Nrf2, an Essential Component of Cellular Stress Response, as a Potential Target of Hormetic Phytochemicals

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ABSTRACT

Living organisms have evolved ubiquitous mechanisms to manage a vast multitude of stressors and noxious conditions. Although Nrf2 mainly plays a major role in cellular antioxidant defence, results from recent studies have highlighted its ubiquitous roles in cellular cytoprotection including anti-inflammatory function. As oxidative stress and inflammatory tissue damage are two major culprits in the pathogenesis of the majority of malignancies and other disorders, Nrf2 is recognized as a potential preventive target. Some chemoprotective and chemopreventive phytochemicals are capable of activating Nrf2 signaling, thereby fortifying cellular defence capacity.

Key words: Nrf2, Keap1, Stress response

INTRODUCTION

Living organisms are subjected to diverse types of stress from both external and internal sources. While excessive stress leads to necrotic or apoptotic death, moderate amounts of noxious stimuli may render the cells tolerant to ongoing or subsequent insults. Such adaptive survival response, termed 'hormesis', accompanies *de novo* synthesis of cytoprotective proteins through activation of distinct stress-responsive signaling. The induction of stress-responsive gene expression represents the first line of cellular defence against a wide array of noxious insults, including oxidative, nitrosative, inflammatory and electrophilic stresses. One of the key signaling molecules involved in cellular stress response is nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) that plays a crucial role in coordinated up-regulation of a battery of antioxidant and other cytoprotective genes that boost cellular adaptive survival response[1,2].

Under unstressed/physiologic conditions, Nrf2 is sequestered in the cytoplasm as an inactive complex with the repressor Kelch-like ECH-associated protein 1 (Keap1), which is a cytoskeleton binding protein. Nrf2 undergoes ubiquitination by the Cul3–Keap1 ubiquitin E3 ligase complex followed by rapid proteasomal degradation. The release of Nrf2 from its repressor and subsequent nuclear translocation are considered to be achieved by alterations in the structure of Keap1. For instance, electrophilic or oxidative stresses covalently modify or oxidize, respectively reactive cysteine residues of Keap1. This causes a decline in the E3 ligase activity and concurrent stabilization of Nrf2[2]. Nrf2, once migrated to the nucleus, binds to the antioxidant response elements (ARE) or electrophile response elements (EpRE) located in the promoter region of genes encoding various antioxidant and phase 2 detoxifying enzymes and other cytoprotective proteins.

ROLES OF NRF2 IN CELLULAR ADAPTIVE/SURVIVAL RESPONSE TO OXIDATIVE AND INFLAMMATORY STRESSES

Oxidative stress can be caused when intrinsic antioxidant capacity is overwhelmed by excessive generation of reactive oxygen species (ROS). ROS, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, contribute to neoplastic transformation either by damaging DNA or indirectly by modulating cellular signal transduction pathways[3]. Moreover, accumulation of ROS *in vivo* generates a state of persistent local inflammation. ROS and pro-inflammatory cytokines are involved in multistage carcinogenesis via genetic and epigenetic mechanisms. Thus, both oxidative stress and inflammation not only can initiate tumorigenesis but also promote the proliferation of damaged cells, creating a tumor microenvironment favorable for the neoplastic transformation of premalignant cells[3].

Living in an environment of various known and unknown sources of ROS, our body has intrinsic ability to guard against oxidative stress-induced cellular damage. The induction of antioxidant enzymes and related cytoprotective proteins represents one of the most important components of cellular defense mechanisms whereby a diverse array of oxidative toxicants and other reactive species can be neutralized or eliminated from the cell

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before they damage genomic DNA. Naturally, cells/tissues are empowered with a panel of antioxidant and detoxifying enzymes, such as NAD(P)H:quinone oxidoreductase-1, superoxide dismutase, glutathione *S*-transferase, glutathione peroxidase, heme oxygenase-1, glutamate cysteine ligase, uridine diphosphate glucuronosyltransferase, etc., which are responsible for protecting DNA and other critical biomolecules from damage that can be caused by ROS and electrophilic toxicants.

In addition to protection against oxidative stress, Nrf2-ARE signaling is also involved in attenuating inflammation-associated pathogenesis, such as autoimmune diseases, rheumatoid arthritis, asthma, emphysema, gastritis, colitis and atherosclerosis[4]. Thus, disruption or loss of Nrf2 signaling causes enhanced susceptibility to both oxidative stress and inflammatory tissue injuries due to inability of damaged cells to mount adaptive survival responses. During the early-phase of inflammation-mediated tissue damage, activation of Nrf2-ARE might inhibit the production of pro-inflammatory mediators including cytokines, chemokines, cell adhesion molecules, matrix metalloproteinases, cyclooxygenase-2 and inducible nitric oxide synthase. It is likely that the cytoprotective function of genes targeted by Nrf2 is attributed to cooperative regulation of the innate immune response and also down-regulation of pro-inflammatory gene expression.

NRF2 AS A PRIME TARGET OF HORMETIC PHYTOCHEMICALS

As mentioned in the previous section, the activation of Nrf2 signaling plays such a pivotal role in cellular protection/tolerance to external stresses from diverse sources. Notably, this physiologically important adaptive stress response mechanism is activated by some chemopreventive and cytoprotective phytochemicals, especially those with antioxidant and/or anti-inflammatory properties, many of which are originally produced by plants as toxins (phytoalexins). It is striking to note that such plant toxins (biopesticides) account for the substantial part of chemicals present in the human diet. Thus, it has been estimated that 99.99% (by weight) of the pesticides in the American diet are chemicals that plant produce to defend themselves[5].

From an evolutionary perspectives, the noxious properties of phytoalexins are important in protecting plants from microbial infection and other harsh environmental conditions including oxidative stress caused by solar irradiation. However, at the subtoxic doses ingested by humans that consume the plants, these same phytochemicals provoke mild stress and consequenty induce adaptive survival responses mediated by stress-response signaling molecule including Nrf2[6] . In line with this notion, some chemopreventive phytochemicals capable of inducing Nrf2-driven antioxidant gene expression can act as prooxidants.

MECHANISMS UNDERLYING NRF2 ACTIVATION BY CHEMOPROTECTIVE PHYTOCHEMICALS

A vast variety of bioactive natural products of plant origin have been reported to activate Nrf2 signaling[7]. These include 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). Despite their structural diversity, there is some commonality in terms of the mechanistic basis for their Nrf2 activating capability. Two of these characteristics in common are prooxidant and electrophilic properties[8].

I. *Phosphorylation of Nrf2 or Oxidation of Keap1 Cysteine Thiols*

Some phytochemicals with pro-oxidant property can alter the redox state of the target cells (Figure 1) through direct generation of moderate amounts of ROS or indirectly through partial depletion of intracellular reduced glutathione (GSH). Both events may cause mild oxidative stress, which activates some protein kinases (protein kinases, PI3-K, MAPKs, etc.) responsible for phosphorylation of Nrf2. This appears to diminish the affinity of Nrf2 for its suppressor Keap1. Alternatively, ROS can oxidize critical cysteine residues of Keap1, which also facilitates the dissociation of Nrf2 from Keap1. In line with this notion, the capability of some flavonoids to induce cytoprotective gene expression correlates well with their prooxidant properties. Thus, those flavonoids that retain a higher intrinsic redox potential to generate ROS were found to be the more potent inducers of EpRE-mediated Nrf2 activation[9]. According to this study, activation of EpRE-Nrf2 signaling by selected flavonoids was accompanied by decreased cellular GSH, indicative of the involvement of ROS.

II. *Covalent Modification of Keap1 Cysteines*

Besides phytochemicals with prooxidant property, those with electrophilic nature can directly modify Keap1 cysteine thiols (Figure 1), thereby increasing the stability and nuclear localization of Nrf2 for EpRE binding. Curcumin, for instance, has two α,β-unsaturated carbonyl moieties, and may hence directly interact with nucleophiles, including Keap1 cysteine thiols that act as a redox sensor. Another example of an electrophilic phytochemical targeting Nrf2-Keap1 signaling is zerumbone, a sesquiterpene derived from tropical ginger, which also bears an α,β-unsaturated carbonyl moiety. Our recent study demonstrated that topical application of zerumbone onto dorsal skin of hairless mice as well as treatment to the mouse epidermal JB6 cells induced activation of Nrf2 and expression of its target protein heme oxygenase-1[10]. In contrast, α-humulene and 8-hydroxy-α-humulene that

lack the α,β-unsaturated carbonyl group, failed to activate Nrf2-mediated heme oxygenase-1 expression.

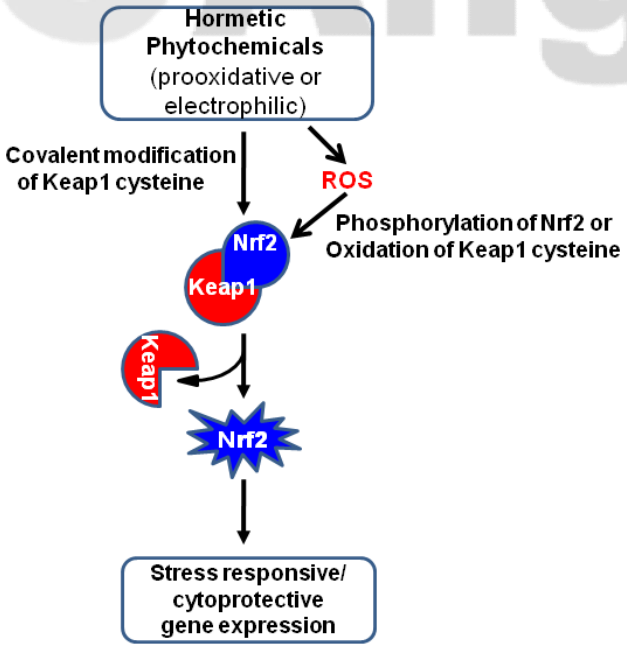

Figure 1. Proposed mechanisms underlying of Nrf2 activation by some hormetic phytochemicals acting as a prooxidant or an electrophile.

Besides the enone-type phytochemicals (e.g., curcumin and xerumbone), some catechol-type polyphenolic compounds can also act as an electrophile via oxidation to a quinone. For instance, carnosic acid derived from rosemary (*Rosmarinus officinalis*) has been reported to activate Nrf2 by binding to specific Keap1 cysteine residues [(11)].

Other categories of electrophilic phytochemicals include isothiocyanates (e.g., sulforaphane) and some organosulfur compounds (e.g., diallyl trisulfide). These compounds are also likely to induce Nrf2 transcriptional activity through modification of specific Keap1 cysteine thiols[(12)].

CONCLUSIONS

The Keap1-Nrf2 pathway is plays a central role in cellular adaptive survival response to a wide array of environmental stresses. Some chemopreventive and chemoprotective phytochemicals that act as prooxidants and/or electrophiles may cause a mild stress, thereby activating Nrf2 signaling. These hormetic phytochemicals are capable of oxidizing or covalently modifying the critical sensor cysteine residues of Keap1, facilitating the release of Nrf2 from this repressor.

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REFERENCES

1. Kensler, T. W., Wakabayashi, N. and Biswal, S. 2007. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Ann. Rev. Pharmacol. Toxicol. 47: 89-116.
2. Taguchi, K., Motohashi, H. and Yamamoto, M. 2011. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. Genes Cells 16: 123-140.
3. Kundu, J. K. and Surh, Y.-J. 2008. Inflammation: gearing the journey to cancer. Mutat. Res. 659: 15-30.
4. Singh, S., Vrishni, S., Singh, B. K., Rahman, I. and Kakkar, P. 2010. Nrf2-ARE stress response mechanism: a control point in oxidative stress-mediated dysfunctions and chronic inflammatory diseases. Free Radic. Res. 44: 1267-1288.
5. Ames, B. N., Profet, M. and Gold, L. S. 1990. Dietary pesticides (99.99% all natural). Proc. Natl. Acad. Sci. USA 87: 7777-7781.
6. Son, T. G., Camandola, S. and Mattson, M. P. 2008. Hormetic dietary phytochemicals. Neuromol. Med. 10: 236-246.
7. Surh, Y.-J., Kundu, J. K. and Na, H.-K. 2008. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. Planta Med. 10: 236-246.
8. Surh, Y.-J. 2011 Xenohormesis mechanisms underlying chemopreventive effects of some dietary phytochemicals. Ann. NY Acad. Sci. 1229: 1-6.
9. Lee-Hilz, Y. Y., Boerboom, A.-M. J. F., Westphal, A. H., van Berkel, W. J. H., Aarts, J. M. M. J. G,, Rietjens, I. M. C. M. 2006. Prooxidant activity of flavonoids induces EpRE-mediated gene expression. Chem. Res. Toxicol. 19: 1499-1505.
10. Shin, J.W., Ohnish, K., Murakami, A., Lee, J. S., Kundu, J. K., Na, H.-K., Ohigashi, H. and Surh, Y.-J. 2011. Zerumbone induces heme oxygenase-1 expression in mouse skin and cultured murine epidermal cells through activation of Nrf2. Cancer Prev. Res. (Phila) 4: 860-870.
11. Satoh, T., Kosaka, K., Itoh, K., Kobayashi, A., Yamamoto, M., Shimojo, Y., Kitajima, C., Cui, J., Kamins, J., Okamoto, S., Izumi, M., Shirasawa, T. and Lipton, S. A. 2008. Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines of Keap1. J. Neurochem. 104: 1116-1131.
12. Hong, F., Freeman, M. L. and Liebler, D. C. 2005. Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. Chem. Res. Toxicol. 18: 1917-1926.