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Self-regulatory role of 4-hydroxynonenal in signaling for stress-induced programmed cell death

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Abstract

Within the last two decades, 4-hydroxynonenal has emerged as an important second messenger involved in the regulation of various cellular processes. Our recent studies suggest that HNE can induce apoptosis in various cells through the death receptor Fas (CD95)-mediated extrinsic pathway as well as through the p53-dependent intrinsic pathway. Interestingly, through its interaction with the nuclear protein Daxx, HNE can self-limit its apoptotic role by translocating Daxx to cytoplasm where it binds to Fas and inhibits Fas-mediated apoptosis. In this paper, after briefly describing recent studies on various biological activities of HNE, based on its interactions with Fas, Daxx, and p53, we speculate on possible mechanisms through which HNE may affect a multitude of cellular processes and draw a parallel between signaling roles of H₂O₂ and HNE.

Keywords

4-Hydroxynonenal; Apoptosis; Cell cycle signalling; Fas; P53; Heat shock factor 1; Daxx

Introduction

Initially thought to be merely a toxic end product of lipid peroxidation, 4-hydroxynonenal (HNE) has now emerged as an important signaling small molecule which is involved in signaling for cell cycle control and the regulation of expression of a multitude of genes, the products of which regulate a variety of cellular processes. Earlier studies on the effects of HNE on DNA, RNA, and protein synthesis, chemotaxis, and effector proteins such as phospholipase C and adenylate cyclase (reviewed in [1]) received little attention and even the earlier reports demonstrating its role in signaling for apoptosis were met with skepticism but in recent years its role on regulation of various signaling pathways is firmly established. The list of studies demonstrating its role in signaling for apoptosis, regulation of gene expression, proliferation, transformation, and its interactions with signaling components in membranes, cytoplasm, and nucleus is consistently growing. Similar to H₂O₂, a major physiologic initiator of signaling processes, HNE is a small molecule, has a relatively short life, is diffusible, and its intracellular concentrations are tightly regulated by enzymes that are induced rapidly under stress conditions that increase its intracellular levels. Formation of HNE is proportional to ROS produced as a result of internal (e.g., metabolic) or external (e.g., UV, chemicals) stress and in recent years credible evidence has accumulated which suggests that at least a part of ROS-induced signaling may be transduced via HNE and that it can be regulated by limiting its intracellular

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concentration [2-4]. These studies have opened a new area in the field of ROS-induced signaling focusing on the regulatory roles of the enzymes involved in the formation and metabolism of HNE. Recent studies in this area have shown that enzymes such as glutathione S-transferases (GSTs), aldehyde dehydrogenases, aldose reductase, glutathione peroxidase, and RalBP1 (Ral-binding protein 1) that are among the major determinants of intracellular levels of HNE can modulate stress-induced signaling for programmed cell death [2-10]. Some of these studies [11-14] seem to have major clinical implications particularly in regard to cancer chemotherapy [11,12], inhibition of tumor growth [13], and sepsis [14] as discussed briefly later in this review.

Recent studies suggest that HNE can induce signaling for apoptosis via multiple pathways, which seem to converge on the activation of JNK and caspase3 [15,16]. Furthermore, there is credible evidence that HNE plays an important role in membrane receptor-mediated signaling and that it can directly interact with transcription factors and transcription repressors. These multiple actions of HNE are consistent with its role in regulation of the expression of numerous genes and modulation of various signaling processes.

Initial pioneering studies on HNE and signaling were covered by Esterbauer in his comprehensive review [1]. A number of recent reviews covering studies on the role of HNE in the modulation of signaling processes are also available [2-4,17-24]. Most of these reviews have focused on the role of HNE in inducing apoptosis, regulation of the expression of genes, and its interactions with target proteins including the components of various signaling cascades. In this review, against a backdrop of relatively recent studies showing the role of HNE in regulation of various cellular processes, we discuss the mechanisms of HNE-induced apoptosis through the extrinsic and intrinsic pathways, its self-regulatory role in this process, its interactions with Fas (CD95), p53, and Daxx, and speculate on probable mechanisms through which HNE affects such a multitude of cellular processes.

4-HNE and signaling

Some of the reported effects of HNE including cell cycle signaling, cell proliferation, transformation, chemotaxis, regulation of gene expression, its interactions with membrane receptors, transcription factors, transcription repressors, and its role in signaling for programmed cell death are listed in Table 1 [25-76]. In recent years, studies in our laboratory have focused on the mechanisms of HNE-induced signaling and its regulation by enzymes which determine the intracellular concentration of HNE. These studies indicate an important role of HNE in signaling for apoptosis induced by stressors including H₂O₂, UV, heat, and oxidant chemicals such as naphthalene or doxorubicin and that apoptosis induced by these agents can be inhibited in cells transfected with enzymes that limit the intracellular concentrations of HNE [7-9,47,77]. For example, the overexpression of GSTA4-4 or GST5.8, which detoxify HNE, inhibits apoptosis induced by xanthine/xanthine oxidase, H₂O₂, UV, and doxorubicin [9]. Furthermore, these studies indicate that lowering of the intracellular concentration of HNE by overexpression of GSTA4-4 results in profound changes in the expression of a multitude of genes and transformation of adherent HLE-B3 into suspended rounded cells which proliferate at a much faster rate [45]. Studies showing that blocking of HNE metabolism by inhibiting RalBP1-mediated transport of GS-HNE, resulting in increased intracellular concentration of HNE, leads to apoptosis in cells of various origins even in the absence of any stressor further suggesting that HNE is involved in stress-induced signaling for apoptosis [11,12,78-80]. Numerous studies by other investigators listed in Table 1 confirm that HNE plays a major role in the signaling for apoptosis. While much remains to be learned about the exact mechanisms of HNE-induced apoptosis, our recent studies discussed in the following sections of this article suggest that not only HNE can induce apoptosis in cells through more

than one pathway but it can self-limit its apoptotic role through its interaction with the nuclear protein Daxx.

Pathways for apoptosis

The pathways for cellular apoptotic signals in general have been suggested to be primarily of two types, extrinsic and intrinsic [81]. Extrinsic apoptosis signaling is mediated by the activation of cell surface death receptors that transmit apoptotic signals after ligation with specific ligands. Fas(CD95) is one such death receptor belonging to the tumor necrosis factor receptor (TNFR) gene superfamily [82-86]. Fas and other members of the TNFR family consist of cysteine-rich extracellular subdomains which recognize and bind to their specific ligands (e.g., the Fas-ligand or Fas-L in the case of Fas-induced signaling) which results in dimerization and activation of the respective death receptor. Subsequent signaling is mediated by the cytoplasmic part of the death receptor, which contains a conserved sequence termed as the death domain (DD). Adapter molecules like Fas-associated protein with death domain (FADD) are recruited to the DD of the activated death receptor, thereby forming the so-called death-inducing signaling complex (DISC). In addition to DD, the adaptor protein FADD also contains a death effector domain (DED), which through a homo-typical interaction, sequesters procaspase-8 in the DISC [83]. Recruitment of procaspase-8 to the DISC leads to its autocatalytic activation and release of active caspase-8, which then processes downstream effector caspases leading to the cell death [83]. The intrinsic pathway of apoptosis due to mitochondrial generated ROS involving p53 is elaborated on later in this section. Both of these pathways converge on the activation of JNK, caspase3, and eventual programmed death of the cell. Recent studies demonstrate that HNE can induce apoptosis in several different cell lines (e.g., HLE B-3, RPE, and Jurkat cells) through the Fas-mediated extrinsic as well as the p53-mediated intrinsic pathway and that the enzymes that regulate HNE concentration in cells can modulate both of these pathways [15,16].

HNE and Fas-mediated signaling

It has been recently demonstrated that HNE induces the expression of Fas (CD95/Apo1) and activates it to mediate the apoptotic signaling in human lens epithelial (HLE B-3), retinal pigment epithelial (RPE), and Jurkat cells through a novel pathway which is distinct from the canonical extrinsic apoptotic pathway involving DISC formation [87]. These studies show that HNE-induced apoptosis in these cells is independent of Fas-L, FADD, procaspase-8, and the components of DISC [16] and suggest that HNE can mimic the effect of the receptor Ligand (Fas-L) and induce Fas-mediated apoptosis through a novel pathway. During stress-induced, Fas-mediated apoptosis, the involvement of a nuclear death-associated protein (Daxx) has also been demonstrated which is translocated to cytoplasm and binds to Fas [87]. It has been suggested that the binding of Daxx to Fas causes activation of ASK1 and subsequent downstream proapoptotic signals for the execution of apoptosis [87,88]. Our recent studies [15,16] show that during the DISC-independent HNE-induced apoptosis of Jurkat cells, HNE does indeed cause the translocation of Daxx to cytoplasm and promotes its binding to Fas. However, the binding of Daxx to Fas inhibits HNE-triggered apoptosis by blocking the signaling from Fas to ASK1, JNK, and caspase3 that is required for apoptosis. This is indicated by studies showing that silencing of Daxx in Jurkat cells makes these cells more sensitive to not only HNE-induced apoptosis but also to stress-induced apoptosis and sensitizes them to agonistic Fas antibodies (CH11) which induce apoptosis via the canonical pathway.

Daxx–HNE interactions

The interactions of HNE with Daxx and the inhibitory role of Daxx suggest that HNE can self-regulate Fas-mediated apoptosis through translocation of Daxx to cytoplasm where it inhibits apoptosis by binding to Fas. Our recent studies showing that HNE induces as well as self-limits

Fas-mediated apoptosis and that it induces heat shock proteins (HSPs) [16,25] strongly suggest a novel role of HNE in the mechanisms of defense in stressed cells implying that besides inducing apoptosis, it also plays a protective role under stress conditions. It is possible that because of the diffusiveness of HNE, a proapoptotic signal initiated by HNE could spread beyond the initial site of the insult to adjacent cells or tissues and HNE-triggered export of Daxx to the cytoplasm may serve as protective mechanism that controls and limits a runaway apoptotic process. These studies showing translocation of the heat shock factor HSF1 in to nucleus leading to the upregulation of HSP70 [16,25]. Taken together with the previously reported induction of glutamate cysteine ligase and GSH biosynthesis [89] in cells exposed to HNE are consistent with the idea of a protective role of HNE. Thus, HNE-induced translocation of Daxx from the nucleus to cytoplasm, and release of free HSF1 in the nucleus may have major implications for the defense mechanisms during stress-mediated signaling, particularly by stressors such as UV radiation, heat shock, or oxidants which invariably increase the cellular concentration of HNE [9].

4-HNE and p53-mediated signaling

Induction of apoptosis by a single compound through multiple pathways is not common but recent studies suggest that in some cells, UV can induce apoptosis via multiple pathways [90]. Since HNE is involved in UV-mediated signaling for apoptosis, it should not be surprising that HNE also induces apoptosis through multiple pathways [91,92]. Apoptotic signaling by different forms of oxidative stress is mediated through a cross-talking set of downstream regulators and effectors of programmed cell death through the intrinsic pathway often involving the p53 tumor-suppressor protein [93]. P53 plays an important role in apoptosis, growth arrest, genomic stability, cell senescence, and differentiation. Because of its short half-life, the constitutive levels of p53 in cells are low. The presence of a transcriptionally inactive latent form of p53 has also been reported in some cells [93-95]. It is generally believed that diverse events such as DNA damage, hypoxia, and oxidative stress activate the p53 gene causing its rapid accumulation, which is proportional to the extent of DNA damage [93,96]. Since HNE concentrations in cells are increased in stressed cells and that it is a common denominator in the mechanisms of apoptosis caused by the diverse forms of oxidative stress including UV [7,9], it is likely to affect the expression and/or activation of p53. If so, HNE-mediated activation of p53 may be one of the mechanisms responsible for HNE-induced apoptosis reported in many cell types [7-9,12-16,46-48,61]. Our recent studies are in accordance with this prediction. It is known that during p53-mediated apoptosis it is activated through phosphorylation [97-99] and its modified and stable form promotes upregulation of the downstream proapoptotic target genes, p21 and Bax [100-103]. Our studies show that HNE causes induction, phosphorylation, and nuclear translocation of p53, activation of p21, Bax, JNK, and caspase3, and leads to the onset of apoptosis in Jurkat and RPE cells [16]. This pathway is inhibited in cells transfected with hGSTA4 due to their ability to rapidly dispose off HNE, suggesting a specific involvement of HNE in p53-mediated apoptosis [unpublished data].

HNE and transcription repressors/factors

Daxx is a transcription repressor, which can regulate the expression of stress responsive genes. It has been shown that Daxx interacts with HSF1 (the nuclear concentration of which is increased following HNE exposure) and upregulates the expression of HSF1-associated genes. Likewise, it is known that interaction of Daxx with other transcription factors such as ETS1, Pax 3, Pax5, smad4, and p53 also affects the expression of the associated genes [104-107]. Thus, HNE can influence a large set of fundamental cellular processes including proliferation, cell cycle events, and apoptosis and can modulate the expression of numerous genes. This is consistent with the reported effect of HNE on many genes including c-myc, AKT, PKC,

TGF β , ASK1, JNK, Nrf2, integrins, connexins, ERK pathway genes, aldose reductase, glycogen synthase kinase, gamma-glutamyl transferase (GGT), adenylate cyclase, RalBP1, and heat shock proteins among others [3,13,25,45,52,57,65,74]. The underlying mechanisms for such a global effect of HNE on gene expression are largely unclear. A plausible mechanism involving HNE in the regulation of the expression of HSF1-associated genes has been speculated. It is known, that in response to stress, the increase in intracellular concentration of 4-HNE triggers RalBP1 (transporter of GS-HNE) to release HSF1 from the RalBP1–tubulin–HSP90–HSF1 complex and its migration to nucleus that leads to the transcription of heat shock proteins. Interestingly, RalBP1 and HSF1 mutually regulate their functions. This is indicated by our unpublished studies showing that in response to stress, their functions are mutually regulated through RalBP1-mediated sequestration of HSF1 into cytoskeleton and HSF1-mediated inhibition of the transport activity of RalBP1 toward glutathione conjugates such as GS-HNE. Thus HNE, being a determinant of GS-HNE concentrations, RalBP1-mediated transport activity can regulate the transcription of HSF1-associated genes [108,109].

In the context of the signaling role of HNE and its glutathione conjugate, GS-HNE, it is important to consider the intracellular concentrations of GSH. If at the time of stress or HNE exposure GSH concentration in cells is low, it is likely that an increased proportion of HNE may be channeled to the reductive or oxidative pathways for the metabolism of HNE. In such a case the enzymes such as aldehyde dehydrogenase and aldose reductase may play a more important role in the regulation of HNE-mediated signaling. However, available evidence suggests that GSH concentration may not be a limiting factor in HNE-induced signaling. Cellular levels of GSH are normally more than three orders of magnitude higher than that of HNE. Moreover, HNE rapidly upregulates the de novo synthesis of GSH by inducing glutamate cysteine ligase [89]. This is consistent with the results of studies showing that in *GSTA4* or *RLIP76*-knockout mice, where the concentration of HNE and GS-HNE is elevated several-fold in tissues, the steady-state levels of GSH are in fact slightly elevated. Nevertheless, further studies are warranted to study the possible correlation between GSH homeostasis and HNE-mediated signaling.

HNE and membrane receptors

Our studies showing the binding of HNE to Fas and subsequent activation of the Fas-L-independent pathway for apoptosis indicating that HNE can affect signaling via a membrane receptor by mimicking the effects of its ligand (fas-L) are consistent with studies showing that HNE can activate membrane tyrosine kinase receptors (TKRs) and mimic the effect of their ligands on the downstream signaling events [19,64,110,111]. Classically, the epidermal growth factor receptor (EGFR) is activated by binding to its ligand EGF. This interaction leads to dimerization of the receptor and activation of its intrinsic tyrosine kinase resulting in the trans-autophosphorylation of tyrosine residues and activation of downstream signaling events [64, 110-112]. Reaction of HNE with EGFR leads to its aggregation [111], which affects the EGFR-mediated downstream signaling events in a manner somewhat similar to EGF [110-112]. The role of the HNE in transducing the membrane receptor-mediated signaling is further confirmed by our recent studies in which we show that the overexpression of RalBP1 in response to the increased level of HNE leads to increased endocytosis of the EGF receptor. RalBP1 along with the POB1 (partner of RalBP1) has been shown to be present in signal transduction complexes for membrane tyrosine-kinase receptors (such as EGF-R) and are phosphorylated [113-116]. The inhibition of endocytosis in the transport-deficient mutants of RalBP1 further supports the idea that the transport and endocytotic functions of RalBP1 are regulated by the cellular concentration of HNE and HNE-SG. The induction of Fas-mediated signaling for apoptosis by HNE suggests that signaling by membrane proteins of different gene families (e.g., death receptors, EGFR, RalBP1) can be propagated by HNE. Further studies on the consequences of the interactions of HNE with the membrane-associated, cytoplasmic, and nuclear proteins

should provide clues to the mechanisms through which HNE causes such a multitude of effects on various cellular processes reported over the years [1-9].

Accumulation of HNE in cells lead to apoptosis—Clinical implications

HNE is continually generated in cells due to the peroxidation of unsaturated lipids. The enzymes such as glutathione S-transferases, aldehyde dehydrogenases, and aldose reductases and transporters such as RalBP1 and MRP1 that catalyze the efflux of its metabolites regulate its intracellular concentrations [2-15]. GSTs catalyze the conjugation of HNE to glutathione (GSH) which is the major pathway for disposition of HNE. The glutathione conjugate of HNE (GS-HNE) can be further acted on by aldehyde dehydrogenase or aldose reductase to yield corresponding acid and alcohol, respectively, but the majority of GS-HNE is transported by RalBP1 for excretion or its further processing to mercapturic acids [11,12,113]. Blocking of the transport of GS-HNE leads to the buildup of GS-HNE and HNE levels in cells as demonstrated in vitro in cell cultures, as well as in vivo in *mGSTA4* knockout and RalBP1 knockout mice. Cancer cells in culture undergo apoptosis in the absence of any stressor (drugs, H₂O₂, UV, etc.) when coated with RalBP1 antibodies to block the transport of the GSH conjugates of HNE or its metabolites. The inhibition of the transport functions of RalBP1 by RalBP1-interacting proteins, POB1 and HSF1, also leads to apoptosis of cancer cells, further supporting the role of HNE and HNE-SG as regulatory signals for various cellular processes [113,116].

Induction of apoptosis in cancer cells by blocking the transport of GS-HNE may have major implications for cancer therapy and it has been shown that in *syn-genic* mice, xenografts of melanoma cells are completely regressed by intraperitoneal injection of anti-RalBP1 antibodies. Likewise, it has been shown that xenografts of human lung cancer and colon cancer cells in nude mice can be completely regressed by anti-RalBP1 immunoglobulin G or by suppression of RalBP1 expression using phosphorothioate antisense against it [11,12]. Among the metabolites of HNE, the involvement of GS-DHN formed by the reduction of GS-HNE by aldose reductase in regulation of NFκB-associated signaling has been demonstrated and it has been shown that blocking the formation of GS-DHN by aldose reductase inhibitors blocks the proliferation of prostate cancer cells [13,14]. Besides cancer, these findings also have clinical relevance to inflammatory diseases including sepsis. While additional work is needed to explore these possibilities, these studies underscore the role of HNE in signaling for not only apoptosis but also for other pathways and place HNE metabolizing enzymes including the transporters involved in the efflux of GS-HNE as viable targets for treatment and prevention of inflammatory diseases.

In vivo significance

HNE-mediated induction and activation of Fas and p53 signaling for apoptosis are perhaps not limited to any specific cell type. This is indicated by our studies showing that increased HNE levels observed in the tissues of *mGstA4-4* knockout mice are accompanied by the induction and activation of both Fas and p53 in all the tissues examined. Furthermore, JNK, on which both these apoptotic pathways converge, is also activated in the tissues of *mGstA4* (–/–) mice. These findings strongly suggest that the effects of HNE on Fas and p53-mediated pathways for apoptosis are not limited to a specific cell type, have in vivo physiologic/pathophysiologic significance, and are relevant to the mechanisms involved in the etiology of degenerative disorders in which a role of chronic oxidative stress is implicated.

Regulatory circuit

Studies showing the effects of HNE on the expression of Fas, p53, and Daxx, and on the compartmentalization of Daxx and p53 discussed earlier in this review, suggest the presence

of a self-limiting regulatory circuit. The physiologic significance and the mechanistic details of the inhibitory Daxx-Fas complex are not completely understood particularly in terms of intracellular concentration of HNE in cells. We have proposed (Fig. 1) a model in which moderate levels of HNE could trigger the translocation of Daxx to the cytoplasm where Daxx would inhibit Fas-mediated apoptosis. However, during severe stress, elevated HNE could cause both, an overexpression of Fas, and its modification by HNE, leading to the onset of Fas-mediated apoptotic signaling. This signaling could reach the threshold necessary to overcome the Daxx inhibition, and lead to massive apoptosis. Because of the diffusibility of 4-HNE, this proapoptotic signal could spread beyond the initial site of the insult to adjacent cells or tissues. Therefore, HNE-triggered export of Daxx to the cytoplasm that serves to control and limit a runaway apoptosis process would be of special importance to adjacent “bystander” cells. This protective role of 4-HNE may be augmented by HNE-mediated translocation of HSF1 into the nucleus and activation of the transcription of heat shock proteins [16].

Recent studies with CRL2571 cells showing ASK1, JNK, and caspase3 activation by HNE are in agreement with numerous previous studies with different cell types where a sustained activation of JNK has been demonstrated during HNE or stress (UV, H₂O₂, superoxide anion, heat, oxidants (DOX))-induced apoptosis [3,7-9,77]. Available evidence suggests that the activation of ASK1 is linked to the cellular redox status regulated by the thioredoxin and thioredoxin reductase system [117]. In this model, the reduced form of thioredoxin binds to ASK1 and inhibits its kinase activity. During oxidative stress, dissociation of thioredoxin from ASK1 causes the activation of ASK1 by oligomerization leading to the activation of JNK and apoptosis [118]. The inhibition of thioredoxin reductase through a direct interaction with HNE has been shown both in vitro and in vivo [28]. It is possible that the HNE-induced activation of ASK1 and JNK may involve modulation of the thioredoxin–thioredoxin reductase system.

H₂O₂ and HNE-induced signaling

A parallel can be drawn between HNE-induced signaling and the signaling mediated by hydrogen peroxide (H₂O₂) [119,120]. Both H₂O₂ and 4-HNE cause proliferation of cells when present at lower concentrations and cause apoptosis at higher concentrations. Both these compounds are reactive small molecules with a biological half-life suitable to the time scales of many biological processes. These characteristics not only allow these compounds to diffuse over distances even across the membranes for transmitting a signal, but also assure a physiologically acceptable rate of signal termination. Furthermore, the moderate reactivity of both H₂O₂ and 4-HNE provides an intricate balance between signaling efficiency and selectivity. The similarities between H₂O₂ and 4-HNE in their suitability as signaling molecules do not necessarily mean that the mechanisms of signaling by these compounds are redundant. Sulfhydryl groups are the main targets of H₂O₂-mediated oxidation. On the other hand, HNE can interact with a broader set of target groups in proteins, including amino groups in addition to sulfhydryls, and has a wider range of reaction types, including Michael addition and Schiff base formation. Therefore during signaling, H₂O₂ and HNE are likely to target different proteins, and the resulting modifications are likely to be chemically and functionally distinct from each other.

The similarities in H₂O₂ and 4-HNE signaling extend to the logic of their respective regulatory circuits. The inhibitory action of Daxx dampens the apoptotic response to HNE at low (subthreshold) but not at high (above-threshold) HNE concentrations, and that the system becomes self-limiting, at least spatially, because diffusion of HNE into adjacent cells elicits Daxx-mediated inhibition of apoptosis. This nonlinear response of the HNE circuit parallels that of H₂O₂ [121] in which below a certain threshold, peroxiredoxin efficiently removes any H₂O₂ that is generated and inhibits signaling. However, when H₂O₂ levels rise above that threshold, peroxiredoxin is oxidatively inactivated, thus opening the “floodgates” and allowing

H₂O₂ signaling to proceed [121]. It is possible that the inhibition of the thioredoxin-thioredoxin reductase system by HNE above a certain threshold concentration may also affect the apoptotic signal transduction through the ASK1-JNK-caspase3 pathway.

While much remains to be learned about the exact mechanisms, it is clear that HNE can influence a large set of fundamental cellular processes including proliferation, cell cycle events, and apoptosis, in part by profoundly affecting the expression of numerous genes [18,45]. Further studies on specific interactions of HNE with various membrane receptors, transcription factors, repressors, and other target proteins may provide clues to the mechanisms responsible for such a global effect of HNE on various cellular processes.

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Abbreviations

HNE, 4-hydroxynonenal; RalBP1, Ral-binding protein1; Daxx, death-associated protein; HSF1, heat shock factor 1; HSP, heat shock proteins; GST, glutathione *S*-transferases, ASK1, apoptosis signaling kinase 1; JNK, c-jun N-terminal kinase.

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Fig. 1.

HNE and signaling for apoptosis: HNE is diffusible and can interact with Fas on plasma membrane, cytoplasmic p53, and nuclear Daxx. HNE binds to Fas and activates the downstream kinases ASK1 and JNK leading to activation of caspase3 and apoptosis. In parallel, HNE interacts with cytoplasmic p53 leading to its activation via phosphorylation and translocation to the nucleus where it promotes transcription of proapoptotic/cell cycle arrest genes Bax/p21. HNE also interacts with the transcription repressor Daxx bound to HSF1, translocates Daxx from nucleus to cytoplasm where it binds to Fas, and inhibits activation of ASK1 to limit apoptosis. Release of free HSF1 leads to upregulated transcription of heat shock proteins.

Table 1**4-HNE as signaling molecule**

Model system	Observed effect(s); Ref. given in parentheses
Jurkat cells	Facilitates cytosolic export of Daxx and its binding to Fas to self-limit Fas-mediated apoptosis [16]
Colon carcinoma RKO cells	Attenuation of HSF-1, induction and stabilization of BCLxL [25]
Human lens epithelial cells	Fas-mediated DISC-independent apoptosis [15]
Vascular smooth muscle cells	Mitogenic response [13]
Rat epithelial type II cells	Induction of γ -GGT via EpRE/Nrf2 [26]
Vascular cells and atherosclerotic lesions	Desensitization of platelet derived growth factor receptor beta [27]
In vitro and In vivo	Inhibition of thioredoxin and thioredoxin reductase [28]
Caco-2 human adenocarcinoma cells	Antiproliferative effects [29]
Human osteosarcoma cells	Proliferation, differentiation, and apoptosis [30]
K562, HL60, HLE B-3	Role in stress-mediated signaling for apoptosis transformation, proliferation, differentiation [3,4,31,32]
Rat hepatocytes	Apoptotic signaling through PKC delta [33]
Rat hepatocytes	Alteration in gene expression [34]
PC12 cells	Adaptive response and enhancement of cell tolerance through induction of thioredoxin reductase via activation of Nrf-2 [35]
HLE B-3 cells	Profound changes in gene expression on depletion of 4-HNE [36]
Pulmonary epithelial cells	Increase in heme oxygenase-1 through activation of the ERK pathway [37]
Nerve cells	Modulation of stress-activated protein kinase pathways [38]
Human fetal liver hematopoietic stem cells	Inhibition of cell proliferation and alteration of differentiation pathways [39]
Human cell lines of varying origin	Modulation of signaling kinases [2]
Macrophages	Inducer of COX-2 [40-42]
Rat pancreatic stellate cells	Activation of the activator protein-1 and mitogen-activated protein kinases [43]
Human neuroblastoma cells	Regulation of glycogen synthase kinase 3 beta [44]
Human lens epithelial cells	HNE depletion leads to phenotypic transformation of adherent cells [45]
Colorectal carcinoma, HL60, HLE B-3 cells	Induction of apoptosis [46-48]
Dentate granule cells	Modulation of voltage gated Ca^{2+} current [49]
Human hepatic stellate cells	Profibrogenic stimulus. Interaction with JNK isoforms [50]
Human erythroleukemic cells	Role in UVA-mediated signaling for apoptosis [9]
Cells of varying origin	Triggers multistep signal transduction for suppression of cellular functions [20]
Hepatic stellate cells	Direct interaction with JNK isoforms [51]

Model system	Observed effect(s); Ref. given in parentheses
Jurkat cells	Down regulation of Akt kinase [52]
Neurons	NGF withdrawal-induced neuronal apoptosis [53]
<i>Chlamydia pneumoniae</i>	Inhibition of IKK/I kappa B-mediated signaling [54]
HBE1 cells	Induction of glutamate cysteine ligase through JNK [55]
Human lung fibroblasts	Activation of epidermal growth factor receptor-linked extracellular signal kinase p44/42 pathway [56]
Retinal pigment epithelial cells	Induction of vascular endothelial growth factor [57]
Macrophages	Activation of PKC beta isoforms [58]
K562 cells	Role in adaptive response of cells to heat and oxidative stress [7]
Vascular smooth muscle cells	Prevents NO production [59]
Vascular smooth muscle cells	NF-kappaB activation and formation of isoprostane [60]
PC12 cells	Activation of JNK pathway [61]
Neurons	Inhibits constitutive and inducible activity of NF-Kappa B [62]
Hepatic stellate cells	Reduces tyrosine phosphorylation [63]
Human epidermoid carcinoma A431 cells	Triggers an epidermal growth factor receptor for growth inhibition [64]
Rat hepatocytes	Regulation of PKC beta isozymes [65]
HeLa cells	Affects expression of c-fos proto-oncogene and proliferation [66]
Rat cerebrocortical neurons	Inhibition of muscarinic acetylcholine and metabotropic glutamate receptor-mediated signaling [67]
Rat hepatocytes	Triggers Ca ²⁺ influx [68]
Human cerebral arteries	Induces relaxation [69]
Neutrophils	Stimulation of chemotaxis [70]
Rat live plasma membrane	Activation of adenylate cyclase [71]
Hepatic tissues	Activation of phospholipase c [72]
Hepatoma cells	Stimulation of heat shock proteins [73]
K562 cells	Inhibition of C-myc [74]
HL60 cells	Cell cycle progression and expression of differentiation-associated antigens [75]
Macrophages	Activates transcription factor AP-1 [76]