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Electrophilic metabolites targeting the KEAP1/NRF2 partnership

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Abstract

Numerous electrophilic metabolites are formed during cellular activity, particularly under conditions of oxidative, inflammatory and metabolic stress. Among them are lipid oxidation and nitration products, and compounds derived from amino acid and central carbon metabolism. Here we focus on one cellular target of electrophiles, the Kelch-like ECH associated protein 1 (KEAP1)/nuclear factor erythroid 2 p45-related factor 2 (NRF2) partnership. Many of these reactive compounds modify C151, C273 and/or C288 within KEAP1. Other types of modifications include *S*-lactoylation of C273, *N*-succinylation of K131, and formation of methylimidazole intermolecular crosslink between two KEAP1 monomers. Modified KEAP1 relays the initial signal to transcription factor NRF2 and its downstream targets, the ultimate effectors that provide means for detoxification, adaptation and survival. Thus, by non-enzymatically covalently modifying KEAP1, the electrophilic metabolites discussed here serve as chemical signals connecting metabolism with stress responses.

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Introduction

Transcription factor nuclear factor erythroid 2 p45-related factor 2 (NRF2) and its main negative regulator Kelch-like ECH associated protein 1 (KEAP1) (Figure 1a) are the principal determinants of cellular redox homeostasis and their partnership is a drug target for prevention and treatment of chronic disease [1]. Under basal conditions, KEAP1, a substrate adaptor for CUL3/RBX1 E3-ubiquitin ligase, continuously targets NRF2 for ubiquitination and proteasomal degradation (Figure 1b). Under conditions of cellular stress, KEAP1 loses its ability to promote the ubiquitination of NRF2, resulting in the stabilization of the transcription factor, its nuclear translocation and enhanced transcription of a network of genes encoding cytoprotective proteins.

Early work from the laboratory of Paul Talalay at Johns Hopkins University, preceding the discovery of NRF2 by nearly two decades, found that numerous structurally diverse small molecules are able to induce cytoprotective enzymes, such as NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), epoxide hydratase, and glutathione *S*-transferases (GSTs) in rodent cells and tissues [2–4]. This was the time of "ligand-protein" interactions, and the structural diversity of the inducers was puzzling. Instead of shape or size, electrophilicity emerged as the only common chemical signature among these diverse structures [5]. One of the inducer classes identified were the isothiocyanates (such as sulforaphane), electrophilic hydrolytic products of a unique class of phytochemicals known as glucosinolates, which are abundant in cruciferous vegetables and thus components of the human diet [6]. Moreover, the inducer activity was dependent on the sulfhydryl reactivity [7] as well as the intracellular concentration [8] of the compounds. Being electron deficient, electrophiles are susceptible to nucleophilic attack, such as that carried out by reactive cysteines in proteins. The discovery of electrophilicity as the only common property among a large number of structurally diverse inducers led Paul Talalay and his colleagues to exclude the involvement of a receptor protein, and predict the existence of a chemical sensor protein endowed with highly reactive cysteine residue(s), a prediction that was met with considerable scepticism at the time.

Abbreviations

15d-PGJ$_2$ 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$
4-HNE 4-hydroxynonenal
ACOD1 aconitate decarboxylase 1
AGE advanced glycation end product
AhR aryl hydrocarbon receptor
CyPG cyclopentenone prostaglandin
DHA docosahexaenoic acid
EFOX electrophilic fatty acid oxo-derivatives from ω-3 fatty acids
EPA eicosapentaenoic acid
Ga3P glyceraldehyde 3-phosphate
GLO1 glyoxalase 1
GSH glutathione
GST glutathione *S*-transferases
HSF1 heat shock factor 1
KEAP1 Kelch-like ECH associated protein 1
Kyn-CKA kynurenine-carboxyketoalkene
LDE lipid-derived electrophile
LTB4DH leukotriene B4 12-hydroxydehydrogenase
MGO methylglyoxal
NQO1 NAD(P)H:quinone acceptor oxidoreductase 1
NRF2 nuclear factor erythroid 2 p45-related factor 2
PKM2 pyruvate kinase M2
PUFA polyunsaturated fatty acid
WDR1 WD Repeat Domain 1

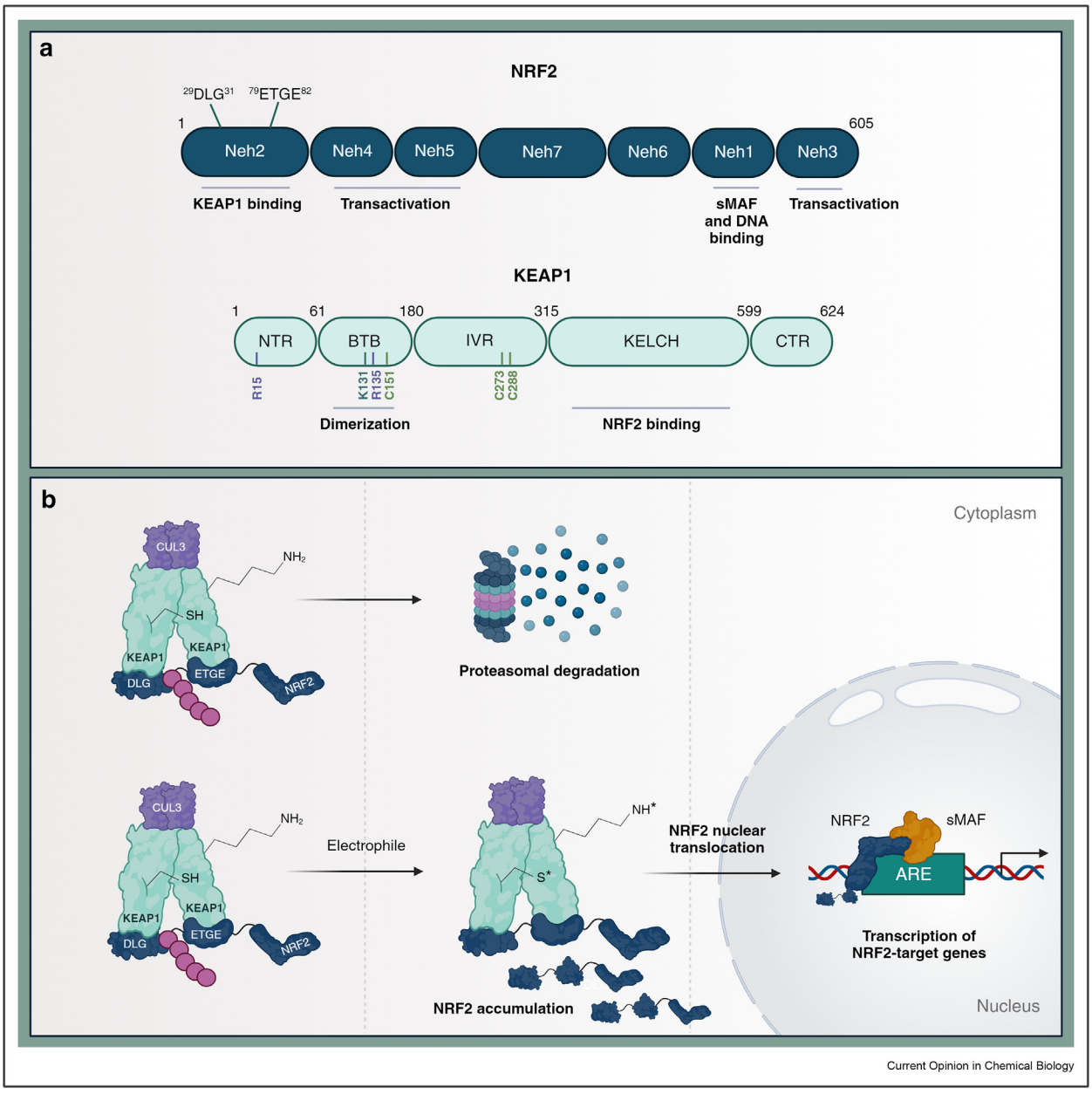
Following the discovery by Masayuki Yamamoto and his colleagues of NRF2 and its repressor KEAP1, as regulators of the expression of these cytoprotective genes [9,10], several sensor cysteines in KEAP1 that are modified by electrophilic inducers were identified, leading to the idea of a "cysteine code", allowing for selectivity, but also ensuring flexibility in achieving a finely-tuned and tightly-regulated NRF2-mediated transcriptional response [11—13]. Most initial investigations were focused on exogenously administered electrophilic compounds, two of which (i.e. the fumaric acid ester, dimethyl fumarate, and the cyanoenone triterpenoid, omaveloxolone) are now clinically used [14,15]. More recently, it has become clear that several endogenously produced electrophilic metabolites can also affect the KEAP1/NRF2 partnership, leading to NRF2 activation and ensuring cytoprotection under conditions of oxidative, inflammatory and metabolic stress. These electrophilic metabolites are the focus of this short review.

Fatty acid-derived metabolites: cyclopentenone prostaglandins, electrophilic fatty acid oxo-derivatives from ω-3 fatty acids (EFOX), 4-hydroxynonenal (4-HNE), acrolein, and nitro-fatty acids

Enzymatic and non-enzymatic oxidation of polyunsaturated fatty acids (PUFAs) produces a plethora of electrophilic lipid peroxidation products with diverse signaling functions (Figure 2a), whereas nitration yields nitroalkene derivatives (Figure 2b). Formation of lipid-derived electrophiles (LDEs) is promoted by inflammatory conditions, and post-translational modification of proteins by protein lipoxidation results in activation of stress signaling pathways, including the KEAP1-NRF2 pathway [16]. In this pathway, KEAP1 is the primary target of LDEs, and the activating species and reactive thiols have been studied extensively. Cyclopentenone prostaglandin (CyPG) 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$ (15d-PGJ$_2$) is synthetized from arachidonic acid via enzymic conversion by cyclooxygenase and PGD2 synthase to prostaglandin D$_2$, followed by non-enzymatic dehydration reactions to yield J-series of prostaglandins [17]. Structural analogues of CyPGs are also formed during non-enzymatic oxidation of arachidonic acid and phospholipids [18,19]. Non-enzymatic oxidation of the ω-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) also give rise to CyPG species analogous to those formed from arachidonic acid [20,21]. Electrophilic oxo-derivatives of DHA and EPA can also be formed enzymatically via cyclo-oxygenase 2-dependent reactions, activating NRF2 [22]. Both enzymatic and non-enzymatic analogs of CyPGs are able to covalently modify KEAP1 and induce NRF2-dependent gene transcription [21,23,24]. With respect to 15d-PGJ$_2$, the thiol targets within KEAP1 have been studied in detail, and it has been concluded that C273 and C288, but not C151, are functionally important residues (Figure 2c) [23,25,26]. In addition to high molecular weight LDEs, oxidation of PUFAs leads to the formation of a variety of aldehydes, such as 4-hydroxynonenal (4-HNE) and acrolein (Figure 2a) [27]. These can also activate NRF2 but are less potent than 15d-PGJ$_2$ [7,23]. The molecular targets of 4-HNE have been studied in detail. 4-HNE appears to primarily target C273 and C288, but it also binds to C151 within KEAP1 [28,29] (Figure 2c). Modification of C288 by acrolein has also been reported (Figure 2c) [28].

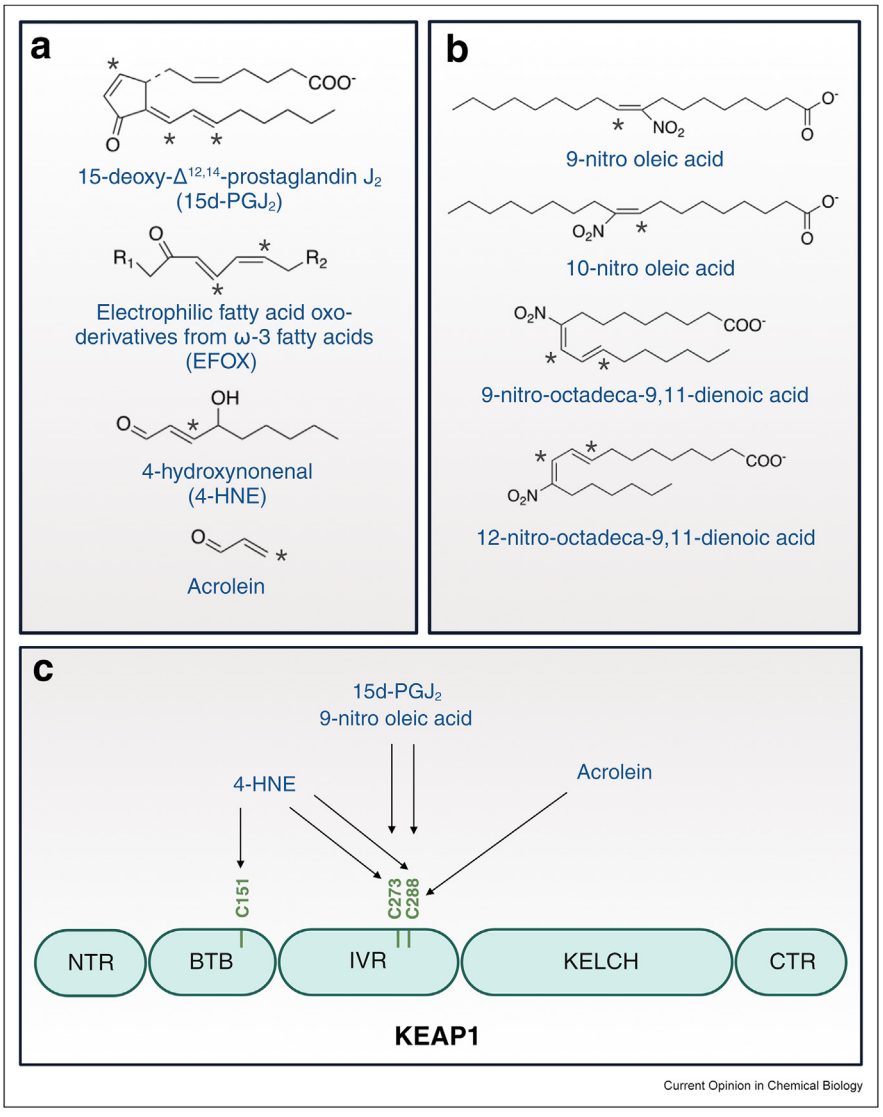
In addition to oxidation, unsaturated fatty acids can undergo nitration via nitrogen dioxide ($\cdot NO_2$)-dependent reactions [30]. The resulting nitroalkene derivatives can undergo reversible Michael addition reactions with cellular low molecular weight and protein thiols [31]. *In vivo*, conjugated linoleic acid is the preferred substrate for fatty acid nitration [32]. While readily nitrated *in vitro*, the *in vivo* levels of nitro-linoleic acid, nitro-oleic acid and nitro-arachidonic acid are within picomolar range or undetectable, much lower than originally reported [33,34]. Many nitroalkenes have been shown to evoke the

Figure 1



(a) Domain structure of human NRF2 and KEAP1. NRF2 has seven domains. The N-terminal Neh2 domain contains the KEAP1 binding motifs DLG and ETGE. The Neh4 and 5 domains are required for NRF2 transactivation. The Neh6 domain contains a phosphodegron and binds to β-TrCP. The Neh1 domain has the DNA-binding motif and the binding region for heterodimerization with a small musculoaponeurotic fibrosarcoma (sMAF) protein. The carboxy-terminal Neh3 domain binds to chromodomain helicase DNA binding protein 6 (CHD6). KEAP1 has five domains: N-terminal region (NTR); Broad complex, tramtrack, and Bric à Brac (BTB) homodimerization domain that also binds to Cullin 3 (CUL3); Intervening region (IVR); Kelch domain (KELCH) that forms a six-bladed β-propeller, through which one of the KEAP1 monomers binds to the ETGE motif of the Neh2 domain of NRF2, and the other monomer binds to the DLG motif; C-terminal region (CTR). The vertical lines indicate the amino acids that are modified by endogenous electrophilic metabolites. **(b) Regulation of NRF2 by KEAP1.** NRF2 binds to the Kelch domains of dimeric KEAP1 via the 'DLG' and 'ETGE' motifs in the Neh2 domain of the transcription factor. In turn, reduced KEAP1 serves as a CUL3/RBX1 E3-ubiquitin ligase substrate adaptor that targets NRF2 for ubiquitination (symbolized as pink circles) and proteasomal degradation. Electrophiles chemically modify specific reactive cysteines, lysines or arginines in KEAP1, impairing its substrate adaptor function. Consequently, NRF2 accumulates, undergoes nuclear translocation, and as a heterodimer with a small MAF transcription factor, binds to specific sequences termed electrophile/antioxidant response elements (EpRE/ARE) in the regulatory regions of NRF2-target genes, and activates their transcription. −SH = reduced cysteine; −S* = modified cysteine; $-NH_2$ = lysine; −NH* - modified lysine.

Figure 2



Examples of fatty acid-derived electrophilic metabolites. (a) 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), electrophilic oxo-derivatives (EFOX) derived from ω-3 fatty acids, 4-hydroxynonenal (4-HNE), and acrolein. R_1 and R_2 indicate variable lateral chains. **(b)** Nitro-fatty acids. **(c)** Cysteine targets in KEAP1 of fatty acid-derived electrophiles. * electrophilic carbon.

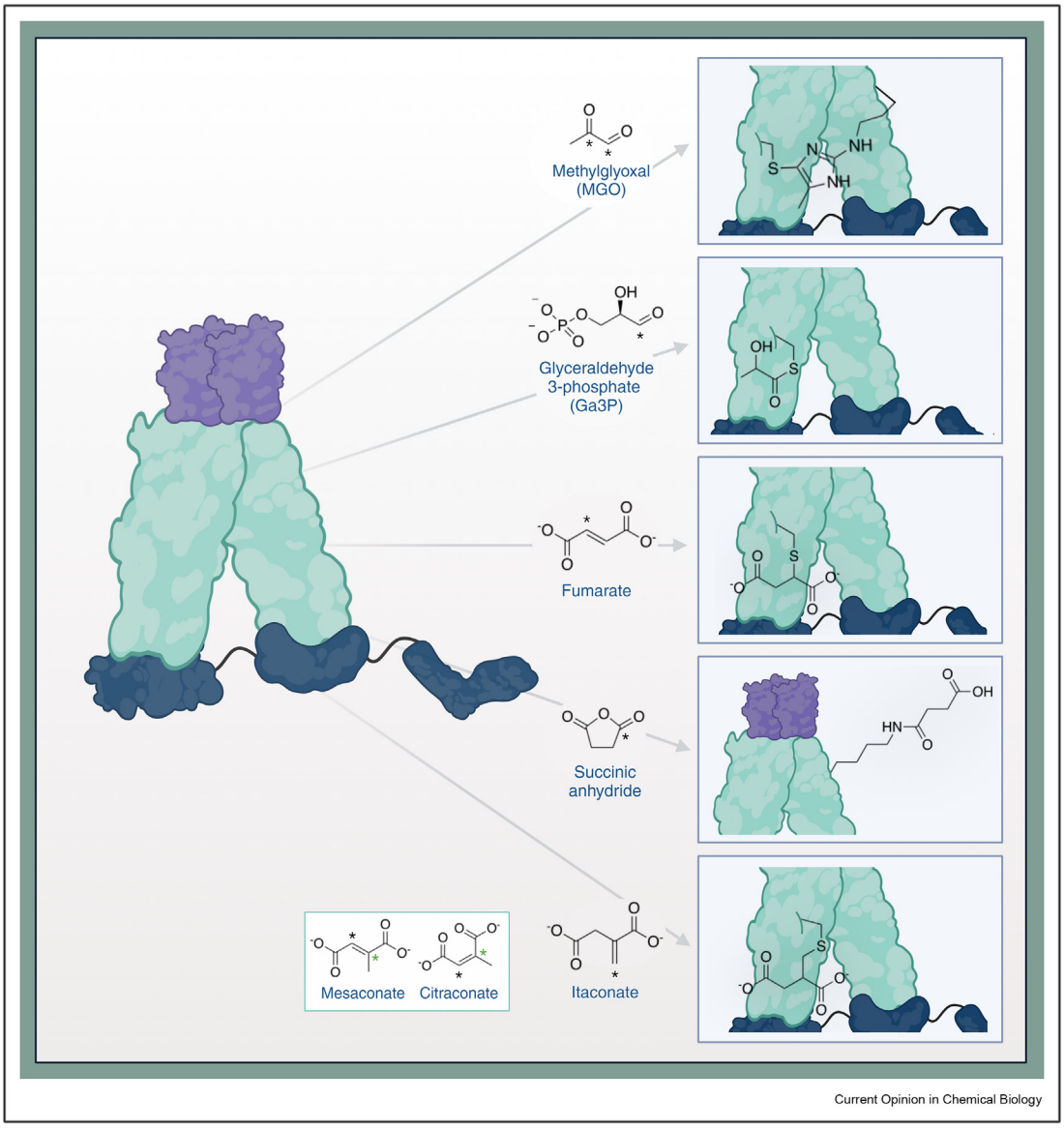
antioxidant response [26,35–38]. The signaling functions and thiol targets of nitro-oleic acid, the prototypical nitro fatty acid, have been studied in detail. The reactivity of nitro-oleic acid with KEAP1 has been examined with recombinant protein as well as in cellular milieu, and it has been concluded that C273 and C288 are the primary targets, whereas C151 is neither particularly reactive nor functionally necessary (Figure 2c) [26].

The effect of many LDEs is limited by leukotriene B4 12-hydroxydehydrogenase (LTB4DH, also called prostaglandin reductase-1 or NADPH alkenal/one oxidoreductase) which catalyzes the hydrogenation of the double bonds in α,β-unsaturated aldehydes and ketones, such as 4-HNE and 15d-PGJ_2 [39,40]. This enzyme has also been identified as the nitroalkene reductase reducing the nitroalkene moiety of nitro-oleic acid, albeit with much lower catalytic rate, yielding the biologically inactive nitro-stearic acid [41]. Interestingly, the expression of LTB4DH is induced by nitro-oleic acid in a NRF2-dependent manner [36], suggesting that induction of LTB4DH provides a feedback inhibitory mechanism modulating the downstream signaling action of LDEs.

Glycolysis-related metabolites: methylglyoxal and glyceraldehyde 3-phosphate

Methylglyoxal (MGO) is a highly reactive electrophilic dicarbonyl compound, a byproduct of glycolysis, which is normally detoxified by the glyoxalase system. MGO is a potent glycating agent of proteins and DNA, leading to the formation of advanced glycation end products (AGEs), and its accumulation has been implicated in the pathogenesis of type 2 diabetes and its complications, as well as in many chronic inflammatory diseases such as cardiovascular disease, neurological conditions, and cancer [42]. The accumulation of MGO causes covalent KEAP1 dimerization by forming methylimidazole intermolecular crosslink between C151 and R15 or R135 of two KEAP1 monomers (Figure 3), resulting in NRF2 activation [43]. Importantly, this activation of NRF2 then serves as a protective negative feedback mechanism,

Figure 3



Modifications of KEAP1 by electrophilic metabolites derived from central carbon metabolism. The glycolytic byproduct methylglyoxal (MGO) activates NRF2 by forming methylimidazole intermolecular crosslink between C151 and R15 or R135 of two KEAP1 monomers, whereas the glycolysis metabolite glyceraldehyde 3-phosphate (Ga3P) causes *S*-lactoylation of C273 of KEAP1. The Krebs cycle metabolite fumarate activates NRF2 by modifying C151 of KEAP1 via *S*-succinylation, whereas the succinyl-CoA byproduct succinic anhydride modifies KEAP1 via *N*-succinylation of K131. Similar to fumarate, cis-aconitate-derived itaconate modifies KEAP1 via 2,3-dicarboxypropylation of C151. The inset shows the itaconate isomers mesaconate and citraconate. * electrophilic carbon. The secondary, less electrophilic carbons in mesaconate and citraconate are shown in green.

facilitating the detoxification of MGO by upregulating the transcription of the NRF2 target glyoxalase 1 (*GLO1*) [44] and increasing the synthesis of glutathione (GSH), an essential component of the glyoxalase system [45]. Additionally, in rapidly proliferating cells, such as mitogen-exposed or cancer cells, NRF2 activation is likely to limit the formation of MGO by channelling glucose metabolism away from glycolysis, through the pentose phosphate pathway, which also ensures the generation of reducing equivalents [46,47].

Accumulation of the sulfhydryl-reactive glycolytic metabolite glyceraldehyde 3-phosphate (Ga3P) activates NRF2 by causing *S*-lactoylation of C273 of KEAP1 (Figure 3) [48]. Ga3P accumulates consequent to inhibition of pyruvate kinase M2 (PKM2), which catalyzes the final step in glycolysis. Physiologically, PKM2 can be inhibited by the amino acids phenylalanine, alanine, tryptophan, and valine, which stabilize the enzyme in an inactive tetrameric form, providing a metabolic sensing mechanism that can react to fluctuating amino acid concentrations [49]. Furthermore, the enzyme activity of PKM2 can be also inhibited by nitrosation and oxidation, modifications that prevent the formation of the active tetrameric form [50]. The enhanced NRF2 activity upon PKM2 inhibition may provide means for a return to homeostatic conditions by transcriptionally increasing the levels of PKM2 [51].

Krebs cycle-related metabolites: Fumarate, succinic anhydride, itaconate, and citraconate

The Krebs cycle metabolite fumarate activates NRF2 by modifying C151 of KEAP1 via *S*-succinylation (Figure 3) [52,53]. Renal cell carcinomas often have mutations in fumarate hydratase, leading to accumulation of fumarate and NRF2 activation. Curiously, a recent study has shown that nicotinamide riboside supplementation (as means for boosting NAD^+) increases the arginine succinate lyase-mediated biosynthesis of arginine and fumarate, activating NRF2, which in turn elevates the antioxidant capacity of adaptive immune cells and blunts inflammatory signalling [54]. Conversely, succinate dehydrogenase subunit A (SDHA) gain-of-function mutations, which have been identified in patients with the primary antibody deficiency disorder persistent polyclonal B cell lymphocytosis, lead to fumarate accumulation and increased NRF2-dependent transcription of pro-inflammatory cytokines [55]. Together, these findings illustrate the complex, context-dependent outcomes of NRF2 activation.

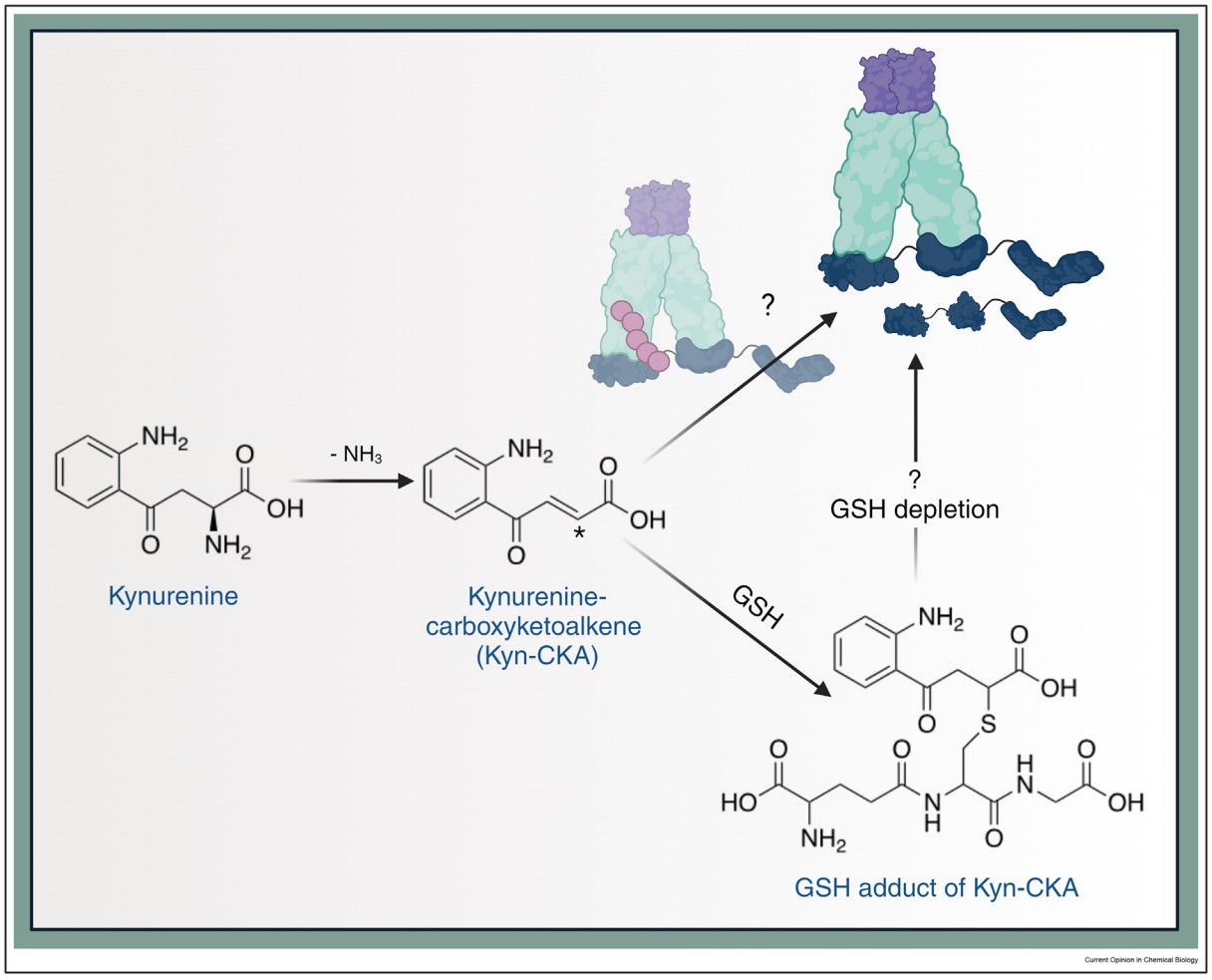
Similar to fumarate hydratase deficiency, depletion of the Krebs cycle enzyme succinyl-CoA synthetase leads to accumulation of another reactive metabolite, the succinyl-CoA byproduct succinic anhydride, which modifies KEAP1 via *N*-succinylation of K131 and inactivates the substrate adaptor (Figure 3) [56]. Whether the resulting NRF2 activation may have a cytoprotective role in mitochondrial diseases associated with succinyl-CoA synthetase impairment is currently unknown.

Another Krebs cycle-derived mildly-electrophilic metabolite, itaconate, alkylates C151 of KEAP1 in a nonenzymatic reaction termed 2,3-dicarboxypropylation (Figure 3) [57]. Itaconate is an anti-inflammatory metabolite, which accumulates during the metabolic reprogramming of activated macrophages. It originates from cis-aconitate consequent to the induced expression of aconitate decarboxylase 1 (*ACOD1*, also known as IRG1) [58]. The resulting NRF2 activation is a critical contributor to the resolution of inflammation; indeed, compared to their wild-type counterparts, NRF2-knockout mice are much more sensitive to endotoxin- and cecal ligation and puncture-induced septic shock [59]. In agreement, ACOD1 deficiency suppresses NRF2 and enhances production of pro-inflammatory cytokines in cardiac macrophages [60], whereas its upregulation improves donor heart preservation and function [61]. In tumour-infiltrating neutrophils, the levels of ACOD1 are increased, and the consequent itaconate production mediates NRF2-dependent cytoprotection, which has a critical role in sustaining the viability of these cells in the tumour microenvironment [62]. Among itaconate and its naturally occurring isomers mesaconate and citraconate (Figure 3, inset), citraconate is the most potent electrophile and NRF2 activator, followed by itaconate and mesaconate (based on glutathione reactivity and lowest unoccupied molecular orbital and electrophilicity index calculations) [63], although the possibility that citraconate or mesaconate modify KEAP1 cysteine(s) has not been investigated.

Amino acid-derived metabolite: Kynurenine-carboxyketoalkene (Kyn-CKA)

Inflammation causes an increase in the levels of the tryptophan-derived metabolite kynurenine consequent to upregulation of the enzymes indoleamine 2,3-dioxygenase-1 (IDO1) and tryptophan 2,3-dioxygenase (TDO2); in turn, kynurenine binds to and activates the aryl hydrocarbon receptor (AhR), exerts anti-inflammatory activity, and contributes to inflammatory disease tolerance [64]. Activation of NRF2 following kynurenine exposure has been demonstrated in cells and in mice [65], although the mechanism by which it occurs is incompletely understood and may involve activation of AhR and/or phosphoinositide 3-kinase (PI3K)/AKT signalling [66,67]. Critically, kynurenine can undergo deamination to generate an electrophilic α,β-unsaturated carbonyl-containing product, kynurenine-carboxyketoalkene (Kyn-CKA) (Figure 4) in cells and *in vivo*. It was recently shown that Kyn-CKA forms adducts with cysteine and GSH, and activates

Figure 4



The amino acid-derived metabolite kynurenine-carboxyketoalkene (Kyn-CKA) as a possible electrophilic modifier of KEAP1. The tryptophan-derived metabolite kynurenine undergoes deamination generating an electrophilic metabolite, kynurenine-carboxyketoalkene (Kyn-CKA), which in turn forms an adduct with reduced glutathione (GSH). Kyn-CKA activates NRF2 in cells and *in vivo*; this is likely a consequence of direct cysteine alkylation of KEAP1 or indirect cysteine oxidation of KEAP1 due to GSH depletion. * electrophilic carbon.

NRF2-dependent gene expression [65]; thus, it is highly likely that Kyn-CKA modifies cysteine sensor(s) in KEAP1. Moreover, in the same study, Kyn-CKA attenuated pro-inflammatory response and was protective against endotoxin-mediated toxicity, although the potential dependence on NRF2 in the protective mechanism has not been investigated.

Concluding remarks

Our knowledge of the effect of electrophiles on the KEAP1/NRF2 partnership has expanded substantially, but a number of challenges still remain. In addition to NRF2, KEAP1 interacts with a multitude of other proteins [14]. The consequences of KEAP1 modifications by electrophiles on these interactions are just beginning to be understood. Thus, the methyl ester of fumarate, dimethyl fumarate, disrupts the interaction of KEAP1 with WD Repeat Domain 1 (WDR1), which subsequently forms a complex with cofilin and actin, and promotes apoptosis in macrophages and neutrophils [68].

Moreover, KEAP1 is not the only target of electrophilic metabolites. Other proteins, such as transcription factor heat shock factor 1 (HSF1), can be also activated, for example by nitro-fatty acids [36,69], providing another layer of cytoprotection, through induction of molecular chaperones that ensure correct protein folding. Multiple protein targets of itaconate have also been identified, including glyceraldehyde 3-phosphate dehydrogenase, aconitate hydratase, lactate dehydrogenase A, annexin A1, gasdermin D, Janus kinase 1, and transcription factor EB [57,70–73]. Itaconate also binds covalently to the catalytic site C191 of *Mycobacterium tuberculosis* isocitrate lyase isoform 1 and inhibits its enzyme activity [74], and

thus has a broad role in the host defence against pathogens. Of note, to overcome the limited membrane permeability of itaconate, 4-octyl itaconate, a cell-permeable derivative of itaconate is commonly used. However, the ester in 4-octyl itaconate activates the molecule and enhances its sulfhydryl reactivity, precluding extrapolation to endogenous conditions and reactivities. Furthermore, sulfhydryl reactivity, although an important factor, is not the only determinant of cellular target (e.g. KEAP1) engagement and subsequent biological (e.g. NRF2 inducer) activity. For example, Zhang et al. [75] determined the rates for both the nonezymatic (second order rate constants) and enzyme-catalyzed conjugations with glutathione of four isothiocyanates (NCS) (allyl-, benzyl-, phenethyl-NCS, and sulforaphane), and found that conjugation of benzyl-NCS is the fastest and that of sulforaphane is the slowest. This was unexpected, because among these isothiocyanates, sulforaphane is in fact the most potent inducer in cells and in mice. A subsequent detailed study revealed that when cells were exposed to nine isothiocyanates that differ in chemical structure, reactivity and inducer potencies, the intracellular concentrations (area under curve), not thiol reactivity, correlated linearly with their inducer potencies [8]. This finding provides an explanation for the high inducer potency of sulforaphane and strongly suggests that the intracellular concentration is a major determinant of biological activity.

The inherent pleiotropism makes it very difficult to establish experimentally which protein target(s) is responsible for the ultimate effect of electrophiles in biological systems. This difficulty is further compounded by the complexity of mammalian organisms, where the expression of the target proteins as well as the generation and the effects of electrophilic metabolites are very likely to be context-, tissue-, and even cell-type specific, and influenced by diet and exposures to pathogens and other environmental factors. Most laboratory studies employ non-physiological concentrations/doses of electrophiles, likely exceeding their endogenous levels and with little consideration of concentration differences and fluctuations in specific cell compartments, which are mostly unknown. Some of these difficulties and limitations are beginning to be overcome by recent advances in the experimental systems employed. Thus, the use of knock-in mice and cells from mice in which C151 in KEAP1 has been replaced with a serine has shown that the cyclic cyanoenone-mediated activation of NRF2 and its downstream cytoprotective activities are abrogated in cells *ex vivo* as well as *in vivo* [76,77]. In the context of cells and model organisms, the development of new technologies, such as T-REX (targetable reactive electrophiles and oxidants), allows for the delivery and quantitative assessment of occupancy of an electrophile locally to a specific protein of interest within the cell, without the overall disturbances associated with potential GSH depletion and enhanced ROS production upon bolus electrophile treatment [78]. Indeed, precision labeling of KEAP1 with 4-HNE in live cells and *C. elegans* employing T-REX has revealed large differences to the modifications observed using bolus electrophile administration, which superstoichiometrically reacts with KEAP1, in addition to numerous other proteins [79]. Moreover, a similar approach in a zebrafish model (Z-REX) allowed the examination of one zKEAP1 paralog at a time, and showed that zKEAP1b augments, whereas zKEAP1a (which carries a C273I substitution) opposes, NRF2 activation by photocaged lipid-derived electrophiles [80]. The use of T-REX has also shown that 4-HNE selectively labels and suppresses the activity of AKT3, but not AKT2, demonstrating the unique ability of this approach to detect not only protein-, but also isoform-specificity [81]. The subsequent development of G-REX (global reactive electrophiles and oxidants) identified novel 4-HNE-sensitive proteins, such as Ube2v2 and Ube2v1, allosteric activators of the E2-ligase UbE2N, which is involved in K63-linked polyubiquitination [82].

Upon summarizing the endogenously produced electrophilic metabolites which have been shown to affect the KEAP1/NRF2 partnership, we have no doubt that many more will be identified in the future. The functional consequences of their effects are comparable to those caused by the more commonly known enzyme-catalyzed post-translational modifications of various signalling proteins. By non-enzymatically covalently modifying KEAP1, as well as other electrophile-sensitive proteins, these reactive metabolites serve as chemical signals connecting metabolism with cellular stress responses.

Author contributions

Albena T. Dinkova-Kostova: Conceptualization, Writing – Original draft preparation, Reviewing and Editing. Anna-Liisa Levonen: Conceptualization, Writing – Original draft preparation, Reviewing and Editing. Henriikka Hakomäki: Figures design and preparation, Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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** of outstanding interest

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