; 42:3474. [PubMed: 12653551]
207. Batthyany C, Schopfer FJ, Baker PR, Duran R, Baker LM, Huang Y, Cervenansky C, Branchaud BP, Freeman BA. J. Biol. Chem. 2006; 281:20450. [PubMed: 16682416]
208. Groeger AL, Freeman BA. Mol. Interventions. 2010; 10:39.
209. Pergola PE, Krauth M, Huff JW, Ferguson DA, Ruiz S, Meyer CJ, Warnock DG. Am. J. Nephrol. 33:469. [PubMed: 21508635]
210. Singh S, Vrishni S, Singh BK, Rahman I, Kakkar P. Free Radical Res. 2010; 44:1267. [PubMed: 20815789]
211. Covey TM, Edes K, Coombs GS, Virshup DM, Fitzpatrick FA. PLoS One. 2010; 5:e13545. [PubMed: 20975834]
212. Yao H, Rahman I. Curr. Opin. Pharmacol. 2009; 9:375. [PubMed: 19615942]
213. Kaminski BM, Steinhilber D, Stein JM, Ulrich S. Curr. Pharm. Biotechnol. 2011 in press.
214. Johnson JJ, Mukhtar H. Cancer Lett. 2007; 255:170. [PubMed: 17448598]
215. Cornblatt BS, Ye L, Dinkova-Kostova AT, Erb M, Fahey JW, Singh NK, Chen MS, Stierer T, Garrett-Mayer E, Argani P, Davidson NE, Talalay P, Kensler TW, Visvanathan K. Carcinogenesis. 2007; 28:1485. [PubMed: 17347138]
216. Yates MS, Tran QT, Dolan PM, Osburn WO, Shin S, McCulloch CC, Silkworth JB, Taguchi K, Yamamoto M, Williams CR, Liby KT, Sporn MB, Sutter TR, Kensler TW. Carcinogenesis. 2009; 30:1024. [PubMed: 19386581]
217. Tran QT, Xu L, Phan V, Goodwin SB, Rahman M, Jin VX, Sutter CH, Roebuck BD, Kensler TW, George EO, Sutter TR. Carcinogenesis. 2009; 30:480. [PubMed: 19126641]
218. Yates MS, Kensler TW. Drug News Perspect. 2007; 20:109. [PubMed: 17440634]
219. Kensler TW, Chen JG, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, Ye L, Coady JL, Wang JB, Wu Y, Sun Y, Zhang QN, Zhang BC, Zhu YR, Qian GS, Carmella SG, Hecht SS, Benning L, Gange SJ, Groopman JD, Talalay P. Cancer Epidemiol. Biomarkers Prev. 2005; 14:2605. [PubMed: 16284385]
220. Kanninen K, Malm TM, Jyrkkanen HK, Goldsteins G, Keksa-Goldsteine V, Tanila H, Yamamoto M, Yla-Herttuala S, Levonen AL, Koistinaho J. Mol. Cell. Neurosci. 2008; 39:302. [PubMed: 18706502]
221. Clements CM, McNally RS, Conti BJ, Mak TW, Ting JP. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:15091. [PubMed: 17015834]
222. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P. Science. 2003; 299:256. [PubMed: 12446870]
223. Burton NC, Kensler TW, Guilarte TR. Neurotoxicology. 2006; 27:1094. [PubMed: 16959318]
224. Satoh T, Lipton SA. Trends Neurosci. 2007; 30:37. [PubMed: 17137643]
225. Shih AY, Li P, Murphy TH. J. Neurosci. 2005; 25:10321. [PubMed: 16267240]
226. Tully AM, Roche HM, Doyle R, Fallon C, Bruce I, Lawlor B, Coakley D, Gibney MJ. Br. J. Nutr. 2003; 89:483. [PubMed: 12654166]
227. Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, Tucker KL, Kyle DJ, Wilson PW, Wolf PA. Arch. Neurol. 2006; 63:1545. [PubMed: 17101822]
228. Nadtochiy SM, Baker PR, Freeman BA, Brookes PS. Cardiovasc. Res. 2009; 82:333. [PubMed: 19050010]
229. Cui T, Schopfer FJ, Zhang J, Chen K, Ichikawa T, Baker PR, Batthyany C, Chacko BK, Feng X, Patel RP, Agarwal A, Freeman BA, Chen YE. J. Biol. Chem. 2006; 281:35686. [PubMed: 16887803]

230. Gorczynski MJ, Smitherman PK, Akiyama TE, Wood HB, Berger JP, King SB, Morrow CS. J. Med. Chem. 2009; 52:4631. [PubMed: 19719236]

231. Li AC, Binder CJ, Gutierrez A, Brown KK, Plotkin CR, Pattison JW, Valledor AF, Davis RA, Willson TM, Witztum JL, Palinski W, Glass CK. J. Clin. Invest. 2004; 114:1564. [PubMed: 15578089]

232. Lancet. 1999; 354:447. [PubMed: 10465168]

233. Ueshima H, Stamler J, Elliott P, Chan Q, Brown IJ, Carnethon MR, Daviglus ML, He K, Moag-Stahlberg A, Rodriguez BL, Steffen LM, Van HL, Yarnell J, Zhou B. Hypertension. 2007; 50:313. [PubMed: 17548718]

234. Marchioli R, Silletta MG, Levantesi G, Pioggiarella R. Curr. Atheroscler. Rep. 2009; 11:440. [PubMed: 19852885]

235. London B, Albert C, Anderson ME, Giles WR, Van Wagoner DR, Balk E, Billman GE, Chung M, Lands W, Leaf A, McAnulty J, Martens JR, Costello RB, Lathrop DA. Circulation. 2007; 116:e320. [PubMed: 17768297]

236. Schenk S, Saberi M, Olefsky JM. J. Clin. Invest. 2008; 118:2992. [PubMed: 18769626]

237. Heilbronn LK, Campbell LV. Curr. Pharm. Des. 2008; 14:1225. [PubMed: 18473870]

238. Shah PK. Circ. Res. 2007; 100:1531. [PubMed: 17556663]

239. Chen YE, Fu M, Zhang J, Zhu X, Lin Y, Akinbami MA, Song Q. Vitam. Horm. 2003; 66:157. [PubMed: 12852255]

240. Staels B. Curr. Med. Res. Opin. 2005; 21 Suppl 1:S13. [PubMed: 15811195]

241. Loke YK, Kwok CS, Singh S. Brit. Med. J. 342:d1309.

242. Lipska KJ, Ross JS. JAMA. 2011; 305:820. [PubMed: 21304068]

243. Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP Jr, Vaughn DJ, Nessel L, Selby J, Strom BL. Diabetes Care. 2011; 34:916. [PubMed: 21447663]

244. Yamamoto K, Itoh T, Abe D, Shimizu M, Kanda T, Koyama T, Nishikawa M, Tamai T, Ooizumi H, Yamada S. Bioorg. Med. Chem. Lett. 2005; 15:517. [PubMed: 15664804]

245. Fu H, Park J, Pei D. Biochemistry. 2002; 41:10700. [PubMed: 12186556]

246. Copeland RA, Pompliano DL, Meek TD. Nat. Rev. Drug. Dis7covery. 2006; 5:730.

247. Rudolph V, Schopfer FJ, Khoo NK, Rudolph TK, Cole MP, Woodcock SR, Bonacci G, Groeger AL, Golin-Bisello F, Chen CS, Baker PR, Freeman BA. J. Biol. Chem. 2009; 284:1461. [PubMed: 19015269]

248. Takahashi M, Uechi S, Takara K, Asikin Y, Wada K. J. Agric. Food Chem. 2009; 57:9141. [PubMed: 19757811]

249. Garg R, Gupta S, Maru GB. Carcinogenesis. 2008; 29:1022. [PubMed: 18321868]

250. Egner PA, Chen JG, Wang JB, Wu Y, Sun Y, Lu JH, Zhu J, Zhang YH, Chen YS, Friesen MD, Jacobson LP, Munoz A, Ng D, Qian GS, Zhu YR, Chen TY, Botting NP, Zhang Q, Fahey JW, Talalay P, Groopman JD, Kensler TW. Cancer Prev. Res. (Phila). 4:384. [PubMed: 21372038]

251. Yates MS, Tauchi M, Katsuoka F, Flanders KC, Liby KT, Honda T, Gribble GW, Johnson DA, Johnson JA, Burton NC, Guilarte TR, Yamamoto M, Sporn MB, Kensler TW. Mol. Cancer Ther. 2007; 6:154. [PubMed: 17237276]

252. Lau A, Villeneuve NF, Sun Z, Wong PK, Zhang DD. Pharmacol. Res. 2008; 58:262. [PubMed: 18838122]

253. Comabella M, Pradillo JM, Fernandez M, Rio J, Lizasoain I, Julia E, Moro MA, Sastre-Garriga J, Montalban X. Eur. J. Neurol. 2009; 16:1197. [PubMed: 19538219]

254. Tsujita T, Li L, Nakajima H, Iwamoto N, Nakajima-Takagi Y, Ohashi K, Kawakami K, Kumagai Y, Freeman BA, Yamamoto M, Kobayashi M. Genes Cells. 2010

255. Cernuda-Morollon E, Pineda-Molina E, Canada FJ, Perez-Sala D. J. Biol. Chem. 2001; 276:35530. [PubMed: 11466314]

256. Cross JV, Foss FW, Rady JM, Macdonald TL, Templeton DJ. BMC Cancer. 2007; 7:183. [PubMed: 17894894]

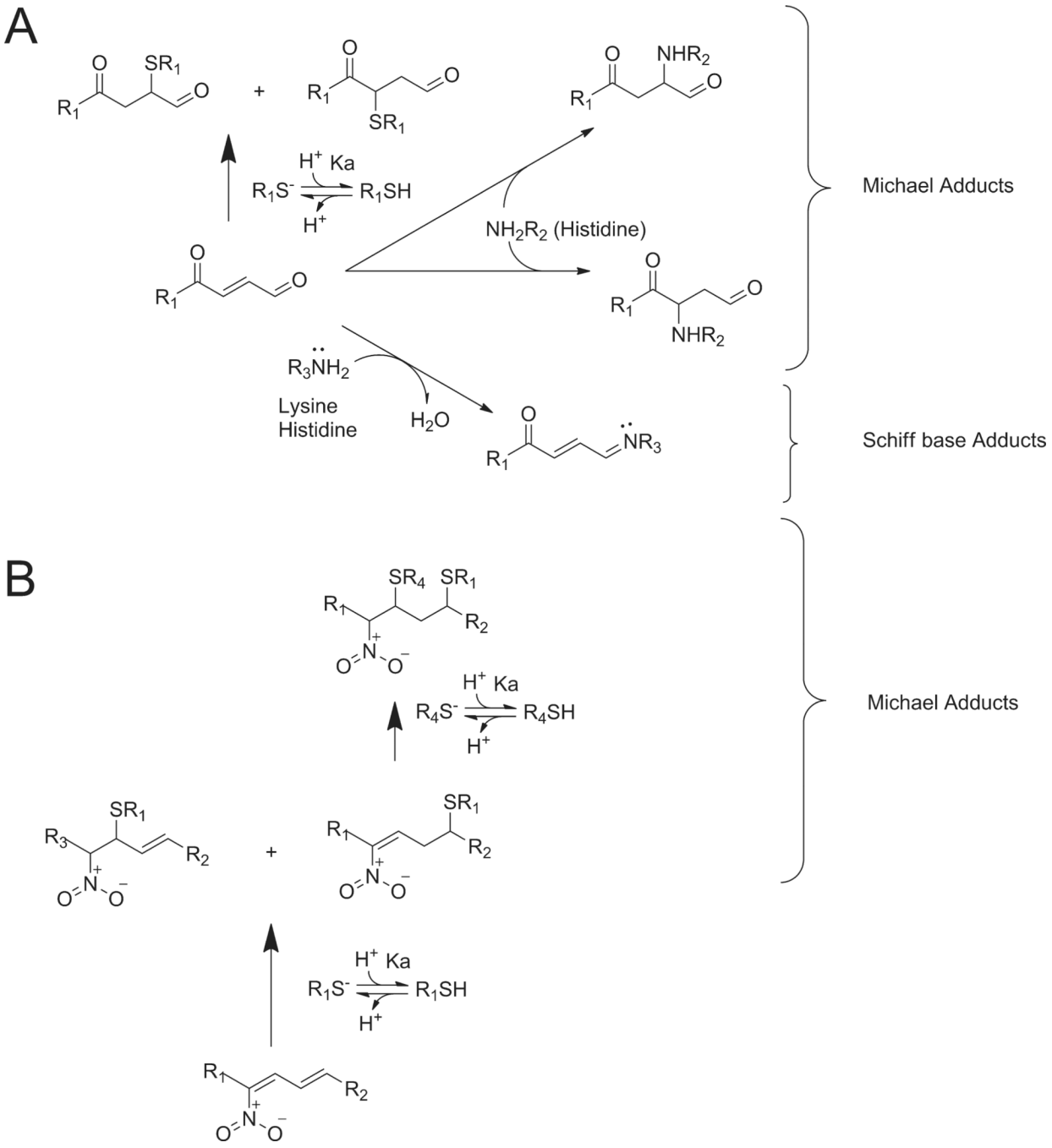

Figure 1.

Reaction scheme of lipid derived electrophiles. (A) Bifunctional electrophiles, a hallmark of lipid breakdown products, react with cellular nucleophiles through Michael addition (α-β-unsaturated carbonyl) and Schiff's base adduct formation (aldehyde). (B) Conjugated nitro-fatty acids react with thiolates at carbons β and δ to form two positional isomers. The adduction at the δ-carbon allows for a subsequent reaction with a nucleophile, allowing cross-linking reactions.

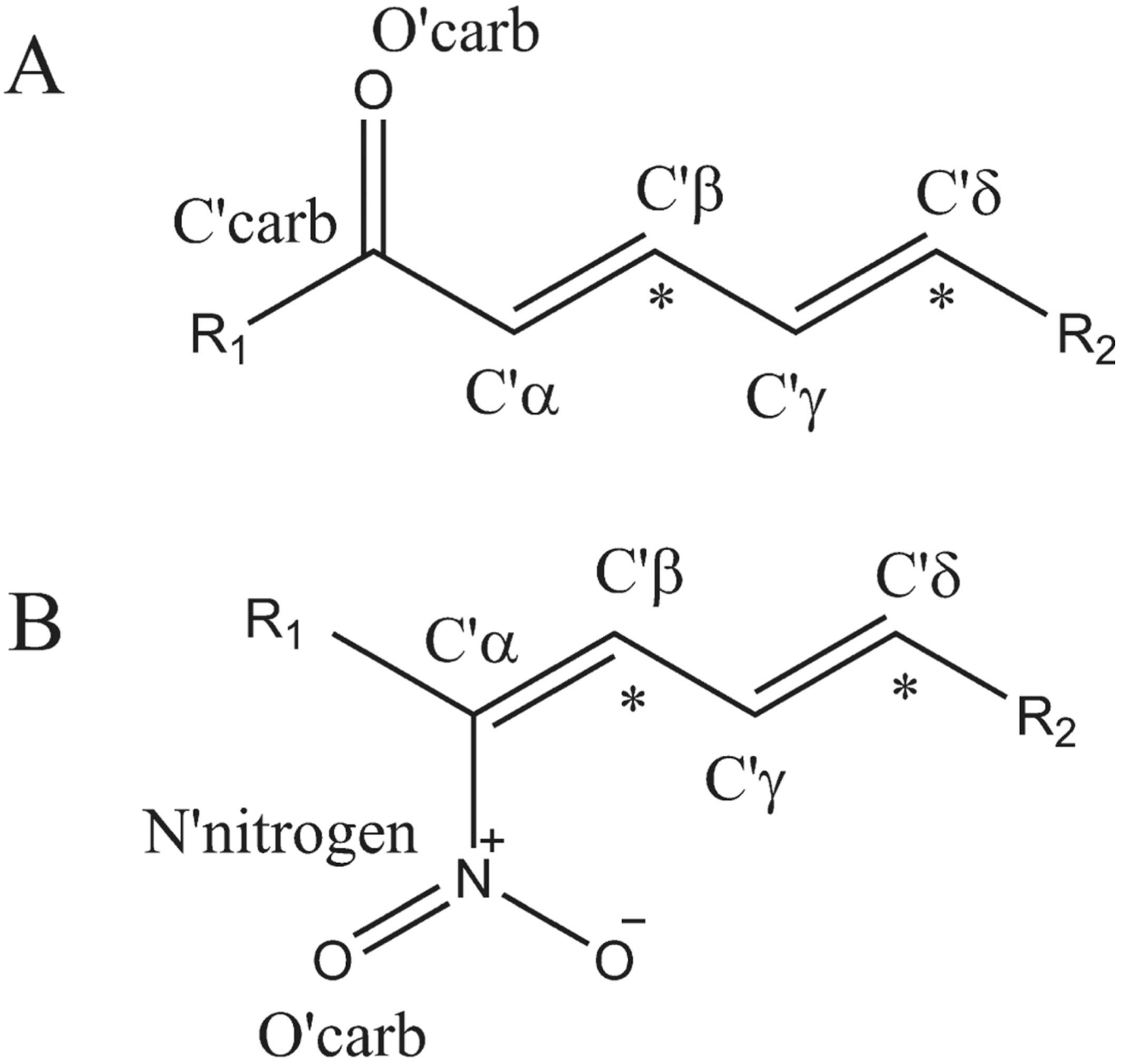

Figure 2.

Structure of α-β-unsaturated ketones and nitroalkenes. (A) Enzymatic and nonenzymatic oxidation of polyunsaturated fatty acids results in the formation of α-β-, γ-δ-unsaturated ketones. Both carbons β and δ are electron poor (shown with an *) and are reactive toward nucleophiles. (B) Nitroalkene structures show a similar conjugation as α-β-unsaturated ketones, and C′β and C′δ are electrophilic. In both cases, the electrophilicity is not lost after an initial reaction with C′δ, allowing for a second nucleophile to react at C′β, permitting cross-linking reactions.

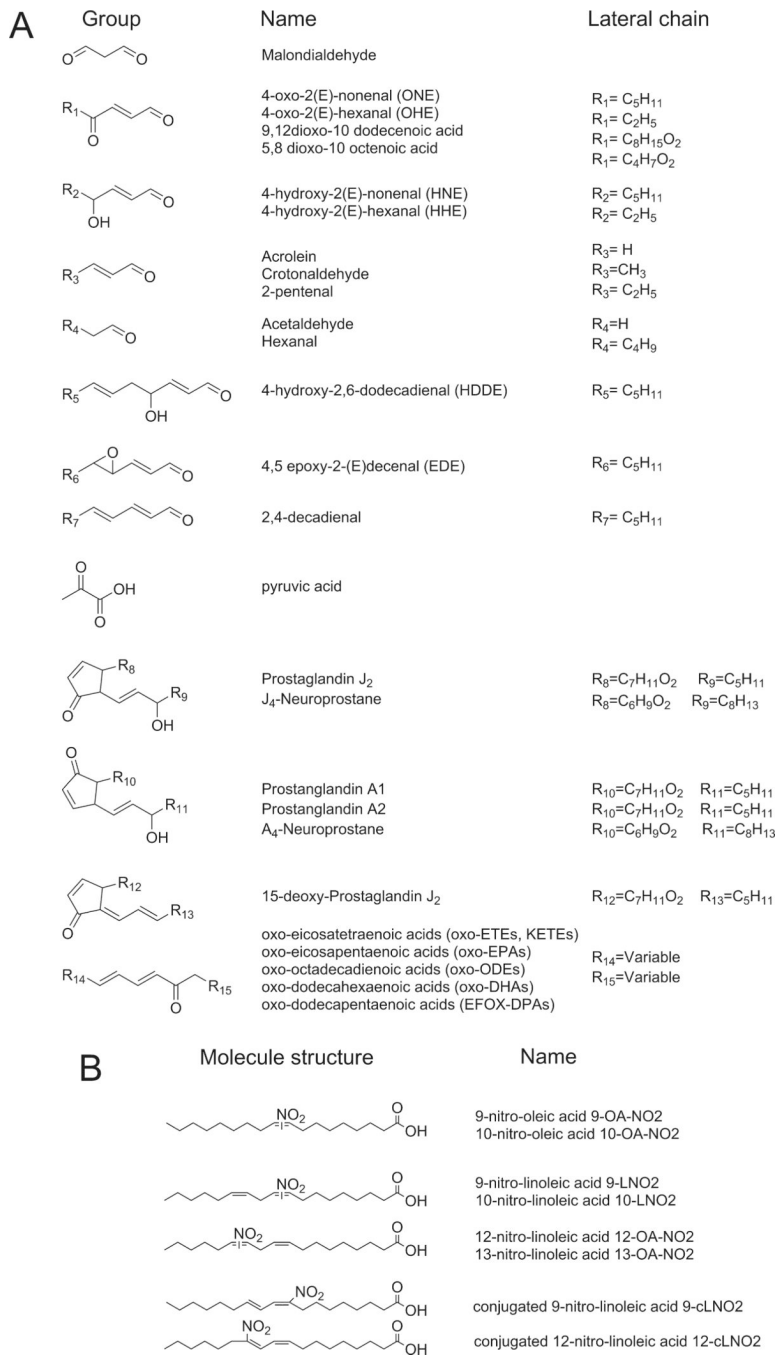

Figure 3.

Different lipid-derived electrophilic groups. (A) Electrophiles containing carbonyl groups. (B) Electrophiles containing nitro groups.

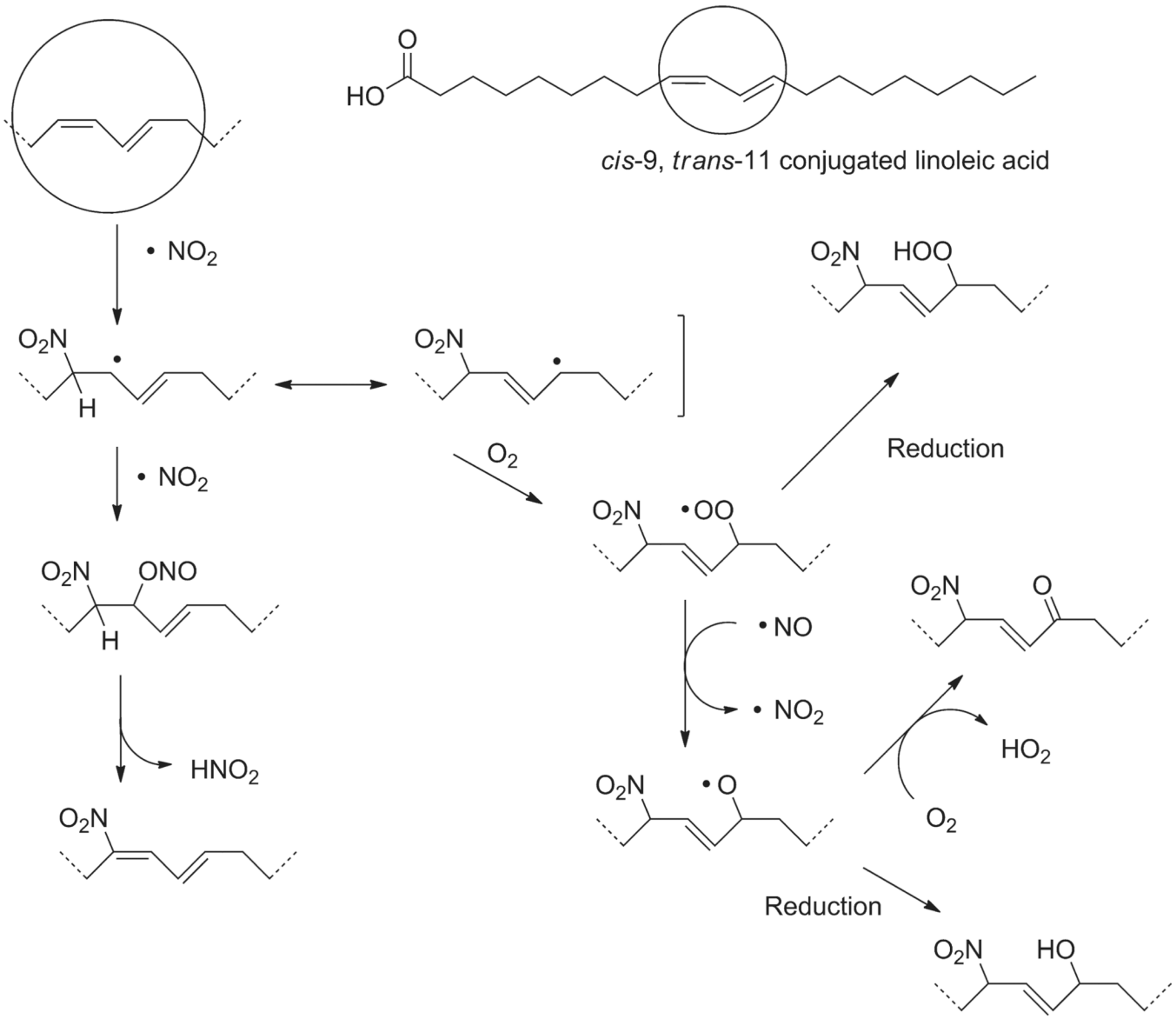

Figure 4.

Nitration of conjugated linoleic acid. The nitration of conjugated linoleic acid proceeds through an initial addition of nitrogen dioxide to the double bond, forming a resonance-stabilized radical. Under low oxygen conditions, a second nitrogen dioxide molecule reacts with the carbon-centered radical, generating an unstable nitrito intermediate that decomposes to form a conjugated nitro-linoleic acid and nitrous acid. In the presence of oxygen, a peroxyl radical is initially formed, that after reduction forms a nitro-hydroperoxy derivative. The peroxyl radical can be reduced to hydroxyl radical, followed by reduction to a nitro-hydroxy derivative or an oxidation to nitro-keto derivative. Although both the presence and absence of oxygen lead to electrophilic products, the final electrophilic groups are different, a nitroalkene formed in the absence of oxygen and an α-β-unsaturated keto in the presence of oxygen.

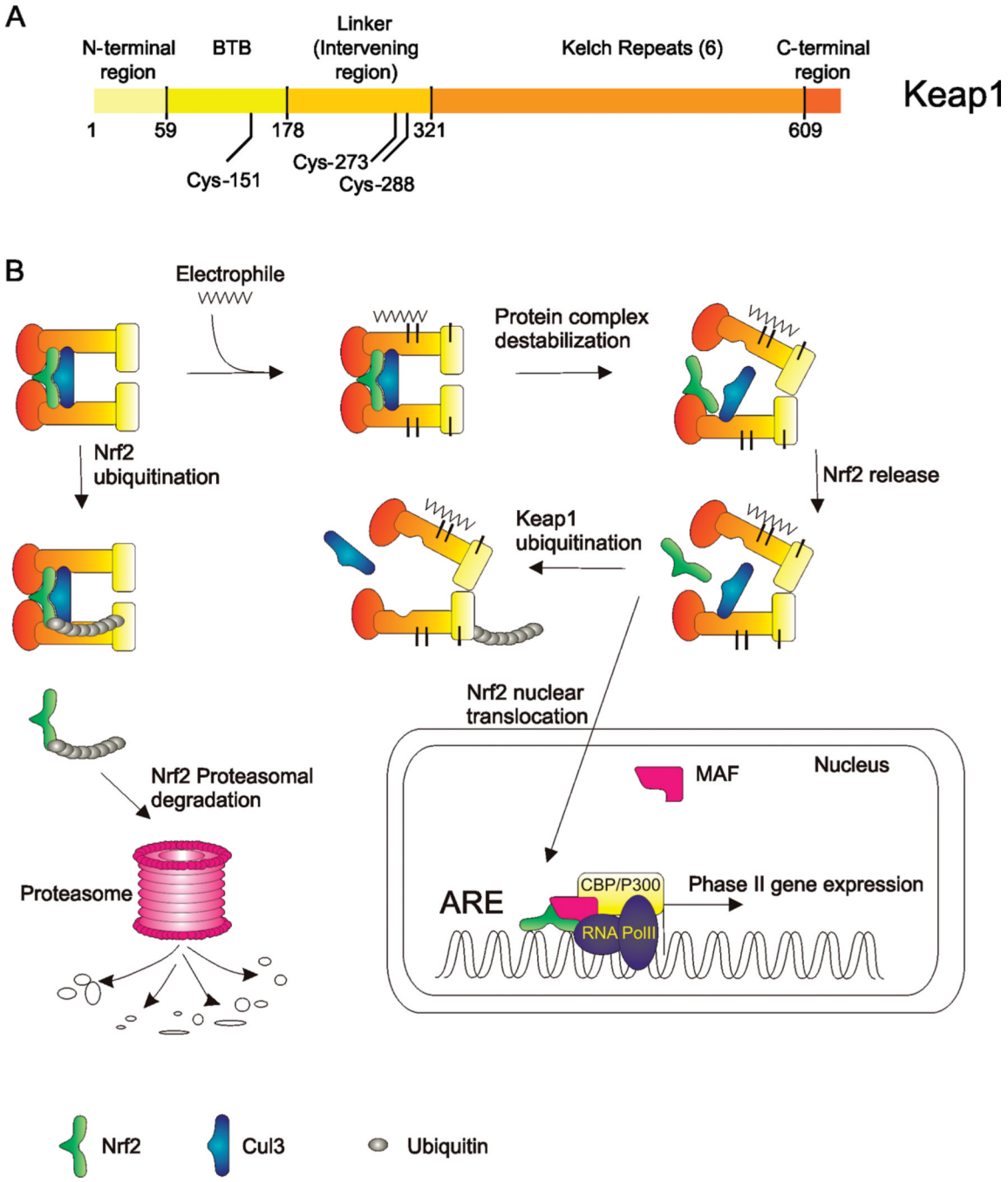

Figure 5.

Keap1-dependent regulation of Nrf2 activity by electrophiles. (A) Functional domains of Keap1. (B) Under normal conditions, Keap1 binds to Nrf2 in the cytosol through the Kelch domain, promoting cullin 3-dependent Nrf2 ubiquitination. The ubiquitinated Nrf2 is degraded by the proteasome. In the presence of electrophiles, reaction with target cysteines in Keap1 occurs, destabilizing the interaction between Keap1 and Nrf2 and switching the ubiquitination reaction from Nrf2 to Keap1. Nrf2 accumulates in the nucleus, activating the expression of phase II gene. Ubiquitinated Keap1 is further degraded.

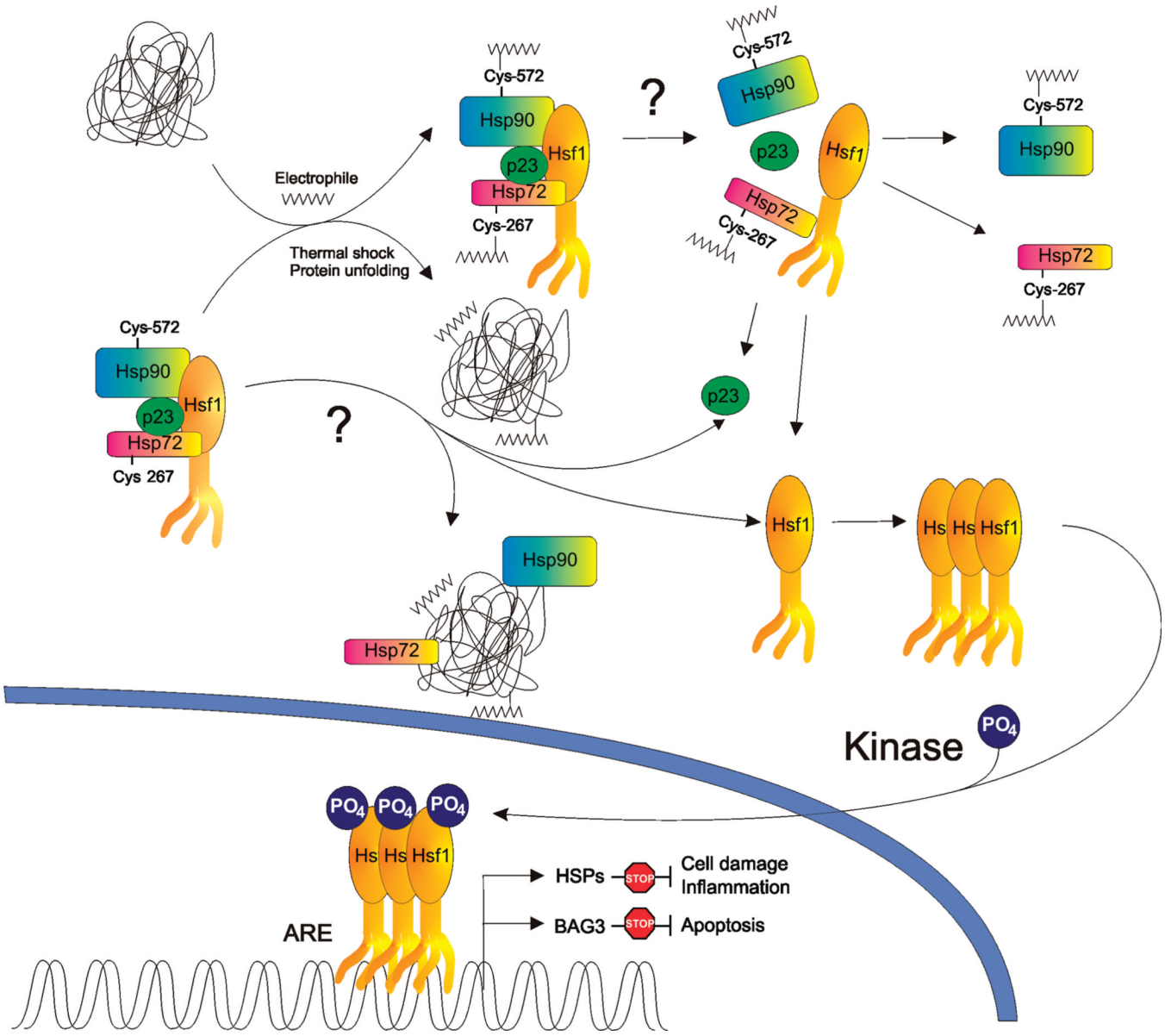

Figure 6.

Dual pathway for the activation of HSF1 by electrophiles. HSPs, specifically HSP90 and HSP72, are basally bound to HSF1. The primary pathway through which electrophiles promote activation of HSF1 involves the electrophilic adduction of nucleophilic residues in HSP90 and HSP72. This destabilizes the interaction of HSP72 and HSP90 with HSF1. Free HSF1 becomes activated in a three-step process involving phosphorylation, trimerization, and nuclear translocation. The HSF1 homotrimer binds to specific heat shock elements (HSEs) to induce the expression of heat shock response genes, which include several HSP chaperones. A secondary pathway through which electrophiles may activate HSF1 is based on the hypothesis that protein electrophilic modifications increase superficial hydrophobicity, attracting HSPs and releasing HSF1.

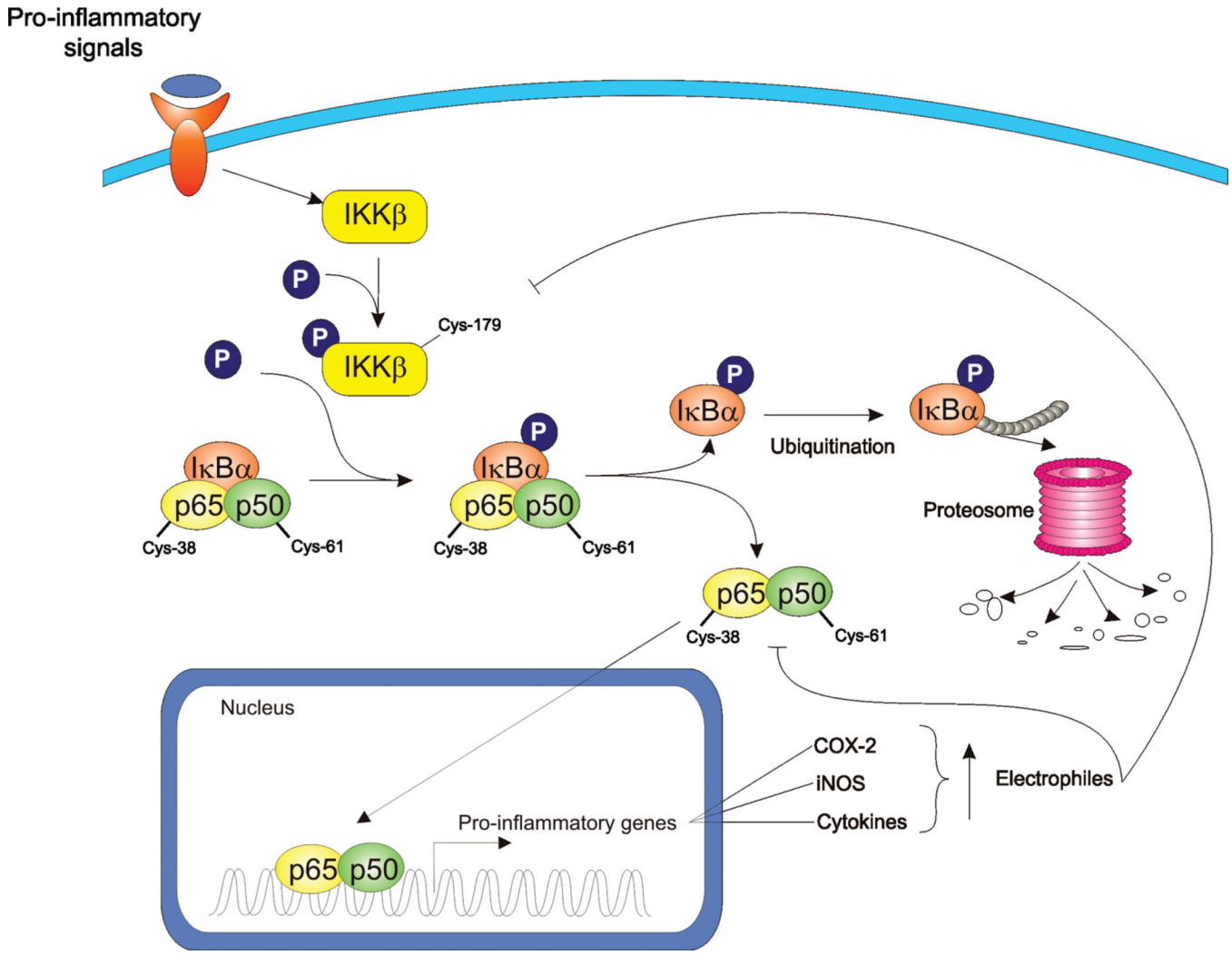

Figure 7.

NF-κB activation by pro-inflammatory stimuli and inhibition by electrophiles. Under basal conditions, IKKβ phosphorylates IκB, releasing the heterodimer p50/p65. Upon nuclear translocation, p65 activates the transcription of a variety of cytokine and inflammatory enzyme-coding genes. Adduction of IKKβ by electrophiles results in its inhibition, impairing NF-κB activation. Moreover, direct adduction of p65 inhibits its nuclear localization by most likely interfering with the dimerization that leads to NfkB dependent gene expression.

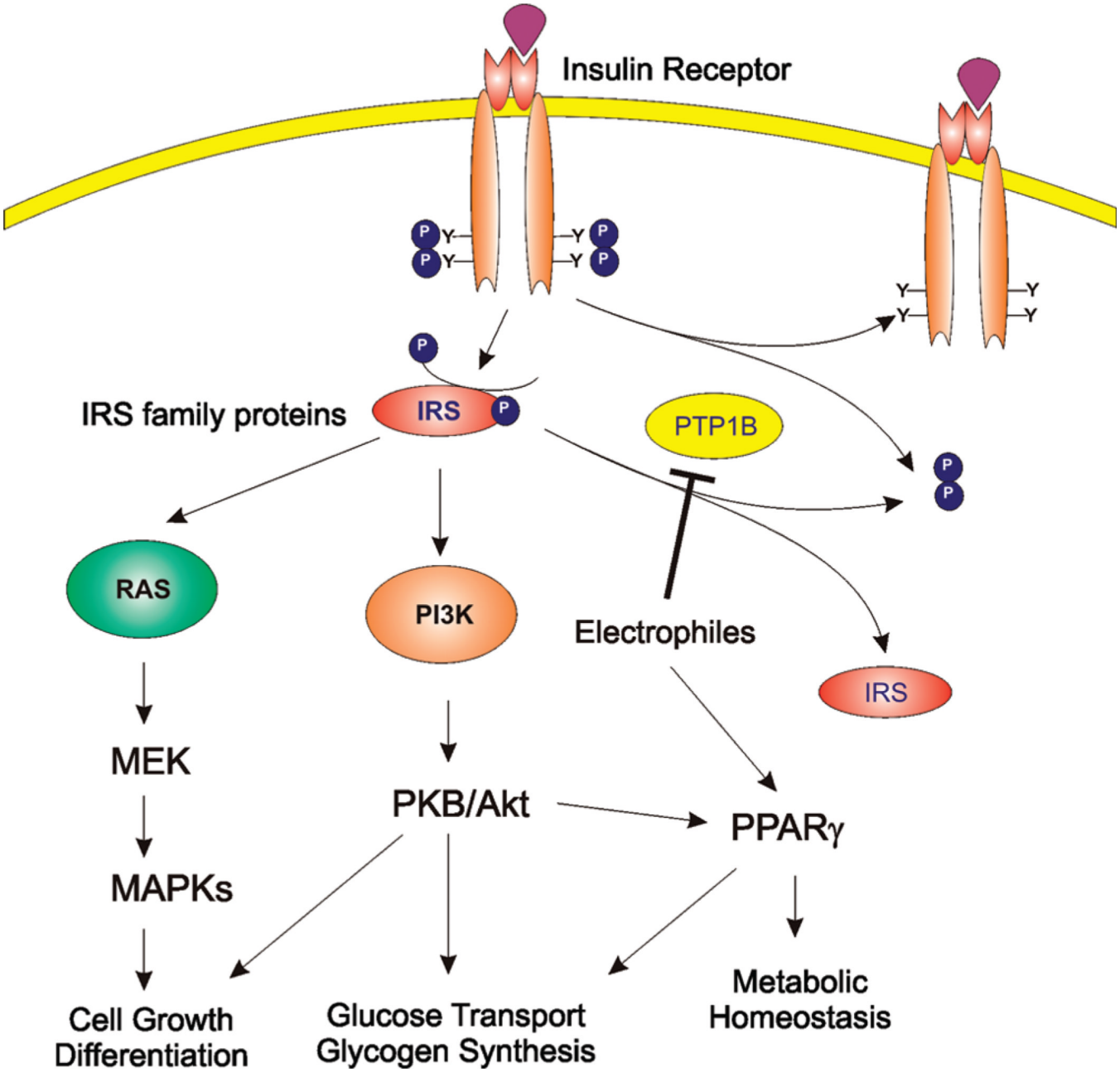

Figure 8.

Regulation of insulin sensitivity by electrophiles through adduction and inhibition of PTP1B. Phosphatases play a critical role in maintaining cellular homeostasis by regulating and limiting phosphorylation and downstream signaling. In particular, the insulin receptor and its signaling pathway are tightly regulated by phosphorylation and dephosphorylation reactions. Activation of the insulin receptor leads to its phosphorylation and triggers the phosphorylation of the IRS protein family, resulting in downstream activation of kinases and modulation of metabolic enzymes and pathways. PTP1B exerts a tight control of these phosphorylation events. Adduction of PTP1B by electrophiles at the catalytic cysteine results in catalytic inhibition, prolonging the effects and intensity of regulated pathways, thus impacting glucose homeostasis and insulin sensitivity. Electrophiles modulate this

pathway both directly (through covalent binding to PTP1B) and indirectly (through activation of the general metabolic regulator PPARγ).

Table 1

In Vivo Generation of Electrophilic Lipids in Health and Disease[a]

				concentration		
electrophile	**inflammatory condition/disease**	**experimental model**	**detection method**	**health**	**disease**	**ref**
OA-NO_2	cardiac ischemia and reperfusion	mouse	LC-ESI-MS/MS	ND[b]	9.5 nmol/L	68
	ischemic preconditioning	rat heart	LC-ESI-MS/MS	215 fmol/mg mitochondrial protein	245 fmol/mg mitochondrial protein	70
LNO_2	cardiac ischemia and reperfusion	mouse	LC-ESI-MS/MS	ND[b]	17.3 nmol/L	68
LNO_2	cardiac ischemia and riperfusion	rat	LC-ESI-MS/MS	3.6 pg/mg protein	186 pg/mg protein	75
	ischemic preconditioning	rat heart	LC-ESI-MS/MS	<50 fmol/mg mitochondrial protein	619 fmol/mg mitochondrial protein	70
15d-PGJ_2	multiple sclerosis	human plasma	ELISA	15 ng/mL	14.8–19.1 ng/mL[c]	253
	carrageenin-induced pleurisy	pleural exudate	ELISA	NM	803 pg/mL	72
A_4/J_4 NPs	Alzheimer's disease	postmortem brain	GC-MS	~100 ng/g brain tissue	295 ng/g brain tissue	31
HNE	Alzheimer's disease	amygdala	HPLC/fluorescence	0.193 nmol/mg	0.486 nmol/mg	77
	early Alzheimer's disease	HPG	LC-ESI-MS/MS	0.4 nmol/mg	1.45 nmol/mg	78
	mild cognitive impairment	HPG		0.4 nmol/mg	1.5 nmol/mg	78
	systemic lupus erythematosus	human red blood cells	Western blot	absent	present	82

[a] Abbreviations: ND, nondetectable; NM, not measured; HPG, hippocampus/parahippocampal gyrus.

[b] Sham-operated animals.

[c] Levels measured in different subtypes of MS patients.

Chem Rev. Author manuscript; available in PMC 2012 March 6.

Table 2

Protein Targets of Electrophilic Lipids and Specific Modified Residues

protein	electrophile	residue	ref
Hsp90	HNE	C-572	129
Hsp72	HNE	C-267	130
Prx6	HNE	Cys-91	174
TrxR	PGA_1	unknown	172
Trx1	HNE, acrolein	Cys-73	170
	15d-PGJ_2	Cys-35, Cys-69	171
ANT	LNO_2	unknown	70
cytochrome c oxidase	HNE	unknown	196
α-ketoglutarate dehydrogenase	HNE	lipoic acid sulfhydryl	197
pyruvate dehydrogenase	HNE	lipoic acid sulfhydryl	197
Keap 1	OA-NO_2	C-273, C-288	111, 254
	15d-PGJ_2	C-273, C-288	99, 113
	HNE	C-288, C-151	105
	SFN	C-288, C-273, C-151	102
PPARγ	4-oxo-DHA	C-285	141
	6-oxo-OTE	C-285	141
	5-oxo-EPA	C-285	141
	5-, 8-, 9-, 11-, 12-, 15-oxo-ETE	C-285	143
	OA-NO_2	C-285	76
IKKβ	15d-PGJ_2	C-179	155
	A_4/J_4-NPs	C-179	31
p65 (NF-κB)	15d-PGJ_2	C-38	158
	LNO_2, OA-NO_2	C-61	157
p50 (NF-κB)	acrolein	C-61, R-307	159
	15d-PGJ_2	C-61,	255
Erk-2	HNE	H-178	184
H-Ras	15d-PGJ_2	C-184	180
MEKK1	isothiocyanate	C-1238	256
PTP1B	1,2-NQ	H-25, C-121, C-215	186
	acrolein	C-215	189
HDAC1	15d-PGJ_2	C-261, C-273	177
	HNE	not well-defined	179
HDAC2	HNE	?	179
GAPDH	OA-NO_2	C-153, C-244, H-108, H-134, H-327	207
	HNE	H-164, C-244, C-281, H-327, K-331	206
Aldolase A	HNE	H-246	204
Enolase	HNE, 15d-PGJ_2	not well-defined	203, 204