Important Role of Antioxidants in Oxidative Stress

Guest Editor: Toshihiko Osawa

Thioredoxin and Thioredoxin Inducers against Oxidative Stress

Hiroshi Masutani,^{1,*} Yoshimi Yamaguchi,¹ Ryoko Otsuki,¹ Nobue Kanoh,² Yuji Kunimoto,² Kazuo Murata,³ and Junji Yodoi¹

¹Department of Biological Responses, Institute for Virus Research, Kyoto University, 53 Shogoin, Kawahara-cho, Sakyo-ku, Kyoto 606-8507, ²Rockfield Co. Ltd., 15-2 Uozakihama-cho, Higashinada-ku, Kobe 658-2204, and ³Redox Bio Science, Inc., 82-1 Shimotsutsumi-cho, Kawabata-dori Marutamachi-sagaru, Sakyo-ku, Kyoto 606-8396, Japan

Received 17 May, 2005; Accepted 12 September, 2005

Summary Thioredoxin is a vital component of the thioredoxin system, maintaining the cellular redox environment and regulating various cellular functions. Studies using thioredoxin transgenic mice showed that thioredoxin is protective against diseases associated with oxidative stress. While thioredoxin expression is ubiquitous and constitutive, we observe the induction in some tissues under stress such as ultraviolet irradiation and reperfusion injury. Nerve growth factor (NGF)-, or heme-induced induction of the thioredoxin gene is regulated through cyclic AMP responsive element (CRE) or the antioxidant responsive element (ARE), respectively. Based on these previous studies, we have performed a screening of extracts from vegetables to identify novel thioredoxin inducers. We showed that sulforaphane and extracts from several kinds of vegetables induce marked activation of the thioredoxin gene through ARE and increase the thioredoxin protein level in K562 erythroleukemia cells and retinal pigment epithelial (RPE) cells. Pretreatment with sulforaphane or the extracts from sprouts of red cabbage reduced cellular damage induced by hydrogen peroxide. We also report the effect of sulforaphane on the photooxidative damage of RPE cells. Thioredoxin inducers and thioredoxin have a cytoprotective role against oxidative stress in gastric mucosal cells. Reduction of disulfide bonds in food allergens was reported to facilitate the cleavage by digestive enzymes. These results provide a basis for developing thioredoxin-inducing and containing functional foods and further investigating thioredoxin inducers beneficial for protection against oxidative stress.

Key Words: thioredoxin, sulforaphane, oxidative stress, antioxidant responsive element (ARE)

Introduction

The cellular reducing environment is provided by two mutually interconnected systems; the thioredoxin system and the glutathione system. Thioredoxin undergoes reversible redox reactions catalyzed by a NADPH-dependent enzyme, thioredoxin reductase through the two-cysteine residues in its active sites [1]. We identified and cloned human

*To whom correspondence should be addressed.

Tel: +81-75-751-4026 Fax: +81-75-761-5766

E-mail: hmasutan@virus.kyoto-u.ac.jp

Abbreviations: ARE, antioxidant responsive element; CRE, cyclic AMP responsive element; DMSO, dimethyl sulfoxide; RT-PCR, reverse transcription-polymerase chain reaction; LDH, lactate dehydrogenase; RPE cell, retinal pigment epithelial cell; NGF, nerve growth factor; HO-1, heme oxygenase-1

thioredoxin [2] and have analyzed its role in the mammalian system. Thioredoxin plays an important role in cellular processes including apoptosis *via* reduction/oxidation (redox regulation) [3, 4]. Families of thioredoxin-dependent peroxidases (peroxiredoxin) scavenge intracellular hydrogen peroxide [5, 6]. Thioredoxin has a protective role against hydrogen peroxide- or tumor necrosis factor α (TNF- α)-induced cellular damage [7, 8]. Homozygous mutants carrying a targeted disruption of the thioredoxin gene died shortly after implantation, suggesting that thioredoxin expression is essential for cell survival and early differentiation in the mouse embryo [9]. Thioredoxin transgenic mice display various phenotypes such as elongated life span and protection against ischemic injury, acute lung failure, diabetes mellitus, and toxicity caused by environmental stressors [10–14]. Since oxidative stress has been implicated in the pathogenesis of these diseases, thioredoxin seems to play an important role in protection against oxidative stress-associated diseases. The identification of thioredoxin inducers may provide a novel therapeutic approach against such oxidative stress-associated diseases. Geranylgeranyl acetone,

an acyclic polyisoprenoid, induces expression of thioredoxin [15–18], raising the possibility that there are unknown natural thioredoxin inducers.

The thioredoxin gene is induced to express by nerve growth factor (NGF) through the cyclic AMP responsive element (CRE) in PC12 cells and thioredoxin plays an important role in neurite outgrowth and NGF signalling [19]. Endoh *et al.* reported neurotrophic activity of thioredoxin for cholinergic neurons [20]. A protective role for thioredoxin against neuronal damage has also been shown. Thioredoxin expression is induced in astroglia after ischemia [21]. Overexpression of thioredoxin in transgenic mice attenuates focal cerebral ischemic injury [11] and excitotoxic hippocampal injury [22]. Decreased expression of thioredoxin in the brain of patients with Alzheimer's disease is also reported [23]. Therefore, these previous results collectively indicate that thioredoxin inducers are beneficial for neuroregeneration and neuroprotection in oxidative stress-associated diseases such as Alzheimer's disease and Parkinson's disease.

The expression of the thioredoxin gene is induced by hemin and *tert*-butylhydroquinone through

Fig. 1. Thioredoxin gene regulation and results obtained from studies using thioredoxin model mice.

the antioxidant responsive element (ARE) [24, 25] in K562 erythroleukemia cells. Sulforaphane [(–)-1-isothiocyanato-4-R-(methylsulfinyl)-butane], an important component of broccoli sprouts, is known to induce the expression of phase II drug detoxification genes through ARE and has a chemoprotective role against chemical-induced carcinogenesis [26]. Isothiocyanates from other vegetables also have been reported to have potent ARE-inducing activity [27, 28]. We here took advantage of these previous works to investigate thioredoxin inducers (Fig. 1).

Enhancement of Thioredoxin Expression by Sulforaphane and Extracts from Vegetables

We assessed first whether activation of the thioredoxin gene is changed by treatment with extracts from various vegetables in K562 cells. We performed a luciferase reporter assay using a vector (pTRX (-1148)-Luc) containing the entire regulatory region of the thioredoxin gene. Extracts from two different strains of broccoli sprout activated the thioredoxin gene, respectively (data not shown). As shown in Fig. 2, sulforaphane induced activation of the thioredoxin gene in pTRX (-1148)-Luc in K562 cells, whereas it had no effect on control pGL3 basic vector. A 12-fold induction by sulforaphane was observed in a reporter vector containing wild-type ARE (pTRXAREWT-Luc). In contrast, the thioredoxin gene was not activated by sulforaphane in a vector containing mutant ARE (pTRXAREm-Luc) (Fig. 2B). These results indicated that this ARE region of the thioredoxin gene plays an important role in the gene expression induced by sulforaphane. In a quantitative real time RT-PCR, the mRNA level of thioredoxin was significantly increased at 24 and 48 h by sulforaphane treatment in K562 cells at a concentration of 10 μ M (data not shown). Treatment with 10 μ M sulforaphane augmented thioredoxin protein expression at 48h in K562 cells, detected by Western blotting (Fig. 2D). To examine whether sulforaphane induce thioredoxin expression *in vivo*, we analyzed the retinal pigment epithelial (RPE) cells, since the expression of thioredoxin in these cells is inducible in some conditions such as reperfusion injury [29]. Peritoneal injection of sulforaphane to mice induced augmentation of thioredoxin expression in the RPE cells [30]. Therefore, the effect of sulforaphane on thioredoxin seems to occur in different kinds of tissues and *in vivo*.

We have detected the thioredoxin-inducing activ-

ity in extracts from several cruciferous plants such as sprouts of red cabbage and other family of plants. The extracts from sprouts of red cabbage had potent thioredoxin-inducing activity (Fig. 2C and D). The thioredoxin-inducing activity of extracts from cruciferous plants seems to be caused by isothiocyanates, while extracts from the other family of plants did not contain isothiocyanates. Therefore, the extracts seem to contain possibly novel thioredoxin inducing substances, different from isothiocyanates. We are now purifying the constituents of the plants that are responsible for the thioredoxin-inducing activity.

The gene expression of redox regulating enzymes such as γ -glutamylcysteine synthetase, glutathione *S*-transferase Ya, NAD(P)H: quinone oxidoreductase (NQO), peroxiredoxin, thioredoxin reductase [31] and hemeoxygenase-1 is regulated through ARE [32] and the transcription factor Nrf2 [33]. γ -Glutamylcysteine synthetase is a key regulatory enzyme of glutathione synthesis [34]. Glutathione *S*-transferase Ya and NAD(P)H: quinone oxidoreductase (NQO) play an important role in drug detoxification [35]. Peroxiredoxin quenches hydrogen peroxide in collaboration with thioredoxin [5, 6]. Hemeoxygenase-1 (HO-1) catalyzes the first and rate-limiting step in the catabolism of heme to reduce heme-induced oxidative stress [36]. These genes have thus the common regulatory element and seem to be coordinately regulated. Since thioredoxin modulates the DNA binding activity of transcription factors including Jun/Fos families of proteins and Nrf2 [25, 37], augmentation of the thioredoxin level

Fig. 2

Fig. 2. Activation of the thioredoxin gene by sulforaphane and extracts from sprouts of red cabbage. (A) Activation of the thioredoxin gene by sulforaphane. K562 cells were transfected with pTRX(-1148)-Luc or PGL3 basic vector [24], then treated with DMSO or 10 μ M sulforaphane (Alexis) for 16 h. For controlling the efficiency of transfection, *Renilla* luciferase gene expression was monitored using pRL-TK vector (Promega). (B) Sulforaphane induced thioredoxin gene expression through ARE. K562 cells were transfected with either pTRX(-1148)-Luc, pTRXAREWT-Luc, pTRX-AREm-Luc [25], or PGL3 basic vector, together with pRL-TK. Each transfected cell was treated with DMSO or 10 μ M sulforaphane for 16 h. (C) Activation of the thioredoxin gene by extracts from sprouts of red cabbage. Extracts from vegetables were prepared as previously reported with a slight modification [52]. Fresh vegetables were homogenized with two volumes of water, subjected to autolysis for 3 h at 37 °C, and freeze dried. The freeze-dried material (4 g) was solved in 140 ml of acetonitrile and shaken for 6 h at 4 °C. The mixture was filtrated by membrane filter and dried by evaporator. The dried material was dissolved in 1 ml of ethanol and used for analyses. K562 cells were treated with DMSO or 10 μ M sulforaphane, 0.05% ethanol, or extracts (0.02%) of sprouts of red cabbage (RCSP). (D) Induction of thioredoxin protein expression by sulforaphane and extracts from sprouts of red cabbage. Expression of thioredoxin protein in K562 cells treated with DMSO, sulforaphane (10 μ M), 0.1% ethanol, or extracts from sprouts of red cabbage (RCSP) (0.01% or 0.02%) for 24 or 48 h was shown. Whole cell lysates (13.5 μ g) were loaded on 15% SDS-PAGE gels and detected using anti-thioredoxin monoclonal antibodies (Redox Bioscience, Inc), followed by peroxidase-conjugated anti-mouse IgG (1:5000) (Amersham Pharmacia Biotech) for 1 h. The epitope was visualized with an ECL Western blot detection kit (Amersham Pharmacia Biotech).

by sulforaphane may potentiate the expression of these redox-regulating genes by redox regulation. Indeed, thioredoxin was reported to facilitate the induction of hemoxygenase-1 [38]. Since oxidative DNA damage is one cause of cancer, suppression of oxidative stress seems beneficial for cancer prevention. In addition, the reduction of harmful toxic substances by facilitation of drug detoxification seems favourable for decreases of oxidative stress and DNA damage. ARE sequences in different genes are varied and some of the genes contain an embedded Jun/Fos binding sequence. The binding proteins to the ARE (Nrf1, Nrf2, NF-E2p45, Bach1, Bach2, and small Maf proteins, Jun/Fos family of proteins, *etc.*) dimerize with each other [39]. The diversity of the ARE sequence and their binding complexes may determine the specificity of ARE-mediated signal of each gene in a different tissue context. The ARE of the thioredoxin gene matches to the functional antioxidant responsive element [40] with a considerable similarity to that of the HO-1 gene. The ARE is regulated by a switch of its binding factors such as NF-E2p45/small Maf, Nrf2/small Maf, and Jun/Fos

in K562 cells [24], whereas it is regulated by Nrf2, Jun family of proteins and small Maf proteins in RPE cells [30]. Therefore, the ARE seems to be regulated differentially in a tissue- and stimulation-dependent manner. The fine molecular mechanism how sulforaphane and other reagents activate ARE has not been fully elucidated, although the regulation through the Keap-1/Nrf2 system are considered to be a central part of the regulation [41–43].

Induction of Thioredoxin by Sulforaphane Pretreatment Rescues Oxidative Stress-Induced Cellular Damage

Thioredoxin is reported to play an important role in protection against oxidative stress [7, 8, 10–14]. We examined whether pretreatment with sulforaphane or extracts from sprouts of red cabbage can reduce oxidative stress-induced damage in K562 cells by analyzing the release of LDH from damaged cells. As shown in Fig. 3, pretreatment with sulforaphane or the extracts for 24 h markedly reduced cellular damage caused by hydrogen peroxide, com-

Fig. 3. Attenuation of hydrogen peroxide-induced cellular damage by sulforaphane. Release of LDH was measured as follows. K562 cells were plated in 96-well plates at a density of 1×10^4 cells per well. Then cells were treated with 0.1% DMSO, 10 μ M sulforaphane, 0.1% ethanol or the indicated concentration of extracts from sprouts of red cabbage (RCSP). After 24 h of incubation at 37°C, cells were treated with 200 μ M H_2O_2 for 24 h. LDH in 100 μ l samples of culture medium was measured using an LDH assay kit (Roche) according to the manufacturer's direction. The data is representative of three independent experiments.

pared with the control. Sulforaphane pretreatment rescued the photooxidative damage induced—decrease in cell numbers, apoptosis, and loss of retinal function as determined by electroretinogram in RPE cells [30]. Other groups also showed that sulforaphane induces the expression of phase II genes and protects against the photooxidative damage of RPE cells [44]. These results suggest that the induction of expression of thioredoxin and other redox-regulating proteins by sulforaphane pretreatment plays a cytoprotective role against oxidative stress elicited by hydrogen peroxide. It should be noted that sulforaphane and isothiocyanates have also apoptosis-inducing activity [45–47]. It is natural to consider that sulforaphane and its analogues are highly active with thiol groups of proteins and behave prooxidant. However, previous studies suggest the effect of these compounds in protection against cancer [48] and the reduction of the risk of cardiovascular problems of hypertension and atherosclerosis [49]. The induction of redox-regulating and phase II enzyme by these compounds may overcome their prooxidant properties.

Mucosal Protection and Attenuation of Food Allergy by Thioredoxin

As shown above, thioredoxin has cytoprotective activity. Treatment of geranylgeranyl acetone, a thioredoxin inducer or thioredoxin, protect against ethanol- or hydrogen peroxide-induced damage of gastric mucosal cells [16]. Thioredoxin inducers as well as thioredoxin itself may be beneficial for protecting cells in the gastrointestinal tract.

Allergens in foods such as eggs, milk, wheat contain intramolecular disulfide bonds. Reduction of the disulfide bonds may facilitate the cleavage of the allergens by digestive enzymes. This idea has already been tested in several antigens in milk, wheat [50] and eggs (Mayumi *et al.* unpublished observations). Thioredoxin, together with thioredoxin reductase and NADPH enhanced the digestibility of β -lactoglobulin and reduced the allergenicity of milk in dogs [51]. Therefore, the use of thioredoxin-enriched materials seems to be a new approach to developing functional foods to counteract allergies (Fig. 4). The materials can be prepared from edible microorganisms such as yeasts. There remain several

Fig. 4. Cleavage of disulfide bonds of food allergens by the thioredoxin system.

obstacles to overcome before the full application of this idea. Thioredoxin exerts its recycling effect on reduction efficiently with thioredoxin reductase and NADPH. How we process the food using a combination of the thioredoxin system should be further investigated. To apply these materials to food, it is important to verify that thioredoxin-enriched materials do not have untoward effects. Such analyses are underway.

Perspectives

Here, we showed that sulforaphane and other undetermined constituents from edible plants induce the expression of thioredoxin. These products may be beneficial for various oxidative stress-associated diseases. Investigation of the mechanism of the function is important to exclude candidates with harmful functions such as DNA damaging properties and endocrine disruption. Thioredoxin-enriched material may also be of use for protecting mucosa and counteracting food allergies. To avoid untoward results and unforeseen outcomes with long-term usage, analyses in various aspects are necessary. It should be also noted that evidence based research is required for the application.

Acknowledgments

We thank Ms. Y. Kanekiyo and Ms. S. Maeji for their secretarial help. We also thank the members of our laboratory and Redox Bio Science Inc. This study was supported by a grant-in-aid for the Research and Development Program for New Bio-Industry Initiatives and by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

- [1] Holmgren, A. and Björnstedt, M.: Thioredoxin and thioredoxin reductase. *Methods Enzymol.*, **252**, 199–208, 1995.
- [2] Tagaya, Y., Maeda, Y., Mitsui, A., Kondo, N., Matsui, H., Hamuro, J., Brown, N., Arai, K., Yokota, T., Wakasugi, H., and Yodoi, J.: ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO J.*, **8**, 757–764, 1989.
- [3] Masutani, H. and Yodoi, J.: Thioredoxin: Overview, in *Methods Enzymol.*, ed. Packer, L. and Sies, H., Academic Press, San Diego, Vol. 347, pp. 279–286, 2002.
- [4] Masutani, H., Ueda, S., and Yodoi, J.: The thioredoxin system in retroviral infection and apoptosis. *Cell Death Differ.*, **12**, 991–998, 2005.
- [5] Fujii, J. and Ikeda, Y.: Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. *Redox Rep.*, **7**, 123–130, 2002.
- [6] Rhee, S.G., Kang, S.W., Chang, T.S., Jeong, W., and Kim, K.: Peroxiredoxin, a novel family of peroxidases. *IUBMB Life*, **52**, 35–41, 2001.
- [7] Nakamura, H., Matsuda, M., Furuke, K., Kitaoka, Y., Iwata, S., Toda, K., Inamoto, T., Yamaoka, Y., Ozawa, K., and Yodoi, J.: Adult T cell leukemia-derived factor/human thioredoxin protects endothelial F-2 cell injury caused by activated neutrophils or hydrogen peroxide. *Immunol. Lett.*, **42**, 75–80, 1994.
- [8] Matsuda, M., Masutani, H., Nakamura, H., Miyajima, S., Yamauchi, A., Yonehara, S., Uchida, A., Irimajiri, K., Horiuchi, A., and Yodoi, J.: Protective activity of adult T cell leukemia-derived factor (ADF) against tumor necrosis factor-dependent cytotoxicity on U937 cells. *J. Immunol.*, **147**, 3837–3841, 1991.
- [9] Matsui, M., Oshima, M., Oshima, H., Takaku, K., Maruyama, T., Yodoi, J., and Taketo, M.M.: Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev. Biol.*, **178**, 179–185, 1996.
- [10] Mitsui, A., Hamuro, J., Nakamura, H., Kondo, N., Hirabayashi, Y., Ishizaki-Koizumi, S., Hirakawa, T., Inoue, T., and Yodoi, J.: Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. *Antioxid. Redox Signal*, **4**, 693–696, 2002.
- [11] Takagi, Y., Mitsui, A., Nishiyama, A., Nozaki, K., Sono, H., Gon, Y., Hashimoto, N., and Yodoi, J.: Overexpression of thioredoxin in transgenic mice attenuates focal ischemic brain damage. *Proc. Natl. Acad. Sci. USA*, **96**, 4131–4136, 1999.
- [12] Hoshino, T., Nakamura, H., Okamoto, M., Kato, S., Araya, S., Nomiyama, K., Oizumi, K., Young, H.A., Aizawa, H., and Yodoi, J.: Redox-active protein thioredoxin prevents proinflammatory cytokine- or bleomycin-induced lung injury. *Am. J. Respir. Crit. Care Med.*, **168**, 1075–1083, 2003.
- [13] Hotta, M., Tashiro, F., Ikegami, H., Niwa, H., Ogihara, T., Yodoi, J., and Miyazaki, J.: Pancreatic β cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. *J. Exp. Med.*, **188**, 1445–1451, 1998.
- [14] Yoon, B.I., Hirabayashi, Y., Kaneko, T., Kodama, Y., Kanno, J., Yodoi, J., Kim, D.Y., and Inoue, T.: Transgene expression of thioredoxin (TRX/ADF) protects against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced hematotoxicity. *Arch. Environ. Contam. Toxicol.*, **41**, 232–236, 2001.
- [15] Hirota, K., Nakamura, H., Arai, T., Ishii, H., Bai, J., Itoh, T., Fukuda, K., and Yodoi, J.: Geranylgeranylacetone enhances expression of thioredoxin and suppresses ethanol-induced cytotoxicity in cultured hepatocytes. *Biochem. Biophys. Res. Commun.*, **275**, 825–830, 2000.

- [16] Dekigai, H., Nakamura, H., Bai, J., Tanito, M., Masutani, H., Hirota, K., Matsui, H., Murakami, M., and Yodoi, J.: Geranylgeranylacetone promotes induction and secretion of thioredoxin in gastric mucosal cells and peripheral blood lymphocytes. *Free Radical. Res.*, **35**, 23–30, 2001.
- [17] Bai, J., Nakamura, H., Hattori, I., Tanito, M., and Yodoi, J.: Thioredoxin suppresses 1-methyl-4-phenylpyridinium-induced neurotoxicity in rat PC12 cells. *Neurosci. Lett.*, **321**, 81–84, 2002.
- [18] Tanito, M., Kwon, Y.W., Kondo, N., Bai, J., Masutani, H., Nakamura, H., Fujii, J., Ohira, A., and Yodoi, J.: Cytoprotective effects of geranylgeranylacetone against retinal photooxidative damage. *J. Neurosci.*, **25**, 2396–2404, 2005.
- [19] Bai, J., Nakamura, H., Kwon, Y.W., Hattori, I., Yamaguchi, Y., Kim, Y.C., Kondo, N., Oka, S., Ueda, S., Masutani, H., and Yodoi, J.: Critical roles of thioredoxin in nerve growth factor-mediated signal transduction and neurite outgrowth in PC12 cells. *J. Neurosci.*, **23**, 503–509, 2003.
- [20] Endoh, M., Kunishita, T., and Tabira, T.: Thioredoxin from activated macrophages as a trophic factor for central cholinergic neurons *in vitro*. *Biochem. Biophys. Res. Commun.*, **192**, 760–765, 1993.
- [21] Tomimoto, H., Akiguchi, I., Wakita, H., Kimura, J., Hori, K., and Yodoi, J.: Astroglial expression of ATL-derived factor, a human thioredoxin homologue, in the gerbil brain after transient global ischemia. *Brain Res.*, **625**, 1–8, 1993.
- [22] Takagi, Y., Hattori, I., Nozaki, K., Mitsui, A., Ishikawa, M., Hashimoto, N., and Yodoi, J.: Excitotoxic hippocampal injury is attenuated in thioredoxin transgenic mice. *J. Cereb. Blood Flow Metab.*, **20**, 829–833, 2000.
- [23] Lovell, M.A., Xie, C., Gabbita, S.P., and Markesbery, W.R.: Decreased thioredoxin and increased thioredoxin reductase levels in Alzheimer's disease brain. *Free Radical. Biol. Med.*, **28**, 418–427, 2000.
- [24] Kim, Y.-C., Masutani, H., Yamaguchi, Y., Itoh, K., Yamamoto, M., and Yodoi, J.: Hemin-induced activation of the thioredoxin gene by Nrf2: A differential regulation of the antioxidant responsive element (ARE) by switch of its binding factors. *J. Biol. Chem.*, **276**, 18399–18406, 2001.
- [25] Kim, Y.C., Yamaguchi, Y., Kondo, N., Masutani, H., and Yodoi, J.: Thioredoxin-dependent redox regulation of the antioxidant responsive element (ARE) in electrophile response. *Oncogene*, **22**, 1860–1865, 2003.
- [26] Fahey, J.W., Zhang, Y., and Talalay, P.: Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. USA*, **94**, 10367–10372, 1997.
- [27] Nakamura, Y., Morimitsu, Y., Uzu, T., Ohigashi, H., Murakami, A., Naito, Y., Nakagawa, Y., Osawa, T., and Uchida, K.: A glutathione S-transferase inducer from papaya: rapid screening, identification and structure-activity relationship of isothiocyanates. *Cancer Lett.*, **157**, 193–200, 2000.
- [28] Morimitsu, Y., Nakagawa, Y., Hayashi, K., Fujii, H., Kumagai, T., Nakamura, Y., Osawa, T., Horio, F., Itoh, K., Iida, K., Yamamoto, M., and Uchida, K.: A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. *J. Biol. Chem.*, **277**, 3456–3463, 2002.
- [29] Ohira, A., Honda, O., Gauntt, C.D., Yamamoto, M., Hori, K., Masutani, H., Yodoi, J., and Honda, Y.: Oxidative stress induces adult T cell leukemia derived factor/thioredoxin in the rat retina. *Lab. Invest.*, **70**, 279–285, 1994.
- [30] Tanito, M., Masutani, H., Kim, Y.C., Nishikawa, M., Ohira, A., and Yodoi, J.: Sulforaphane induces thioredoxin through the antioxidant-responsive element and attenuates retinal light damage in mice. *Invest. Ophthalmol. Vis. Sci.*, **46**, 979–987, 2005.
- [31] Hintze, K.J., Wald, K.A., Zeng, H., Jeffery, E.H., and Finley, J.W.: Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles *via* an antioxidant response element. *J. Nutr.*, **133**, 2721–2727, 2003.
- [32] Motohashi, H., Shavit, J.A., Igarashi, K., Yamamoto, M., and Engel, J.D.: The world according to Maf. *Nucl. Acids Res.*, **25**, 2953–2959, 1997.
- [33] Itoh, K., Igarashi, K., Hayashi, N., Nishizawa, M., and Yamamoto, M.: Cloning and characterization of a novel erythroid cell-derived CNC family transcription factor heterodimerizing with the small Maf family proteins. *Mol. Cell Biol.*, **15**, 4184–4193, 1995.
- [34] Griffith, O.W. and Mulcahy, R.T.: The enzymes of glutathione synthesis: γ -glutamylcysteine synthetase. *Adv. Enzymol. Relat. Areas Mol. Biol.*, **73**, 209–267, xii, 1999.
- [35] Dinkova-Kostova, A.T. and Talalay, P.: Persuasive evidence that quinone reductase type 1 (DT diaphorase) protects cells against the toxicity of electrophiles and reactive forms of oxygen. *Free Radical. Biol. Med.*, **29**, 231–240, 2000.
- [36] Yachie, A., Niida, Y., Wada, T., Igarashi, N., Kaneda, H., Toma, T., Ohta, K., Kasahara, Y. and Koizumi, S.: Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J. Clin. Invest.*, **103**, 129–135, 1999.
- [37] Packer, L. and Yodoi, J. (ed.): Redox Regulation of Cell Signaling and Its Clinical Application, Marcel Dekker, New York, 1999.
- [38] Wiesel, P., Foster, L.C., Pellacani, A., Layne, M.D., Hsieh, C.-M., Huggins, G.S., Strauss, P., Yet, S.-F., and Perrella, M.A.: Thioredoxin facilitates the induction of heme oxygenase-1 in response to inflammatory mediators. *J. Biol. Chem.*, **275**, 24840–24846, 2000.
- [39] Nguyen, T., Sherratt, P.J., and Pickett, C.B.: Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu. Rev. Pharmacol. Toxicol.*, **43**, 233–260, 2003.

- [40] Wasserman, W.W. and Fahl, W.E.: Functional antioxidant responsive elements. *Proc. Natl. Acad. Sci. USA*, **94**, 5361–5366, 1997.
- [41] McWalter, G.K., Higgins, L.G., McLellan, L.I., Henderson, C.J., Song, L., Thornalley, P.J., Itoh, K., Yamamoto, M., and Hayes, J.D.: Transcription factor Nrf2 is essential for induction of NAD(P)H:quinone oxidoreductase 1, glutathione *S*-transferases, and glutamate cysteine ligase by broccoli seeds and isothiocyanates. *J. Nutr.*, **134**, 3499S–3506S, 2004.
- [42] Kobayashi, A., Kang, M.I., Okawa, H., Ohtsuji, M., Zenke, Y., Chiba, T., Igarashi, K., and Yamamoto, M.: Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol. Cell Biol.*, **24**, 7130–7139, 2004.
- [43] Zhang, D.D., Lo, S.C., Cross, J.V., Templeton, D.J., and Hannink, M.: Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol. Cell Biol.*, **24**, 10941–10953, 2004.
- [44] Gao, X. and Talalay, P.: Induction of phase 2 genes by sulforaphane protects retinal pigment epithelial cells against photooxidative damage. *Proc. Natl. Acad. Sci. USA*, **101**, 10446–10451, 2004.
- [45] Nakamura, Y., Kawakami, M., Yoshihiro, A., Miyoshi, N., Ohigashi, H., Kawai, K., Osawa, T., and Uchida, K.: Involvement of the mitochondrial death pathway in chemopreventive benzyl isothiocyanate-induced apoptosis. *J. Biol. Chem.*, **277**, 8492–8499, 2002.
- [46] Singh, S.V., Srivastava, S.K., Choi, S., Lew, K.L., Antosiewicz, J., Xiao, D., Zeng, Y., Watkins, S.C., Johnson, C.S., Trump, D.L., Lee, Y.J., Xiao, H., and Herman-Antisiewicz, A.: Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. *J. Biol. Chem.*, 2005 (in press).
- [47] Choi, S. and Singh, S.V.: Bax and Bak are required for apoptosis induction by sulforaphane, a cruciferous vegetable-derived cancer chemopreventive agent. *Cancer Res.*, **65**, 2035–2043, 2005.
- [48] Talalay, P. and Zhang, Y.: Chemoprotection against cancer by isothiocyanates and glucosinolates. *Biochem. Soc. Trans.*, **24**, 806–810, 1996.
- [49] Wu, L., Noyan Ashraf, M.H., Facci, M., Wang, R., Paterson, P.G., Ferrie, A., and Juurlink, B.H.: Dietary approach to attenuate oxidative stress, hypertension, and inflammation in the cardiovascular system. *Proc. Natl. Acad. Sci. USA*, **101**, 7094–7099, 2004.
- [50] Buchanan, B.B., Adamidi, C., Lozano, R.M., Yee, B.C., Momma, M., Kobrehel, K., Ermel, R., and Frick, O.L.: Thioredoxin-linked mitigation of allergic responses to wheat. *Proc. Natl. Acad. Sci. USA*, **94**, 5372–5377, 1997.
- [51] del Val, G., Yee, B.C., Lozano, R.M., Buchanan, B.B., Ermel, R.W., Lee, Y.M., and Frick, O.L.: Thioredoxin treatment increases digestibility and lowers allergenicity of milk. *J. Allergy Clin. Immunol.*, **103**, 690–697, 1999.
- [52] Zhang, Y., Talalay, P., Cho, C.G., and Posner, G.H.: A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. USA*, **89**, 2399–2403, 1992.