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Xenohormesis mechanisms underlying chemopreventive effects of some dietary phytochemicals

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A wide variety of phytochemicals present in our diet, including fruits, vegetables, and spices, have been shown to possess a broad range of health-beneficial properties. The cytoprotective and restorative effects of dietary phytochemicals are likely to result from the modulation of several distinct cellular signal transduction pathways. Many dietary phytochemicals that are synthesized as secondary metabolites function as toxins, that is, "phytoalexins," and hence protect plants against insects and other damaging organisms and stresses. However, at the relatively low doses consumed by humans and other mammals, these same toxic plant–derived chemicals, as mild stressors, activate adaptive cellular response signaling, conferring stress resistance and other health benefits. This phenomenon has been referred to as xenohormesis. This review highlights the xenohormesis mechanisms underlying chemopreventive effects of some dietary chemopreventive phytochemicals, with special focus on the nuclear transcription factor erythroid 2p45 (NF-E2)–related factor 2 (Nrf2) as a key player.

Keywords: hormesis; xenohormesis; phytochemicals; adaptive response; chemoprevention

> *All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and remedy.* Paracelsus (1493–1541)

Living organisms constantly cope with a broad spectrum of noxious stimuli or adverse conditions, and the adaptation to external stressors—physical, chemical, biological, and social/psychological—is an essential principle for survival. Interestingly, the response to a stressor is not necessarily linear with regards to the dose, but rather *U*- or *J*-shaped. Thus, exposure to a low level of stressful stimulus that is detrimental at higher levels can confer tolerance or resistance to a subsequent insult by the same or related stressor agent or condition. Such an adaptive stress response has been identified as an evolutionarily conserved process. The term *hormesis* defines a nonlinearity biphasic biological response where exposure to a low dose/level of an environmental toxicant or noxious condition results in a potentially beneficial effect, whereas a high dose/level has an adverse effect. In the field of biomedical disciplines, hormesis is referred to as an adaptive response or preconditioning of cells and organisms to a moderate/intermittent stress.[1,2] Hormesis represents a fundamental concept in evolutionary theory, explaining how life on earth has adapted to harsh environment. To survive environmental hazards, organisms have developed distinct cellular signaling pathways that mediate hormetic responses. These include transcription factors and their upstream kinases, which regulate the expression of genes encoding a battery of stress resistance and cytoprotective proteins (e.g., protein chaperones, heat-shock proteins, antioxidant and phase-2 detoxifying enzymes, etc.).[3]

Xenohormesis hypothesis: evolutionary adaptation to foreign stressors for survival advantage

Organisms have evolved to sense stress signaling molecules produced by other species in their environment. In this way, organisms can properly be prepared in advance for deteriorating environmental conditions. This interspecies hormesis is referred to as xenohormesis, which describes a phenomenon

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where an organism senses chemical cues from other species about the status of environment or food supply and responds to them in a way that is beneficial.[4,5]

Mutualism between plants and animals supports a coevolutionary impetus for xenohormesis. Plants and animals share a high degree of sequence homology between their stress response signaling pathways.[6] For instance, when plants are subjected to harsh environment, such as a drought, microbial infection, attack by insects and pests, etc., they produce chemicals that help endure such stressful conditions or protect them from further environmental hazards. Since animals normally depend upon plants for their food supply, they have adapted to sense the bioactive substances produced by stressed plants in order to gauge changing external conditions.[6] In this context, those chemical substances (phytochemicals) produced from plants for self-defense in response to stress or other adverse conditions are inherently phytoalexins (plant toxins). These xenohormetic phytochemicals, which alert animals to adversity, can stimulate their stress response and eventually fortify cellular defense capacity (*vide infra*).

Cellular stress responsive gene induction by xenohormetic phytochemicals

The stress response of plants has been evolving for almost one billion years. Because most plants cannot physically move around, they must endure environmental stresses in place. This sedentary life of plants may explain the complexity of their stress response.[6] Plants produce toxins to protect themselves against fungi, insects, and animal predators. Consistent with this notion, cultivated plant foods contain on average fewer natural toxins than do their wild counterparts.[7] When plants are under stressful conditions, there might be a marked increase in their accumulation of natural pesticides (biopesticides), occasionally to the levels that can be acutely toxic to humans. As such plant toxins constitute the substantial part of chemicals present in the human diet, it has been estimated that 99.99% (by weight) of the pesticides in the American diet are chemicals that plants produce to defend themselves.[8]

Xenohormesis can explain how environmentally stressed plants produce bioactive compounds that can confer stress resistance and survival benefits to animals that consume them. Animals take advantage of exploiting the information contained in products of sophisticated stress response of plants, which has developed as a result of their stationary lifestyle.[6] Indeed, the majority of known health-beneficial effects of edible plants are attributable to the pharmacologically active substances of plants' stress response. Although the noxious properties of xenohormetic phytochemicals are detrimental to microorganisms, insects, and pests eating plants, at the subtoxic doses ingested by humans as part of their diet, the same compounds are considered to induce mild cellular stress responses.[9,10] This, in turn, activates adaptive stress response signaling pathways, leading to increased expression of genes mostly encoding cytoprotective proteins including antioxidant enzymes, phase-2 detoxifying enzymes, protein chaperones, growth factors, mitochondrial proteins, etc. For instance, the oxidative stress caused by some flavonoids with prooxidant activity can contribute to their health-promoting activity by inducing important antioxidant enzymes, pointing to a beneficial effect of a supposed toxic chemical reaction.[11]

Although there has been a paucity of solid clinical data to support the xenohormesis hypothesis in the nutritional field, accumulating evidence from recent studies suggests that xenohormetic mechanisms may underlie health-beneficial effects of some edible phytochemicals in humans. As xenohormetic phytochemicals can improve our body's functions by stimulating our cellular stress response, they can be applied in drug development and the nutritional enhancement of diet.[6] Specific examples of signal transduction molecules activated by phytochemicals that exert xenohormetic effects include Nrf-2, AMP-activated protein nkinase, histone deacetylases of the sirtuin family (e.g., SIRT-1), FOXO, and transient receptor potential vanilloid receptor.[3,10]

Nrf2 as an essential component of xenohormetic circuit

Living organisms have evolved ubiquitous mechanisms to manage a vast multitude of stressors and noxious conditions. Animals that consume plants as a primary source of their diet have apparently developed mechanisms to eliminate phytoalexins or neutralize their potentially deleterious effects. One of the most essential components of physiologically important stress response signaling pathways is the

redox-sensitive transcription factor Nrf2, which is considered the cellular redox sensor. Multiple lines of evidence support that the known health-beneficial effects of low doses of phytochemicals largely depend on their ability to activate Nrf2 signaling.[9]

Under physiologic conditions, Nrf2 is normally sequestered in the cytoplasm as an inactive complex with the repressor Kelch-like ECH-associated protein 1 (Keap1). The release of Nrf2 from its repressor and subsequent nuclear translocation are most likely to be achieved by alterations in the structure of Keap1. Nrf2, once migrated to the nucleus, forms a heterodimer with another protein, such as small Maf, which in turn binds to the antioxidant response elements or more correctly electrophile response elements (EpRE), located in the promoter region of genes encoding various antioxidant and phase-2 detoxifying enzymes. Some chemopreventive and cytoprotective agents target Keap1 by oxidizing or covalently modifying one or more of its specific cysteine thiols, thereby facilitating dissociation of Nrf2 from Keap1 and nuclear translocation.[12] In addition, upstream signaling kinases, such as p38, protein kinase C, extracellular signal-regulated protein kinase, c-Jun N-terminal kinase, and phosphoinositide-3-kinase, can activate Nrf2 through phosphorylation at its specific serine residues. This may also facilitate the nuclear localization of Nrf2.

Nrf2 has evolved over millennia from primitive origins. Thus, there exist homologues of mammalian Nrf2 even in lesser-developed invertebrate species, including *Caenorhabditis elegans* and *Drosophila*. In particular, those regions essential for the regulating stability and DNA binding of Nrf2 are remarkably conserved. During evolution, organisms might have selected a distinct form of Nrf2 for optimal defense capacity to manage a broad spectrum of external insults.[13]

The primary function of Nrf2 is to protect cells and organisms from oxidative stress by upregulating the *de novo* synthesis of diverse antioxidant enzymes and cytoprotective proteins, such as heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1, those involved in glutathione metabolism (e.g., glutamate cysteine ligase, glutathione *S*-transferase, glutathione peroxidase), and thioredoxin. Nrf2 also plays a role in facilitating the elimination of some electrophilic toxicants by inducing the expression of phase-2 detoxifying enzymes. The list of stress response and cytoprotective proteins whose expression is primarily regulated by Nrf2 has been expanding.

Although Nrf2 mainly plays a major role in cellular antioxidant defense, results from recent studies have highlighted its anti-inflammatory function (Fig. 1).[14] As oxidative stress and inflammatory tissue damage are two major culprits in the pathogenesis of the majority of human malignancies, Nrf2 is recognized as a potential target for cancer chemoprevention. Some representative chemopreventive phytochemicals capable of activating Nrf2 signaling and their underlying mechanisms are described in the following section.

Activation of Nrf2 by chemopreventive and cytoprotective phytochemicals

There are numerous phytochemicals that have been reported to activate Nrf2 signaling.[15] Though their chemical structures are diverse, ranging from flavonoids (e.g., epigallocatechin gallate and quercetine) to stilbenes (e.g., resveratrol and piceatannol), diferuloylmethanes (e.g., curcumin and caffeic acid phenethylester), and organosulfur compounds (e.g., allilcin and diallyl trisulfide), there is some commonality responsible for Nrf2 activation. Two of these characteristics in common are prooxidant and electrophilic properties.[11,16]

Electrophilic and oxidant phytochemicals, which exert beneficial health effects at low doses, can alter the redox state of the target cells (Fig. 1). This can be achieved by direct generation of reactive oxygen species (ROS) or indirectly by decreasing intracellular reduced glutathione (GSH). Mild oxidative stress can activate protein kinases responsible for the activation of the Nrf2 through phosphorylation of the specific amino acids localized in this transcription factor. As a result, there will be upregulation of cytoprotective gene expression. Alternatively, ROS can oxidize critical cysteine thiol groups present in Keap1, which facilitates the dissociation of Nrf2 from a complex with its negative regulator Keap1 for nuclear translocation. Interestingly, the ability of antioxidant flavonoids to activate an EpRE-mediated response correlates well with their prooxidant properties. Thus, it has been reported that flavonoids with a higher intrinsic redox potential to generate oxidative stress and redox cycling are the most potent inducers of EpRE-mediated

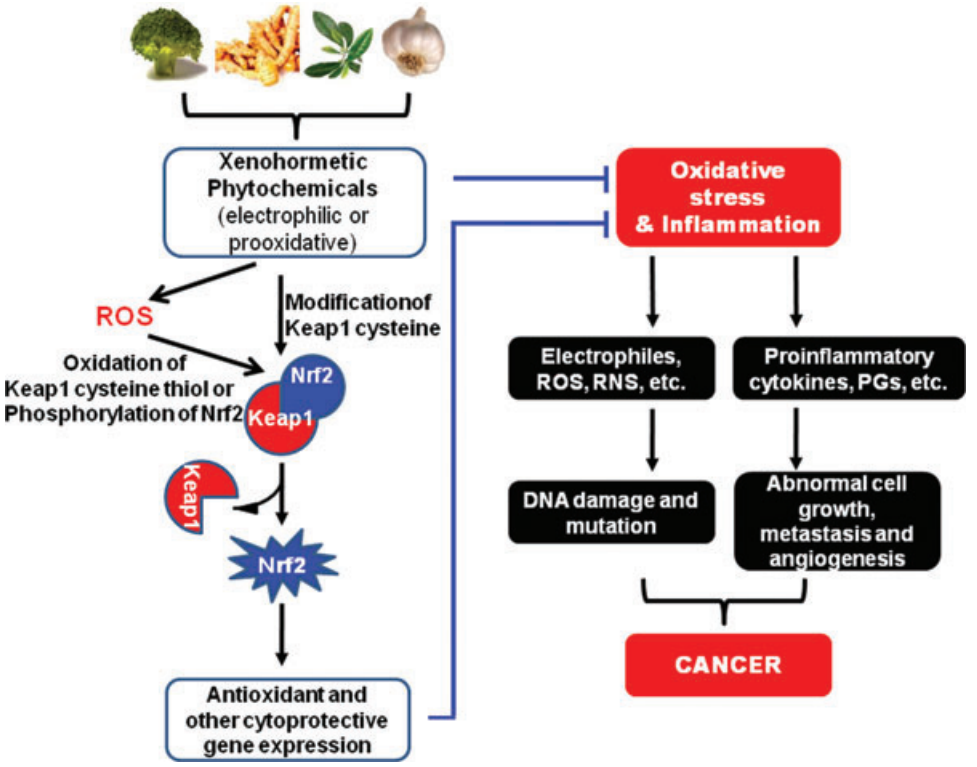

Figure 1. **Activation of Nrf2-Keap1 signaling by xenohormetic phytochemicals with cancer chemopreventive potential.**

cytoprotective gene expression.[11] According to this study, Nrf2-activation by flavonoids was accompanied by decreased cellular GSH, lending support to an prooxidative mechanism.

Some electrophilic phytochemicals, such as curcumin, are capable of directly modifying Keap1 cysteine thiol groups. Curcumin bears the α,β-unsaturated carbonyl moiety and hence can act as a Michael reaction acceptor capable of interacting with nucleophiles, such as protein thiols. Catalytic hydrogenation of curcumin at both double bonds conjugated with carbonyl groups produces tetrahydrocurcumin, which cannot act as a Michael reaction acceptor. Unlike curcumin, tetrahydrocurcumin failed to activate EpRE-mediated HO-1 expression in rat smooth muscle cells.[17] Oral administration of curcumin resulted in enhanced nuclear translocation and EpRE binding of Nrf2 and subsequently HO-1 expression in rat liver, but these effects were very marginal in tetrahydrocurcumin-treated animals.[18] Another example of an electrophilic phytochemical targeting Nrf2-Keap1 signaling is zerumbone, a sesquiterpene derived from tropical ginger, which also contains an α,β-unsaturated carbonyl moiety. Zerumbone was found to suppress chemically induced papilloma formation in mouse skin.[19] Our recent study has revealed that topical application of zerumbone onto dorsal skin of hairless mice induces activation of Nrf2 and expression of HO-1.[20] Treatment of mouse epidermal JB6 cells with zerumbone caused a marked increase of Nrf2 nuclear translocation as well as the promoter activity of HO-1, and also enhanced binding of Nrf2 to the antioxidant response element. Notably, α-humulene and 8-hydroxy-α-humulene, the structural analogues of zerumbone that lack the α,β-unsaturated carbonyl group, failed to activate Nrf2 and were unable to increase HO-1 expression. Unlike zerumbone, these nonelectrophilic analogues could not suppress the phorbol ester-induced JB6 cell transformation as well.[20]

Besides the enone-type phytochemicals including curcumin and xerumbone, those with the catechol moiety are also electrophilic as they undergo oxidative conversion to a quinone. For instance, carnosic acid, a naturally occurring catechol-type

polyphenolic diterpene derived from rosemary (*Rosmarinus officinalis*), has been reported to activate the Nrf/Nrf2 transcriptional pathway by binding to specific Keap1 cysteine residues.[21] Many noncatechol-type polyphenols undergo oxidative conversion to produce a catechol metabolite, which, through redox cycling, can directly modify target proteins including Keap1 via (*S*)-alkylation or provoke oxidative stress by interacting with GSH.

Other categories of electrophilic phytochemicals include isothiocyanates (e.g., sulforaphane) and some organosulfur compounds (e.g., diallyl trisulfide). Sulforaphane, abundant in broccoli sprouts, has been reported to strongly induce carcinogen detoxifying enzymes, predominantly through activation of Nrf2.[22] Sulforaphane-induced activation of Nrf2 signaling was largely attributed to its Keap1 thiol modification, especially at cysteine 151, which is supposed to facilitate release of Nrf2 from the inactive complex with Keap1. However, a later study by Egner *et al.* demonstrated that thiol modification of Keap1 cysteine 151 by sulforaphane failed to cause direct dissociation of Nrf2 from Keap1, but rather resulted in structural changes in Keap1.[23] This led to polyubiquitination and subsequent proteasomal degradation of Keap1, thereby allowing Nrf2 to escape from Cul3-dependent proteasomal degradation, providing a new insight into the mechanism underlying Nrf2 activation by sulforaphane.

Garlic oil contains several organosulfur compounds, such as diallyl sulfide, diallyl disulfide, and diallyl trisulfide. When each of these garlic-derived organosulfur compounds was treated to human hepatoma HepG2 cells, diallyl trisulfide elicited the most pronounced effects in terms of inducing nuclear translocation and transcriptional activity of Nrf2 and antioxidant gene expression.[24] Co-treatments with thiol-reducing antioxidants, such as *N*-acetylcysteine and GSH, attenuated diallyl trisulfide–induced EpRE activity and Nrf2 accumulation.

Conclusion

Xenohormesis can explain how plants challenged with environmental stressors, such as microbes, insects, animal predators, drought, and excess solar illumination, produce bioactive substances that, as chemical cues, alert animals to adversity. When consumed by animals, the same compounds stimulate an adaptive survival response, conferring stress resistance and health benefits.[6] One of the key molecules that plays a central role in cellular adaptive response to a wide array of external stressors is the redox-sensitive transcription factor Nrf2.

If Nrf2 plays such a pivotal role in the physiological stress response, is sustained upregulation or activation of this transcription factor beneficial? Oxidative and electrophilic stressors alter the cellular redox state, thereby rapidly activating Nrf2. However, it remains to be proven if such a situation occurs in response to chronic exposure of cells to low-dose dietary phytochemicals, which renders the target cells better able to respond to a subsequent harmful challenge.[16]

While timely transient activation of Nrf2 signaling is pivotal to boost cellular defense against acute toxicity, unnecessarily elevated Nrf2 activity may not be necessarily beneficial, and may even be detrimental to organisms. While low levels of Nrf2 activity predispose cells to chemical carcinogenesis, inappropriately overactivated Nrf2 may play a role in the progress of cancer.[25] Moreover, the overactivation of Nrf2 is associated with increased resistance to anticancer therapeutic regimens and confers survival advantage to some cancerous or transformed cells.[26] In this context, Nrf2 is a double-edged sword in regulating redox regulation in cancerous versus normal cells, and its functions may also follow the hormetic dose response.

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Conflicts of interest

The author declares no conflicts of interest.

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