Harnessing the Antioxidant Power with ARE-Inducing Compounds

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The Nrf2-ARE system is a potential therapeutic target for cancers and neurodegenerative diseases. In this issue, Hur et al. (2010) report the discovery of a novel modifiable Nrf2-ARE activator from the unbiased high throughput screening of a small molecule library.

The inhibitory effects of chemical agents on the carcinogenic activities of others were first reported in the early twentieth century. Following this discovery, the cancer chemopreventive potentials of a variety of chemical compounds have been identified (Holtzclaw et al., 2004). Among them, those of naturally occurring phytochemicals found in vegetables and fruits, such as broccoli, grapes, ginger, and turmeric, have captured public attention because they seem intuitively safer than synthetic compounds. The identification of phytochemicals responsible for cancer chemopreventive effects has been carried out (examples include sulforaphane from broccoli, resveratrol from grapes, zerumbone from ginger, and curcumin from turmeric), and in vivo targets of these compounds have been extensively studied. One of the major targets of these phytochemicals is the gene induction of phase 2 detoxification enzymes, such as glutathione-*S* transferases and NAD(P)H:quinone oxidoreductase (NQO1), which can detoxify and/or excrete many harmful compounds including carcinogens. The important issue here is that these compounds indirectly obviate carcinogens by inducing cellular defense mechanisms, so that their effects can last even after the compounds themselves have disappeared from cells.

A consensus sequence 5′-TGACnnn GC-3′, which is essential for this induction, was found in the phase 2 detoxification genes. It was named antioxidant response element (ARE) because the most potent inducers in the early era were phenolic food antioxidants such as *tert*-butylhydroquinone (tBHQ). Later, transcription factor Nrf2 was identified as the only *trans*-acting factor that can bind to ARE and transactivate the phase 2 genes (Kobayashi and Yamamoto, 2006). Under basal conditions, Nrf2 is rapidly degraded by proteasome, and little induction of target genes is observed. This degradation is controlled by Keap1, an Nrf2-specific adaptor protein for the Cul3 ubiquitin ligase complex. Nrf2-ARE activating compounds block Keap1-dependent Nrf2 ubiquitination, thus leading to the stabilization and nuclear accumulation of Nrf2 and the subsequent induction of Nrf2 target genes.

The critical roles of Nrf2 in cancer chemoprevention have been demonstrated using Nrf2-knockout mice (Kwak and Kensler, 2010). Mice pretreated with Nrf2-ARE activating compounds, such as oltipraz, show Nrf2-dependent reduction of tumor formation. In addition to cancer chemopreventive effects, the neuroprotective potentials of Nrf2-ARE activators have recently become a hot topic since antioxidant proteins, such as heme oxygenase 1 and superoxide dismutase 1, have been identified as Nrf2 targets (Vargas and Johnson, 2009). Oxidative stress has been implicated in the pathogenesis of several neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Nrf2 activation protects both neurons and astrocytes from oxidative damage in many different acute paradigms, and it is a promising therapeutic target in chronic neurodegeneration. It is therefore considered worthwhile to develop highly active and low toxic Nrf2-ARE activating compounds.

In this issue, Hur et al. (2010) report on the identification of Nrf2-ARE activating compounds from an unbiased screen of a chemical library. There are two important points in this paper. First, this is the first report of a large-scale systematic screen for Nrf2-ARE activators. Screening was carried out by means of a human neuroblastoma cell line and an ARE-dependent reporter gene. One hundred and seventeen small molecule activators were isolated from 1.2 million compounds. Among them, seven available lead compounds were further characterized, and a chloropyrimidinone, named here AI-1 (ARE-inducing compound 1), was selected for further characterization because of its decreased toxicity in comparison to others (Figure 1). AI-1 showed similar inducing activities as the tBHQ of the ARE-reporter and endogenous NQO1 genes. Resistance to hydrogen peroxide in neuroblastoma cells was elevated by AI-1 treatment. Biotin-modified AI-1 was demonstrated to bind directly to Cys151 of Keap1 both in vivo and in vitro, thus suggesting that Keap1 Cys151 is a target site for AI-1 in the Nrf2-ARE system. Cys151, which is located near the Cul3-binding site in Keap1, is a well-known target for many Nrf2-ARE activating compounds, such as sulforaphane, tBHQ, and ebselen (Kobayashi et al., 2009). AI-1-binding at Cys151 weakened the interaction between Keap1 and Cul3, thus inhibiting the ubiquitination of Nrf2 and facilitating the stabilization and nuclear accumulation of Nrf2. Second, the extensive molecular dissection of AI-1 was carried out, which clarified the critical and tolerable chemical structures in AI-1 (Figure 1). The finding of tolerable structures is valuable, since the compound thus became modifiable to more useful or effective activators. For example, a biotin group was able to be added to AI-1 without obviating

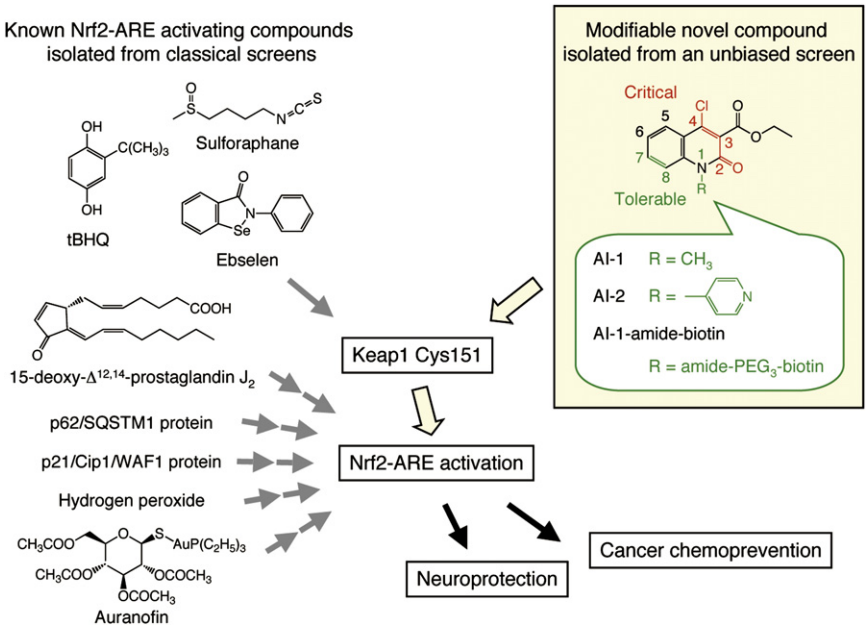

Figure 1. Cancer Chemoprevention and Neuroprotection by Nrf2-ARE Activating Compounds

Most of the known potent Nrf2-ARE activating compounds are unmodifiable, while compounds isolated from the unbiased screens of chemical libraries such as AI-1 (Hur et al., 2010) can be modified to be more useful or effective activators. The Nrf2-ARE system has multiple activating pathways that provide us with an opportunity to design a variety of cancer chemopreventive and neuroprotective drugs.

the Nrf2-ARE activating activity (AI-1-amide-biotin), which is a helpful tool for analyzing protein interactions. It also raises the possibility of upgrading the lead compound to less toxic and more target-specific activators. Indeed, AI-2 which has more potent Nrf2-ARE activating activity than AI-1 was successfully generated by modifying the N1 side group.

This exciting work opens up a new frontier of drug discovery based on the Nrf2-ARE system. Further screening may make it possible to overcome the problems of off targets in the case of AI-1, which was shown in this paper to bind HDACs and PP2A, as well as many other proteins. To expand the scale of screening, it will be helpful to determine the 3D structure of the Keap1 protein containing Cys151 and utilize this information for in silico screening. It is noted that the target sites of Nrf2-ARE activators are not restricted to Cys151 of Keap1 (Figure 1) (Kobayashi et al., 2009). Some activators, such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, target different sites in Keap1. Hydrogen peroxide and the anti-rheumatic drug auranofin may target other proteins than Keap1 or Nrf2. Recently, some proteins such as p62/SQSTM1 and p21/Cip1/WAF1 have been shown to directly target the Nrf2-interacting site of Keap1 or the Keap1-interacting site of Nrf2, respectively (Komatsu et al., 2010; Chen et al., 2009). These multiple sensing pathways of Nrf2-ARE activation therefore provide us with a chance to design a variety of Nrf2-ARE activating drugs that are suitable for the clinical conditions and severity of various diseases. The current success of an unbiased high-throughput screen for Nrf2-ARE activators will encourage the future development of new strategies in drug discovery for cancer chemoprevention and neuroprotection.

REFERENCES

Chen, W., Sun, Z., Wang, X.J., Jiang, T., Huang, Z., Fang, D., and Zhang, D.D. (2009). Mol. Cell *34*, 663–673.

Holtzclaw, W.D., Dinkova-Kostova, A.T., and Talalay, P. (2004). Adv. Enzyme Regul. *44*, 335–367.

Hur, W., Sun, Z., Jiang, T., Mason, D.E., Peters, E.C., Zhang, D.D., Luesch, H., Schultz, P.G., and Gray, N.S. (2010). Chem. Biol. *17*, this issue, 537–547.

Kobayashi, M., and Yamamoto, M. (2006). Adv. Enzyme Regul. *46*, 113–140.

Kobayashi, M., Li, L., Iwamoto, N., Nakajima-Takagi, Y., Kaneko, H., Nakayama, Y., Eguchi, M., Wada, Y., Kumagai, Y., and Yamamoto, M. (2009). Mol. Cell. Biol. *29*, 493–502.

Komatsu, M., Kurokawa, H., Waguri, S., Taguchi, K., Kobayashi, A., Ichimura, Y., Sou, Y.S., Ueno, I., Sakamoto, A., Tong, K.I., et al. (2010). Nat. Cell Biol. *12*, 213–223.

Kwak, M.K., and Kensler, T.W. (2010). Toxicol. Appl. Pharmacol. *244*, 66–76.

Vargas, M.R., and Johnson, J.A. (2009). Expert Rev. Mol. Med. *11*, e17.

