Free Radical Research, August 2006; 40(8): 775–787

informa
healthcare

REVIEW

Part of the Series: From dietary antioxidants to regulators in cellular signaling and gene regulation

Sulforaphane and selenium, partners in adaptive response and prevention of cancer

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Accepted by Professor H. Sies

(Received 7 March 2006)

Abstract
The association of decreased cancer risk with intake of cruciferous vegetables and selenium is stronger than that reported for fruits and vegetables in general. An active constituent in *cruciferae* is sulforaphane. Chemopreventive effects of both, sulforaphane and selenium have been attributed to an antioxidant action which certainly is too simplicistic. Sulforaphane induces via activation of the Nrf2/Keap1 system phase 2 enzymes that protect against carcinogens and oxidants. Induced enzymes comprise the selenoproteins thioredoxin reductase-1 (TrxR1) and gastrointestinal glutathione peroxidase (GI-GPx, GPx2), which contain antioxidant response elements (ARE) in their promoter regions. Translational realisation of the enhanced transcripts depends on adequate selenium supply, which explains the synergism of Nrf2 activators and selenium. Regarding tumorigenesis the role of TrxR1 is ambiguous: it is essential for fast tumor cell growth but also diminishes vascularisation of tumors. The anticarcinogenic role of GI-GPx is evident from enhanced gastrointestinal tumor formation in gpx2/gpx1 double KO mice.

Keywords: *Sulforaphane, selenium, colon cancer, adaptive response, GI-GPx, TrxR1*

Background: five-a-day campaign versus more specific dietary strategies for cancer prevention

Cancer is a major cause of mortality throughout the world. An estimated 10 million new cases and more than 6 million deaths from cancer occurred in 2000 [1]. Between 2000 and 2020, the cancer incidence is predicted to increase by 29% in the developed world [1], or globally by about 50% [2]. Apart from smoking and infection, which are established risk factors, diet has been significantly associated with cancer and it has been estimated that 30–40% of all cancers can be prevented by adequate diets, physical activity and maintenance of appropriate body weight [3].

Our diet contains (pro)carcinogens but it also contains protective factors. In 1997, the World Cancer Research Fund (WCRF) from the American Institute for Cancer Research reported of convincing evidence for a protection from multiple forms of cancer by a greater intake of fruits and vegetables [3]. One year later the Chief Medical Officer's Committee on Medical Aspects of Food and Nutrition Policy of the United Kingdom (COMA) reached similar conclusions [4]. Altogether, early evidences were

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2006 Informa UK Ltd
DOI: 10.1080/10715760600722643

convincing so that the "five-a-day" campaign was initiated.

However, not all cancers respond to a high intake of fruits and vegetables and recent reports from prospective cohort studies do not support the protective role of fruits and vegetables that were reported in the retrospective epidemiological and case–control studies (reviewed in Ref. [5]). The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, e.g. did not find any significant association between breast cancer risk and fruit and vegetable consumption [6], whereas in another cohort study evidences for a prevention of colorectal cancer were more convincing [7]. Reasons for the seemingly conflicting results were looked for in the dietary questionnaires and in biased recall and matching cases to controls in retrospective studies, but also in random errors in the measurement of diet or limited variability of dietary intakes in cohorts that might have masked an association in the prospective studies [5].

One of the reasons for the inconsistencies might be that the general intake of fruits and vegetables is not the adequate parameter to predict prevention. Instead, some families of fruits and vegetables could be more effective than others. For example, in the Health Professional Follow-up Study, bladder cancer was only weakly associated with a low intake of fruits and vegetables [8], whereas a high intake of cruciferous vegetables decreased bladder cancer by significant 51% [9] and also breast cancer risk in premenopausal women [10]. A meta-analysis of 7 cohort studies and 87 case–control studies revealed that all cohort studies and 67% of the case–control studies reported on an inverse association between total crucifer intake and cancer risk, with cabbage, cauliflower and broccoli being the most efficient ones [11]. Thus, the association between cancer and cruciferous vegetables is stronger than the association between cancer and fruits and vegetables in general.

A relationship between low intake of selenium and cancer risk was already reported in 1969 [12]. Many *in vitro*, animal and prospective studies (reviewed in Refs. [13,14]) have confirmed this relationship. So far, however, there is only one double-blind, randomized, placebo-controlled clinical trial that supports a chemopreventive role of selenium [15]. In this Nutritional Prevention of Cancer (NPC) trial, 1312 subjects with a history of non-melanoma skin cancer were included taking either placebo or 200 μg selenium per day from selenium-enriched yeast in which the chemical form of selenium is primarily selenomethionine. After 4.5 years of treatment no effect on the primary end point, recurrence of skin cancer, was observed. However, selenium significantly reduced total cancer mortality, and cancer from prostate, colon and lung. In the follow-up study to the end of blindness, the effect on total and prostate cancer remained [16]. Thus, selenium can at least influence the incidence of particular cancers. In fact, systematic evaluation of all dietary agents reported to suppress colon cancer in rodents and of dietary prevention studies in humans qualify selenium as first among the compounds that are supposed to prevent cancer in humans [17] (http://www.inra.fr/reseau-nacre/sci-memb/corpet/indexan.html).

Cruciferous vegetables accumulate glucosinolates which have been made responsible for their chemopreventive effect. In addition, they are able to accumulate selenium in various forms. It has, thus, been tested whether selenium-enriched broccoli was more effective than selenite, selenate or broccoli alone in the reduction of cancer incidence in an experimental model of colon cancer susceptibility. This was indeed the case [18,19]. One of the most efficient glucosinolate-derived isothiocyanates is sulforaphane. It induces phase 2 detoxifying and antioxidant enyzmes that in part explains its anticarcinogenic functions. The recent findings that sulforaphane is able to induce the selenoproteins thioredoxin reductase-1 (TrxR1) [20–22] and the gastrointestinal form of glutathione peroxidases (GI-GPx) [23] brings selenium and sulforaphane into a novel focus. We will, therefore, compile the present knowledge on selenium as essential factor for selenoprotein biosynthesis and on sulforaphane as inducer of the adaptive response with the ultimate goal to unravel potential links between both dietary factors. They have for long been considered as antioxidants but now appear to work synergistically in the prevention of cancer by novel mechanisms.

Cruciferae

Classification

The family of *Cruciferae* or *Brassicaceae* belongs to the order of *Capparales* of the class of *Magnoliopsida* or Dicots and includes the genera *Brassica*, *Sinapis*, *Rorippa* and *Armoracia* with a large number of species with chemopreventive potential (Table I). The *Brassica oleracea* species contains a high number of variants, like Brussel sprouts, cauliflower, (chinese) cabbage, collards, and kale. Kohlrabi and rutabaga belong to the turnip species (*B. rapa*). Mustard is distributed over several genera (Table I). Broccoli was derived from wild cabbage (*B. oleraceae*). During cultivation it has become so complex that it was systematically divided into several groups: common broccoli (*Botrytis* group), and sprouting broccoli (*Italica* group). Crucifers prefer temperate regions such as the Mediterranean region where they reach maximum diversity.

Table I. Systematic classification of selected cruciferous vegetables.

order: Capparales
family: Brassicaceae/Cruciferae
genus: *Brassica*
species: *B. oleracea* (cabbage)
B. napus (rape)
B. rapa (turnip, tendergreen mustard)
B. nigra (black mustard)
B. campestris (wild mustard, yellow)
genus: *Sinapis*
species: *S. alba* (white mustard)
genus: *Rorippa* (also called Nasturtium)
species: *R. nasturtium-aquaticus* (water cress)
genus: *Armoracia*
species: *A. rusticana* (horseradish)

Glucosinolates and metabolites

Glucosinolates, the effective components in *cruciferae*, consist of a β-D-thioglucose group, a sulfonated oxime moiety and a variable side-chain derived from amino acids (Met, Phe, Trp or branched chain amino acids). Based on the chemical nature of the side chains, glucosinolates can be divided into different classes: aliphatic, aromatic, indolyl or allylic glucosinolates (Figure 1). Glucosinolates are hydrolyzed in a reaction catalyzed by myrosinase to glucose and the respective aglycone. The aglycone is generally unstable and undergoes a spontaneous rearrangement (Lossen-rearrangement) accompanied either by the loss of sulfate to yield an isothiocyanate, such as sulforaphane, phenethyl- or allyl-ITC (Figure 1) or at low pH by a proton-dependent desulfuration resulting in a nitrile and elemental sulfur (Figure 2). Elimination of sulfate can also lead to the formation of thiocyanates. Myrosinase is provided by the plants themselves or by the gastrointestinal microflora. In plants, it is released from cells disrupted during harvesting, mastication or chopping. Cooking destroys the enzymatic activity. Since mammalian tissues are devoid of myrosinase, glucosinolates in cooked vegetables require bioactivation by myrosinase produced by intestinal bacteria [24].

Sulforaphane. Sulforaphane (SFN), the isothiocyanate released from glucoraphanin, has chemopreventive properties as shown in a large number of experimental animal studies. Various mechanisms for the preventive effects have been discussed:

- Initiation of cell differentiation, cell cycle arrest, and apoptosis [25,26] would prevent tumor growth. Disruption of microtubulin polymerization has been proposed to explain cell cycle arrest [27]. More recently, an inhibition of histone deacetylase (HDAC) by SFN-metabolites, SFN-cysteine and SFN-N-acetyl cysteine, has been observed *in vitro* [28] and *in vivo* [29]. The block in HDAC activity led to a de-repression of $p21^{CIP1/Waf1}$ and finally to a decrease in the overall rate of cell growth and tumor development in Apc^{min} mice [29]. Inhibition of HDAC is a novel mechanism of how sulforaphane can influence gene expression.
- Inhibition of NFκB activation would be anti-carcinogenic by the inhibition of inflammation as risk factor. As underlying mechanism the interaction of sulforaphane with thiols of factors required for NFκB activity, such as GSH, thioredoxin, or Ref-1 has been discussed [30,31].
- One of the most intensively investigated effect of sulforaphane is the induction of phase 2 enzymes, which in some model systems is accompanied by the inhibition of phase 1 enzymes [32]. Phase 2

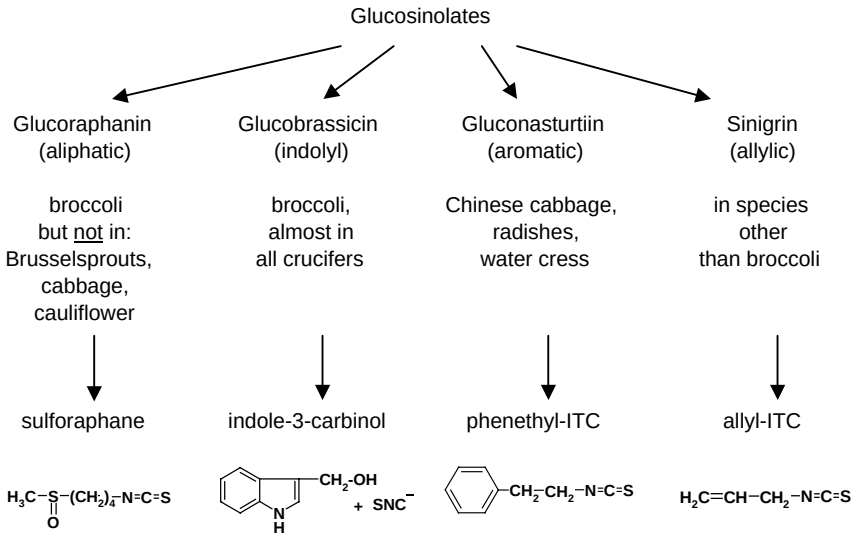

Figure 1. Glucosinolates, occurrence in crucifers and released isothiocyanates.

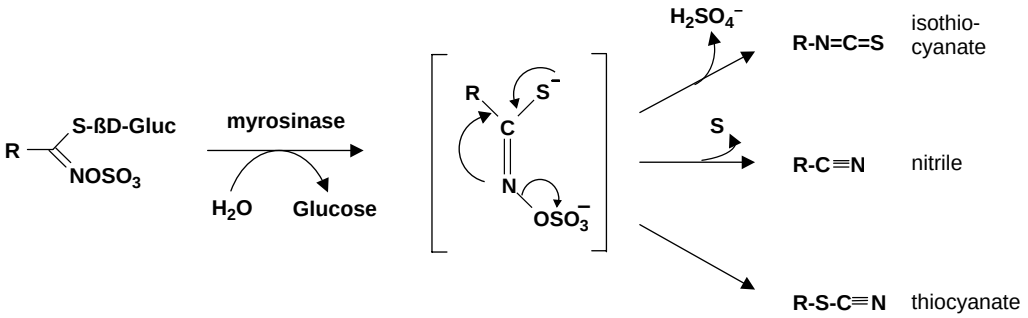

Figure 2. Common degradation products of glucosinolates.

enzymes counteract the pro-carcinogenic actions of phase 1 enzyme products by reducing the electrophilicity of reactive carcinogens via conjugation with endogenous ligands such as glutathione and glucuronic acid. In so far, up-regulation of the phase 2 system supplies to an organism a powerful arsenal to cope with electrophilic and oxidative toxicants and facilitates their elimination and/or inactivation [33]. Phase 2 enzymes are induced by the activation of the transcription factor Nrf2 which will be discussed in more detail below.

The Nrf2/Keap1 system

Nrf2 is the NF-E2-related factor 2, a member of the NF-E2 family of basic leucine zipper transcription factors (b-ZIP). It binds to the "antioxidant response element" (ARE) better called the "electrophile responsive element" (EpRE), which is present in the promoters of genes encoding phase 2 enzymes and enzymes of the antioxidant system (Table II), and activates their transcription [34]. Nrf2-deficient mice display reduced expression levels of phase 2 enzymes and accordingly an increased susceptibility to carcinogens [35]. Evidently, Nrf2 is the key transcription factor in the regulation of detoxification.

Nrf2 interacts with Keap1, the Kelch-like ECH-associated protein-1. Keap1 contains 25 cysteine residues conserved in human, rat, and mouse, which could serve as redox sensors [36] but also bind zinc, as has recently been demonstrated [37]. In mice, crucial cysteines have been identified as Cys 273 and Cys 288 [38]. The common view of the Nrf2/Keap1 interplay is that Keap1 retains Nrf2 in the cytoplasm and prevents is activation. To this end Keap1 homodimerizes via its BTB (broad comlex, tramtrack and bric-a-brac) domain and interacts with Nrf2 and with actin via its Kelch repeats (reviewed in Ref. [39]). This complex of Keap1, Nrf2 and actin localizes in the perinuclear space. The release of Nrf2 and its subsequent nuclear translocation is achieved by a change in the conformation of Keap1 via modification of one or more of the crucial cysteine residues. More recent studies, however, revealed that this scheme is still too simplicistic. Keap1 does not merely sequester Nrf2 in the cytoplasm, it also regulates its degradation. Keap1-bound Nrf2 becomes ubiquitinylated and is degraded by the ubiquitine-proteasome pathway. Eliminated Nrf2 is constantly replaced by newly synthesized one [40,41]. In this view, Keap1 functions as a substrate adaptor that bridges Nrf2 to the Cul3-Rbx1-E3 ubiquitin ligase complex [42–44]. The complex does not only ubiquitinylate Nrf2 but also Keap1 [45].

Free Nrf2 is more stable and less frequently degraded. Free Nrf2, either released from Keap1 or newly synthesized and not yet bound, translocates into the nucleus. Several signals mediating its nuclear transport have been discussed.

- Phosphorylation of Nrf2 by PKC [46,47] facilitating its dissociation from Keap1. Ser-40 has been identified as phosphorylation site.

Table II. Targets for Nrf2.

Targets for Nrf2	Reference
Phase 2 detoxifying enzymes	
UDP-glucuronosyl transferase 1A6	[120]
aflatoxin B1 aldehyde reductase	[121]
microsomal epoxide hydrolase	[122]
glutathione-S-transferases	[34,123]
NADPH quinone oxidoreductase	[34,124,125]
Redox-active proteins	
heme oxygenase 1	[126,127]
ubiquitin/PKC-ξ-interacting protein A170	[128]
peroxiredoxin 1	[128,129]
heavy and light chain of ferritin	[130,131]
thioredoxin	[93]
thioredoxin reductase-1*	[20–22]
dihydrodiol dehydrogenase	[132]
cyclooxygenase-2	[133]
GSH-related enyzmes	
γ-glutamyl-cysteine synthetase	[134]
cystine/glutamate exchange transport system X_c^-	[135]
gastrointestinal glutathione peroxidase (GI-GPx, GPx2)*	[23]
leukotriene B4 dehydrogenase	[136,137]

* Selenoproteins.

- Actin depolymerization controlled by PI3K-related signals [48,49] causing the translocation of Nrf2 and actin. The switch-off signal is actin repolymerization which allows Nrf2 and actin to exit the nucleus.
- Mediation of the nuclear transport by nuclear importin and exportin proteins [50]. These proteins recognize nuclear localization signals (NLS) or nuclear export signals (NES) on cargo proteins in a Ran GTPase-dependent manner. Surprisingly, these signals have not only been found in Nrf2 but also in Keap1. In Nrf2, an NES [51,52] locates in the leucine zipper dimerization domain and an NLS in the C-terminus [52]. The NLS directs Nrf2 to the nucleus. The NES shuttles Nrf2 via Crm1 (chromosome region maintenance)/exportin [53] and has to be masked, probably by heterodimerization with small Maf proteins, to maintain Nrf2 in the nucleus. In Keap1, an NES lies between the BTB and the Kelch domain [53]. Blocking of Crm1/exportin by leptomycin B results in a nuclear localization of both Nrf2 and Keap1 indicating that Keap1 obviously enters the nucleus together with Nrf2 and is shuttled between the cytoplasm and nucleus via Crm1/exportin. The meaning of the transport of Keap1 into the nucleus is not clear yet. It has been suggested that either Keap1 picks up Nrf2 from the nucleus or enters the nucleus to present Nrf2 to the nuclear proteasome for degradation.

Thus, Keap1 can oppose Nrf2 activation in 3 ways: (1) cytoplasmic sequestration, (2) targeting Nrf2 for proteasomal degradation and (3) facilitating the export of Nrf2 from the nucleus. Whatever the precise mechanism of Nrf2 activation will turn out to act *in vivo*, activation of Nrf2 can be obtained by a change in the conformation of Keap1 in any of the scenarios. This can be achieved by (i) exposure of cells to thiol modifying/oxidizing agents reacting with critical cysteine residues [54] (ii) displacement of zinc from the coordinating cysteines [37], (iii) ubiquitinylation of Keap1 resulting in its proteasome-independent degradation [45], or (iv) inhibition of the ubiquitinylating effect of Keap1 [55]. All these events can be mediated by compounds with oxidative or electrophilic properties (see below). Under oxidative conditions Keap1 is released from the cytoskeleton and from the degradation machinery. This favours the nuclear localization of Nrf2 leading to the activation of the respective target genes. Induction is terminated by the export of Nrf2, re-binding to Keap1 and proteasomal degradation.

Nrf2 activators. Many Nrf2 activators (Table III) are derived from the diet (reviewed in [56]). Their chemical structure is varied but they have in common to be electrophilic, to modify thiols, or to chelate metal ions [57–59]. Isothiocyanates most probably modify Keap1 by thiol modification via a Michael addition [36,38], although a direct binding to Keap1 SH groups has only been shown for 15-deoxy-Delta12,14-PGJ_2 [60]. However, a direct interaction might not necessarily be required. ITCs, especially sulforaphane, not only react with protein thiols but much faster with glutathione thereby shifting the cellular redox status to a more oxidized one. In consequence, proteins become modified at cysteine residues as a novel regulatory principle [61–63]. Keap1 might be one of the first sensors responding to an altered cellular redox state and might become modified by oxidation rather than by covalent modification. High concentrations of sulforaphane also induce apoptosis which can be explained by the same shift in the cellular oxidation state [64,65].

Table III. Activators of Nrf2.

Activators of Nrf2	References
isothiocyanates	[58]
dithiol-thiones (oltipraz)	[58,138]
oxidizable hydroquinones	[58]
Michael reaction acceptors	[139]
trivalent arsenicals	[125]
heavy metals	[125]
vicinal dimercaptanes	[125]
carotenoids/polyenes	[140]
hydroxynonenal	[129]
(-)epigallocatechin-3-gallate	[141]
(-)epicatechin-3-gallate	[141]
oxidized LDL	[129]
shear stress	[142]
ER stress	[143]
heme	[144]
hydroperoxides	[125]
15d-PGJ_2	[145]

Selenium

How selenium is anti-carcinogenic is not clear at all. It has often been declared as an antioxidant. However, selenium is an element which can occur in many different chemical forms, most of which do not have antioxidant functions [66]. In mammals, selenium is an integral part of selenoproteins in the form of selenocysteine (SeCys). SeCys is encoded by TGA and is incorporated into the growing peptide chain during translation by a unique mechanism (reviewed in [67,68]). The human selenoproteome consists of 25 selenoproteins [69] from which 5 are glutathione peroxidases, 3 thioredoxin reductases and 3 deiodinases. The function of most selenoproteins is far from being clear. Even within the family of glutathione peroxidases, which all reduce hydroperoxides, each individual member appears to have additional

individual functions [70–73]. In view of the mutagenic potential of peroxides, optimization of GPx-activity has amply been discussed as the underlying mechanism for the protective effect of selenium. However, to become incorporated into selenoproteins selenium compounds have to be metabolized to selenide [74] which is not possible for all selenium compounds. Thus, other mechanisms than up-regulation of selenoproteins might be involved in their beneficial effect and the need for supra-nutritional selenium intake to observe a chemopreventive effect appears to support this assumption [75]. However, full expression of selenoprotein P requires greater selenium intake than that needed for optimal plasma GPx expression [76], indicating that an adequate marker for the selenium status has not yet been found and that there might be selenoproteins only responding to high levels of selenium. So far, investigations of the regulation of selenoprotein expression have mainly been focused on the availability of selenium needed for translation. Regulation of the transcription has been considered with less emphasis, a situation which will change after the recent findings that selenoproteins can be induced by activators of the Nrf2/Keap1 system.

Selenoproteins induced by sulforaphane

Thioredoxin reductase. Thioredoxin reductases (TrxRs) are a family of NADPH-dependent selenoflavoproteins ubiquitously found in mammalian tissues. So far, 3 isoforms are known, the classical cytosolic form (TrxR1), the mitochondrial form (TrxR2) and the testes-specific thioredoxin and glutathione reductase (TGR) (for review see Ref. [77]). TrxRs reduce Trx-S_2 to Trx-$(SH)_2$ using NADPH as reduction equivalents. TrxR is a homodimeric protein that contains two distinct redox centers in each subunit. The first one resembles typical disulfide reductases in comprising FAD with associated cysteines. The second one is situated at the C-terminus and consists of Gly–Cys–Sec–Gly–COOH [78]. Its role is to transfer the reduction equivalents from the central redox center to the substrate.

The Trx/TrxR couple acts as a protein disulfide reductase system that contributes to the redox regulation of transcription factor activity, cell growth and inhibition of apoptosis. Trx is a key factor for DNA synthesis by directly transducing electrons to ribonucleotide reductase [79]. The continuous reduction of thereby oxidized Trx is indispensable for cell proliferation. In so far, the Trx/TrxR system is absolutely required for healthy cells. However, the beneficial effects of the system might change to its opposite during the growth and progression phase of tumors. The Trx/TrxR system is often up-regulated in cancer cells. But, whereas an enhanced Trx level has been associated with aggressive tumor growth and poor prognosis [80], the role of TrxR in cancer is not entirely clear. TrxR is highly expressed in a number of human tumors [81,82] and during carcinogenesis [83]. Inhibition of TrxR1 indeed prevented cancer cell growth *in vivo* [82] and TrxRs have been suggested as potential targets for anticancer drugs [84]. In the reduced form of TrxR and at physiological pH ($-SH/-Se^-$), the selenide is easily attacked by electrophiles and alkylating agents. If the first electrophilic molecule is bound, a second can alkylate the neighbouring cysteine as has been shown for the binding of curcumin to TrxR [85]. In this way, the activity of TrxR is irreversibly lost. A number of electrophilic anticancer drugs have been tested for their ability to inhibit TrxR activity and found to be effective [86]. Inhibition of TrxR activity may contribute to the anticancer effect of these drugs but may also explain their toxicity to healthy cells. Thus, inhibition of TrxR is a double-edged sword.

The direction of Trx/TrxR research mentioned so far is surprising since the antioxidant thioredoxin system has initially been considered to prevent tumor initiation, as it prevents oxidative DNA damage. Also, discrepancies between *in vitro* and *in vivo* investigations on the effect of TrxR1 levels on tumor growth have been reported [87]. Further, overexpression of TrxR1, and to a lesser extent also TrxR2, decreased cell growth and induced the expression of epithelial differentiation markers in HEK-293 cells [88]. Chemical inhibition of TrxR in primary bovine mammary endothelial cells led to an increase in VEGF and VEGF receptor expression, cell migration, proliferation and angiogenesis [89], which are effects that are indispensable for tumor growth. Thus, TrxRs might be both anti-carcinogenic by dampening neovascularization of neoplastic tissue and promoting differentiation or pro-carcinogenic by facilitating proliferation in general. Their precise role in tumorigenesis therefore will depend on the stage of the tumor development.

Recent manipulation of the Trx/TrxR system shed new light on its role in carcinogenesis. Treatment of HUVEC with the α, β-unsaturated aldehyde acrolein did not only inhibit TrxR activity, as expected, but also initiated a recovery of activity after several hours due to an induction of the enzyme [90]. As outlined above, electrophiles such as acrolein are potent activators of Nrf2, and a functional ARE is indeed present in the promoter of TrxR1. Activators of Nrf2, including sulforaphane, induced TrxR1 but not TrxR2 [20–22]. Also cadmium [91], arsenite, H_2O_2 and DEM [92] were effective. Whereas, cadmium activated Nrf2 by the inhibition of its proteasomal degradation, the other compounds rather acted by thiol modification. TrxR1 induction by sulforaphane was synergized by selenium supplementation [20], indicating that the increase in mRNA can be used to translate more protein if selenium is available. Yet Nrf2 activators not

only induce TrxR1 but also thioredoxin [93]. The induction of the entire Trx/TrxR system by a transcription factor that is generally accepted to trigger the adaptive response suggests that the phenomenon is rather meant to prevent tumor initiation than to inhibit the growth of an existing tumor. In view of the high concentrations of Trx and TrxR in many tumor cells, it remains to be investigated whether the up-regulation is a remnant from the attempt to counteract tumor initiation upon certain stimuli or a mechanism to sustain tumor growth.

GI-GPx. The gastrointestinal glutathione peroxidase (GI-GPx, GPx2) has first been identified to be exclusively expressed in the gastrointestinal system and suspected to act as barrier against hydroperoxide absorption. Yet it soon became obvious that its expression was not limited to the GI system. GI-GPx was found in several cancer cell lines and was up-regulated in pre-neoplastic lesions in skin cancer [94] and in early stages of adenomatous polyposis [95].

GI-GPx$^{-/-}$ mice appear to have a normal phenotype [96], but mice deficient in both, cGPx (GPx1) and GI-GPx, showed retarded growth after weaning, exhibited severe ileocolitis starting at day 11 of age [97], and developed intestinal cancer [98]. Interestingly, the development of both, ileocolitis and tumors depended on, or were essentially aggravated by, colonisation of the intestine by bacteria. The intimate relationship of GI-GPx and the gastrointestinal flora is further evidenced by an induction of GI-GPx expression upon colonisation of the intestine [99]. These observations point to a pivotal role of GI-GPx in counteracting and developing tolerance to inflammatory stimuli of the intestinal microflora.

An endogenous inducer of GI-GPx has so far not been recognized. Chu et al. [100] first identified several caudal homeobox protein binding sequences and two retinoic acid responsive elements in the GI-GPx gene and showed that endogenous GI-GPx could be induced by retinoic acid in some (MCF-7) but not all (HT29) cells. More recently, Morbitzer and Herget [101] discovered that GI-GPx was down-regulated in hepatoma cells infected with hepatitis C virus subgenomic RNA. Induction of GI-GPx by retinoic acid suppressed the HCV replicon suggesting a therapeutically interesting inverse relationship of GI-GPx levels and viral replication.

In several microarray studies, GI-GPx transcripts were elevated together with phase 2 enzyme transcripts upon exposure to the Nrf2 activator sulforaphane or to hyperbaric oxygen [102,103]. Based on the GI-GPx promoter description of Kelner et al. [104], the 5′ region of GI-GPx was cloned and analysed for transcription factor binding sites and the functionality of putative antioxidant responsive elements (ARE) was investigated by Banning et al. [23]. From the two AREs identified, the ATG-proximal ARE proved to be indispensable for endogenous and sulforaphane-induced GI-GPx expression. The enhancement of a GI-GPx promoter-driven reporter gene expression by transfection with Nrf2 and suppression thereof by transfection with Keap1 finally identified GI-GPx as a target for Nrf2 [23].

GI-GPx is the second selenoprotein found to be regulated by the Nrf2/Keap1 system. Like TrxR1 GI-GPx belongs to the antioxidant defence system, but like TrxR1 it might have alternative functions in the redox regulation of enzymes. GI-GPx is a selenoprotein ranking highest in the hierarchy of selenoproteins investigated so far [105]. Whereas the mRNA from low ranking selenoproteins, like cGPx (GPx1), is degraded when selenium becomes limiting, the mRNA of GI-GPx can even increase and becomes preferentially translated when selenium supply is restored [105]. The ranking of TrxR1 is lower, but overexpression of TrxR1 led to a decrease in cGPx, indicating that enhanced TrxR1 transcripts withdraw selenium from the biosynthesis of obviously less important selenoproteins [88].

Remains the question when it makes sense to increase the mRNA of a protein that is preferentially synthesized anyway. The most plausible answer might be under selenium-limiting conditions when the expression of even high-ranking selenoproteins is decreased. The limited amounts of selenium could then be used to translate an enhanced level of mRNA. The ability of trace amounts of GI-GPx to prevent ileocolitis and, in consequence tumor formation was convincingly demonstrated in mice with the genotypes *Gpx1*$^{-/-}$ *Gpx2*$^{+/-}$ and *Gpx1*$^{+/-}$ *Gpx2*$^{-/-}$ that were grown under restricted selenium supply [106]. One allele of *Gpx2* was sufficient for complete protection even under selenium restriction.

The function of an up-regulated GI-GPx might be rather anti-inflammatory than intrinsically anti-carcinogenic, i.e. it rather prevents tumor initiation by inflammatory mediators than tumor progression, as outlined by Chu et al. [107]. Abundant evidence exists for linking inflammation and cancer. An essential percentage of malignancies are caused by infectious Agents, including hepatitis B and C virus (liver cancers), human papilloma viruses (cervical cancer), and *Helicobacter pylori* (stomach cancer). Vaccinations could be the key to prevent these types of cancers. Also chronic inflammation obviously facilitates cancer development, e.g. Crohn's disease (cancer of the small intestine), or ulcerative colitis (colon cancer) (reviewed in [108]). The link might be activated NFκB shown to be crucial for malignant transformation [109–112], enhanced expression of cyclooxygenase-2 (COX-2) [113], and/or induction/activation of lipoxygenases [114]. Accordingly, increased levels of PGE_2 and leukotriene B_4 are found at sites of inflammation and of

tumor formation. The role of GI-GPx in this scenario has not yet been investigated. However, in analogy to the other glutathione peroxidases, an inhibition of the activity of lipoxygenases and cyclooxygenases appears to be a plausible explanation (reviewed in Ref. [73]). As overexpression of PHGPx (GPx4) in tumorigenic L292 cells prevented PGE_2 production, cell proliferation, and solid tumor growth in nude mice [115], a similar function of GI-GPx may be postulated.

Combination of sulforaphane and selenium

The possible synergism that might be obtained by feeding selenium together with sulforaphane sounds promising. Synergy has been observed in the enhanced up-regulation of TrxR1 protein by sulforaphane in the presence of selenium in HepG2 cells [20] but so far not *in vivo*. Vegetables serving as good sources for a high selenium and high sulforaphane supply again are *cruciferae* since they do not only accumulate glucosinolates but also selenium. Accordingly, increasing the selenium content in broccoli should raise the power of glucosinolates. Broccoli grown on selenium-fertilized ground indeed inhibited the formation of chemically induced preneoplastic lesions in rat colon [18], of spontaneous development of intestinal tumors in mice [116] and of mammary tumors in rats [19]. But a recent *in vitro* study failed to explain the *in vivo* findings by the proposed interaction of sulforaphane and selenium in the adaptive response. Surprisingly, the sulforaphane content of broccoli grown on substrates with high, medium and low selenium was inversely correlated with the selenium content [117]. Indeed, selenium fertilisation of broccoli changed the pattern of phenolic compounds and the content of glucosinolates, especially that of glucoraphanine from which sulforaphane is the breakdown product [118]. In consequence, the extract of selenium-enriched broccoli did not induce an adaptive response, as measured by NQO1 expression, whereas it optimized the selenium-dependent antioxidant systems, as was evident from a slight increase of TrxR1 activity and a rise of the primary antioxidant selenoenzyme cGPx [117]. The latter effect was associated with an optimum protection against oxidative DNA damage in terms of DNA strand breaks. This observation might explain why selenium-enriched broccoli exerts chemopreventive efficacy *in vivo* despite a marginal or absent activation of the adaptive response.

Selenium thus contributes to the prevention of cancer in two ways: It optimizes the cellular antioxidant system in a largely ARE-independent way (the cGPx promoter does not contain an ARE element) and it synergizes with plant-derived electrophiles such as sulforaphane in full expression of the adaptive response. In order to exploit both protective mechanisms, selenium fertilisation of cruciferous plants is evidently not the way of choice. Instead, the glucosinolates and the selenium, which is required for their activity, should be supplied from different alimentary sources.

Conclusions

Emerging evidence tends to rule out any direct antioxidant action as principle of tumor prevention by *cruciferae*-derived antioxidants such as sulforaphane and by alimentary selenium compounds. Instead, many of such plant-derived compounds of which sulforaphane is just a well investigated prototype, activate the Nrf2/Keap1 system and thereby induce protective phase 2 enzymes. These comprise two selenoproteins, TrxR1 and GI-GPx, the latter according to the KO mice experiments appear to be anti-carcinogenic. For full expression of the adaptive response by sulforaphane, adequate or enhanced selenium supply is mandatory.

Plant-derived micronutrients that proved to be antioxidants *in vitro* activate the adaptive response through Keap1 modification *in vivo*. They can do so in different ways: (i) by direct alkylation of essential SH groups due to their electrophilic potential, (ii) via autoxidation-derived superoxide and peroxide formation resulting in Keap1 oxidation, or (iii) by shifting the cellular redox balance towards an oxidative state with similar consequences. Similarly, the preventive effects of selenium may result from different mechanisms: (i) optimization of expression of antioxidant selenoproteins such as cGPx, (ii) oxidative activation of Keap1 via an oxidative stress, as is induced by supranutritional selenium supplementation [119] and (iii) by cooperation with plant-derived Nrf2 activators in establishing the adaptive response. Inducing the adaptive response triggered by a mild oxidative stress via autoxidation of antioxidants or S-alkylation of Keap1 by electrophilic plant compounds may therefore be considered as kind of vaccination to render the organism more resistant to severe oxidative stress or exposure to strong alkylants, which both are clearly pro-carcinogenic. The emerging interdependence of selenium biochemistry and expression of adaptive response is one example for the relevance of a balanced diet and should pave the way to more rational dietary recommendations for the prevention of cancer.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, DFG, Priority program: Selenoproteins, SPP1087, Br778/5-3.

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