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Postgraduate Symposium Epigenetic and antioxidant effects of dietary isothiocyanates and selenium: potential implications for cancer chemoprevention

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There is evidence from epidemiological studies suggesting that increased consumption of cruciferous vegetables may protect against specific cancers more effectively than total fruit and vegetable intake. These beneficial effects are attributed to the glucosinolate breakdown products, isothiocyanates (ITC). Similarly, selenium (Se) consumption has also been inversely associated with cancer risk and as an integral part of many selenoproteins may influence multiple pathways in the development of cancer. This paper will briefly review the current state of knowledge concerning the effect of Se and ITC in cancer development with a particular emphasis on its antioxidant properties, and will also address whether alterations in DNA methylation may be a potential mechanism whereby these dietary constituents protect against the carcinogenic process. Furthermore, we will discuss the advantages of combining ITC and Se to benefit from their complementary mechanisms of action to potentially protect against the alterations leading to neoplasia. Based on this review it may be concluded that an understanding of the impact of ITC and Se on aberrant DNA methylation in relation to factors modulating gene-specific and global methylation patterns, in addition to the effect of these food constituents as modulators of key selenoenzymes, such as gastrointestinal glutathione peroxidase-2 (GPx2) and thioredoxin reductase-1 (TrxR1), may provide insights into the potential synergy among various components of a plant-based diet that may counteract the genetic and epigenetic alterations that initiate and sustain neoplasia.

Cancer chemoprevention: Epigenetics: Selenoproteins: Thioredoxin reductase-1: Gastrointestinal glutathione peroxidase-2

Diet and lifestyle play an important role in cancer aetiology and it has been estimated that specific dietary patterns and constituents are key environmental components that may contribute to the development of one-third of human cancers⁽¹⁾. During the early 1990s, available evidence suggested that increased consumption of fruits and vegetables may be protective against cancer at some sites⁽²⁾. However, the evidence for a large preventive effect was based primarily on data from case-control studies, which are prone to recall bias⁽³⁾. Then in the late 1990s, when the results of prospective cohort studies of diet and cancer began to emerge, their outcomes did not confirm

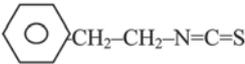
the strong inverse associations that had been observed in the earlier case-control studies and provided limited support for a protective effect of increased fruit and vegetable consumption⁽¹⁾.

However, a weak association between total fruit and vegetable intake and overall cancer risk does not exclude the possibility that a small target group of fruits and vegetables, or a specific compound present in some of these foods may exert a protective effect⁽⁴⁾. In this respect, the emerging evidence for a variety of potentially important components present in plant-based foods that possess cancer-preventive properties has stimulated interest in the concept of

Abbreviations: DNMT, DNA methyl transferase; GPx2, gastrointestinal glutathione peroxidase-2; HDAC, histone deacetylase; ITC, isothiocyanate; SeMet, L-selenomethionine; SeMSC, Se-methylselenocysteine; SFN, sulforaphane; TrxR1, thioredoxin reductase-1.

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Table 1. Glucosinolates and chemical structures of isothiocyanates (ITC) found in commonly eaten cruciferous vegetables

Glucosinolates	Associated ITC	Chemical structure of ITC and main plant source
Glucoraphanin	Sulforaphane	$\begin{array}{c} \text{CH}_3\text{-S-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N=C=S} \\ \\ \text{O} \end{array}$ Broccoli, Brussels sprouts
Glucoiberin	Iberin	$\begin{array}{c} \text{CH}_3\text{-S-CH}_2\text{-CH}_2\text{-CH}_2\text{-N=C=S} \\ \\ \text{O} \end{array}$ Broccoli, Brussels sprouts, cabbage
Glucoerucin	Erucin	$\text{CH}_3\text{-S-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N=C=S}$ Rocket
Sinigrin	Allyl-ITC (AITC)	$\text{CH}_2=\text{CH-CH}_2\text{-N=C=S}$ Mustard and horseradish
Gluconasturtiin	Phenethyl-ITC (PEITC)	 $\text{C}_6\text{H}_5\text{-CH}_2\text{-CH}_2\text{-N=C=S}$ Watercress, radish, turnip

chemoprevention. Particular attention has focused on citrus fruits, dark-green vegetables and cruciferous vegetables that have been shown to influence various stages in the development of cancer⁽⁵⁾ by preventing the genotoxic damage of cellular DNA upon exposure to endogenous or exogenous carcinogens (initiation phase), inhibition of clonal expansion of initiated cells by induction of apoptosis and modulation of signal transduction (promotion phase) and blockade of tumour with invasive and metastatic potential (progression phase). Hence, consumption of cruciferous vegetables has been more strongly associated with cancer protection than total vegetable consumption in both animal models and from available prospective cohort data. Numerous studies suggest that phytochemicals in cruciferous vegetables are responsible for their chemoprotective effects, and among them, the glucosinolate breakdown products, isothiocyanates (ITC), are believed to be responsible for this anti-carcinogenic action (Table 1)⁽⁶⁾. Another dietary compound that has been shown to have cancer-chemopreventive roles is Se, which is an essential trace element that occurs in different chemical forms. Although *in vitro* cell culture studies and *in vivo* studies using animal models suggest that Se is effective for cancer prevention, the inconsistent results obtained in Se clinical trials indicate that a more focused approach to understand the mechanisms of different forms of Se on antioxidant and anticancer activity is needed. Also, the fact that abundant experimental evidence has shown anti-carcinogenic effects of Se in individuals with apparently full selenoenzyme expression⁽⁷⁾ suggests that the direct chemical properties of Se and its metabolites could be involved in their anti-carcinogenic activities. Therefore, as well as analysing the antioxidant properties of Se, this review will address whether alterations in DNA methylation may be a potential mechanism whereby dietary Se protects against colon carcinogenesis. In addition, we will discuss the advantages of combining ITC and Se to benefit from their complementary mechanisms of action to potentially

prevent the accumulation of alterations during neoplastic transformation that lead to uncontrolled cell growth and loss of genomic stability.

Molecular basis of the chemoprotective effects of isothiocyanates

Understanding the chemoprotective mechanisms of ITC is important not only because these compounds block the formation of a wide variety of carcinogen-induced tumours in rodents but also because ITC and their glucosinolate precursors are widely available in human dietary plants and are consumed in considerable quantities⁽⁸⁾. Glucosinolates are relatively biologically inert, but can degrade to a range of bioactive compounds, such as ITC and indoles, on hydrolysis by the plant-based enzyme myrosinase. However, in the human diet, the myrosinase in cruciferous vegetables is often heat-inactivated during cooking, and in this situation glucosinolates can also be hydrolysed less efficiently by the colonic microflora⁽⁹⁾. Verhoeven *et al.* reviewed the evidence for *Brassica* consumption and cancer risk, and reported that 67% of all studies showed an inverse association between total *Brassica* vegetable intake and risk of cancer at various sites⁽¹⁰⁾ and a wide range of studies in human subjects, animals and *in vitro* have confirmed this finding⁽⁵⁾. For instance, cruciferous vegetables have been found to reduce morphological markers of colon cancer risk in dimethylhydrazine-treated rats⁽¹¹⁾. Also, a prospective study carried out in Japan to investigate associations between fruit and vegetable consumption and risk of oesophageal squamous cell carcinoma, showed that only cruciferous vegetables were associated with a significantly decrease in risk (hazard ratio per 100 g/d 0.44 95% CI 0.23, 0.82)⁽¹²⁾. This reduced cancer risk has been attributed to the ability of ITC to influence the process of carcinogenesis partly by modulation of phase I and II enzymes, induction of apoptosis and cell-cycle arrest^(13,14).

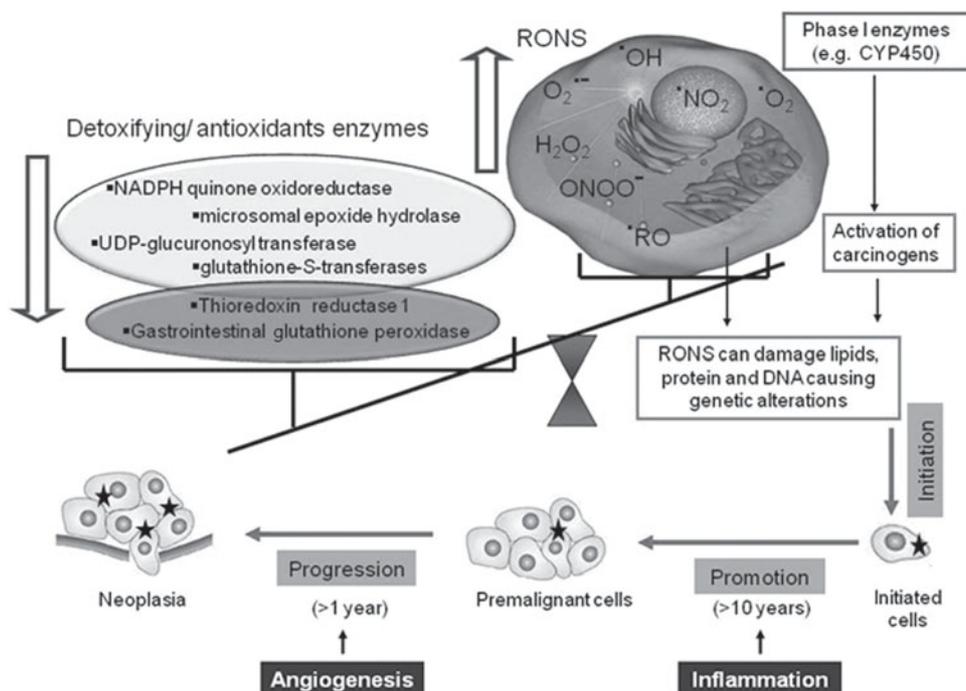


Fig. 1. Role of oxidative stress and antioxidant systems in a simplified scheme of carcinogenesis. Reactive oxygen and nitrogen species (RONS) are constantly generated in cells as unwanted by-products of aerobic metabolism and this together with the inflammatory microenvironment of the tissue acts as a predisposing factor to multistage carcinogenesis⁽¹⁹⁾. Although a low physiologic level of RONS is scavenged efficiently by the cellular antioxidant defence system, an imbalance between the generation of RONS and cellular antioxidant capacity generates a state of oxidative stress that contributes to carcinogenesis.

Inhibition of phase I enzymes is thought to be a preventive measure against chemically induced carcinogenesis. Procarcinogens are usually converted into highly reactive intermediates that form adducts with DNA, RNA and protein. These transformations are catalysed by cytochrome P450 enzymes. DNA adducts that persist unrepaired can generate mutations in critical genes such as oncogenes and tumour suppressor genes^(15,16). A dose-dependent inhibition of cytochrome P450, family 1, subfamily A, polypeptide 1 and cytochrome P450, family 2, subfamily B, polypeptide 1/2 by sulforaphane (SFN) was observed in rat hepatocytes, and also the expression of cytochrome P450, family 3, subfamily A, polypeptide 4 (the major cytochrome P450 enzyme in human liver) was markedly decreased at both mRNA and activity levels⁽¹⁷⁾.

Induction of antioxidant and detoxifying enzymes by isothiocyanates

Much of the evidence suggests that a decrease in the expression of antioxidant enzymes together with an increase in the production of free radical species might render cells susceptible to permanent damage and initiate the sequence of events leading to cancer (Fig. 1). On the other hand, elevation of phase II enzymes and other antioxidant systems in specific tissues exposed directly to bioactive food components, such as the colon, may confer cytoprotection against the toxicity of electrophiles and reactive oxygen species⁽¹⁸⁾. These groups of functionally diverse phase II enzymes

include glutathione transferases, UDP-glucuronosyltransferases and NAD(P)H:quinone oxidoreductase 1. The modulation of phase II gene expression and enzyme activity by ITC has been assessed in a number of cell lines of different origin, but the most commonly used are derived from liver hepatoma, such as human HepG2 and mouse Hep1c1c7.

Stimulation of these enzymes is one of the most important components of cellular defence mechanisms and the ability of ITC to promote their activation is now understood to be enabled by the Keap1-NF-E2-related factor-2-antioxidant responsive element pathway. However, the list of detoxifying or antioxidant enzymes stimulated by ITC is not only limited to the phase II group, as the NF-E2-related factor 2-antioxidant responsive element signalling pathway also targets other redox-active proteins and glutathione-related selenoenzymes such as thioredoxin reductase-1 (TrxR1) and gastrointestinal glutathione peroxidase (GPx2), respectively, that will be examined in the following section. Studies have revealed that SFN and its glutathione conjugate were found to increase significantly in a time-dependent manner both UDP glucuronosyltransferase 1 family, polypeptide A1 and glutathione S-transferase α 1 mRNA levels in HepG2 ($P < 0.005$) and HT-29 ($P < 0.05$) cells⁽²⁰⁾. In another study, when mouse Hep1c1c7 cells were incubated with 2.5 μ M SFN, an increase in NAD(P)H:quinone oxidoreductase 1 activity was observed, reaching a maximum induction of three-fold over control⁽²¹⁾. Also, perfusion of broccoli extracts to human jejunum (equivalent

to about 1.2 g dry weight broccoli) resulted in an induction of glutathione *S*-transferase α 1 and UDP glucuronosyltransferase 1 family, polypeptide A1 in exfoliated enterocytes; the changes in gene expression were also confirmed in Caco-2 cells, where SFN was responsible for the induction of glutathione *S*-transferase α 1 (three-fold)⁽²²⁾.

Cell-based models have been used extensively for screening and detection of novel cancer chemopreventive agents from food compounds. However, the induced level and type of phase II enzyme varies between different cell lines as reported in a study that used seven widely adopted cell lines, including Hepa1c1c7, HepG2, MCF-7, MDA-MB-231, LNCaP, HeLa and HT-29, where the effects of 25 μ M SFN on the enzymatic activity of glutathione transferase, NAD(P)H:quinone oxidoreductase 1, aldo-keto reductase and glutathione reductase were evaluated⁽²³⁾.

This tissue-specific response has been confirmed *in vivo* after analysing the ability of six plant-derived ITC (allyl-ITC, isoberverin, erucin, SFN, iberin and cheirolin) to increase tissue levels of NAD(P)H:quinone oxidoreductase 1 and glutathione transferase in a variety of rat tissues at doses of 40 μ mol/kg per d. The results revealed different levels of enzyme induction in the duodenum, forestomach and urinary bladder out of fifteen tissues analysed, and such responses differed depending on the ITC employed⁽²⁴⁾. These differences observed at the cell and tissue level may be explained by the degree at which different ITC accumulate in cells⁽²⁵⁾.

Selenium and cancer prevention heterogeneity: metabolism and antioxidant functions

Although dietary Se intake was shown to be inversely associated with cancer mortality as early as the 1960s⁽²⁶⁾, it was not until 1996 that an intensive effort was launched to try to understand the mechanism of action of Se as a cancer-preventive agent after Clark *et al.*⁽²⁷⁾ reported results from The Nutritional Prevention of Cancer study, where they showed that supplementation with selenised yeast decreased cancer incidence by almost 50%. However, further trial results have been contradictory and heterogeneous among organs⁽²⁸⁾ and as a consequence the American Institute for Cancer Research and the World Cancer Research Fund have concluded in its latest report that there is limited evidence that food containing Se protects against colorectal and stomach cancer. Nevertheless, for other sites, such as prostate, such foods probably do decrease risk of cancer⁽¹⁾. Another disappointing outcome arose from the Selenium and Vitamin E Cancer Prevention Trial, which demonstrated that Se (200 μ g/d from L-selenomethionine (SeMet)), vitamin E, or Se+vitamin E did not prevent prostate cancer in healthy population groups of men in the study and also had no effect on secondary endpoints, which included lung cancer and colorectal cancer⁽²⁹⁾.

Nonetheless, there is extensive evidence that monomethylated forms of Se such as methylselenol are critical metabolites for the chemopreventive effect of Se⁽³⁰⁾. The major forms of Se occurring in food are the organic forms SeMet (found in plants and animal sources) and selenocysteine (found in animal sources). SeMet can also be

metabolised via the multi-step trans-sulfuration pathway to selenocysteine, in turn degraded to hydrogen selenide for subsequent methylation by methyltransferases that give rise to methylselenol (Fig. 2)^(31,32). In this respect, Se-methylselenocysteine (SeMSC; present in plants of the Brassica family) and γ -glutamyl-Se-methylselenocysteine (present in plants of the Allium family), which are known to be converted to methylselenol (Fig. 2)⁽³³⁾, have been found to account for the anti-carcinogenic effect of Se-enriched broccoli and garlic, respectively, and have proved to be more effective in reducing colon and mammary tumorigenesis in rodents than selenate, selenite or Se-enriched yeast, which contains mostly SeMet^(34,35).

This protective effect attributed to Se could be explained by its multiple cellular functions including cell cycle regulation, immune surveillance, apoptosis, cancer cell migration and angiogenesis⁽³⁷⁾. Moreover, some of its associated chemopreventive properties may relate to the crucial fact that Se is a component of the amino acids selenocysteine and SeMet, which are incorporated into twenty-five genes encoding over thirty mammalian selenoproteins⁽³⁸⁾. Selenoproteins that might be relevant to cancer risk include glutathione peroxidase-1 (GPx1), GPx2, Sep15, SelP and TrxR1⁽³⁹⁾.

Considering that different forms of Se supplementation, either as inorganic Se (sodium selenite) or as organic forms such as SeMSC may have different efficacies in the expression of different selenoproteins and that ITC have been found to promote the induction of TrxR1 and GPx2 due to the presence of an antioxidant responsive element in their gene promoter, we have evaluated the effects of the ITC SFN and iberin, which contains one alkyl group less than SFN, together with different forms of Se (selenite and SeMSC) on the expression of these selenoproteins in Caco-2 cells to establish whether a combination of ITC+Se offer additive or synergistic effects on their regulation. Our results have shown that ITC in combination with either form of Se induced more protein expression of TrxR1 and GPx2 than either compound did individually⁽⁴⁰⁾. The importance of these findings relates to the fact that in the pre-initiation and/or early stages of colon carcinogenesis the disruption of cellular defence mechanisms (consisting of a battery of detoxifying or antioxidant enzymes) would make cells more susceptible to DNA damage by both unwanted by-products of normal cellular metabolism (Fig. 1), or other environmental sources of reactive oxygen species, which in rapidly dividing cells, such as in the colonic epithelium, may escape repair mechanisms resulting in somatic mutations⁽⁴¹⁾. Therefore, an increase in the cellular antioxidant defence mechanisms may prevent the deleterious effects of free radicals that would otherwise affect important biomolecules and render colon cells susceptible to the accumulation of genetic alterations that lead to cancer (Fig. 1). It has been previously suggested that the up-regulation of these selenoproteins by ITC and Se results from an independent mechanism. While the former works transcriptionally through the antioxidant responsive element located in the promoter region of the genes, the latter acts by post-transcriptional mechanisms that involve the provision of an adequate supply of selenocysteine for incorporation into TrxR1 or Gpx2 that delays its

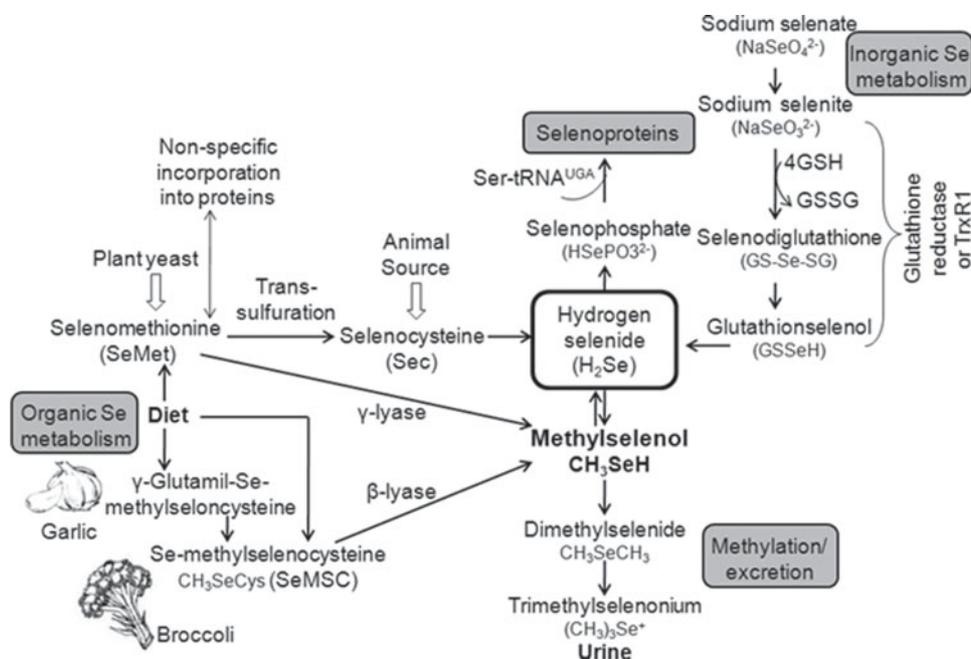


Fig. 2. Pathways of Se metabolism. Se salts undergo reductive metabolism with glutathione reductase and NADPH or can be directly reduced by thioredoxin reductase (TrxR1) to hydrogen selenide (H_2Se). Selenomethionine and selenocysteine can generate methylselenol through H_2Se . The latter is the source for incorporation into selenocysteine-containing proteins by co-translational incorporation of selenocysteine by seryl-transfer RNA at UGA codons. Diet may also enrich the methylselenol pool by passing the H_2Se pool, by using direct precursors such as Se-methylselenocysteine (SeMSC) or γ -glutamyl-Se-methylselenocysteine, which is converted to SeMSC first and then acted upon by a β -lyase to also give methylselenol. Methylation of H_2Se constitutes an excretion pathway for Se^(7,36).

degradation^(42–44). Similar results in relation to the up-regulation of TrxR1 by co-addition of different forms of ITC and Se have been observed in other studies that have employed different cell lines^(42,45).

Nevertheless, data emerging from *in vivo* and *in vitro* studies suggest that TrxR1 and GPx2 are up-regulated in cancer. These observations provide evidence that TrxR1 and GPx2 are critical for self-sufficiency in growth of malignant cells, in which these selenoproteins act predominantly as pro-cancer proteins^(46,47). In addition to its role in the activation of antioxidant and detoxifying enzymes, NF-E2-related factor 2 has been suggested to have a positive role in cancer tumorigenesis and chemoresistance⁽⁴⁸⁾. Thus, ITC+Se-mediated increase in selenoprotein expression, such as TrxR1 and GPx2, may be more relevant in normal cells as a cancer-preventive measure to inhibit malignant transformation prior to the initiation of carcinogenesis. However, a long-term exposure of dietary ITC and/or Se might potentially lead to other modifications to the human genome that will ultimately impact upon gene expression and cancer susceptibility. With these possibilities in mind we have investigated whether modifications to epigenetic marks may be a potential mechanism whereby these dietary constituents inhibit the development of neoplastic colonic lesions.

Dietary modulation of epigenetic modifications and cancer

Epigenetics refers to heritable changes in the pattern of gene expression without any alteration in the nucleotide

sequence⁽⁴⁹⁾. In human subjects and other mammals the most frequent epigenetic modification of DNA bases involves cytosine, which is modified reversibly by adding a methyl group (CH_3) to its C_5 position. This modification occurs only on cytosines that precede a guanine in the DNA sequence, referred to as the CpG dinucleotide⁽⁵⁰⁾. Short regions of 0.5–4 kb in length, known as CpG islands, are rich in CpG content. These islands are typically found in or near promoter regions of genes where transcription is initiated. In normal somatic cells, the vast majority of CpG dinucleotides in the genome are methylated, whereas CpG islands often remain unmethylated, allowing gene expression to occur. In cancer cells, this pattern of CpG methylation becomes disrupted, with high level of methylation within promoter regions of genes causing abnormal gene silencing, in addition to global hypomethylation of genomic DNA, which promotes chromosomal instability, translocation and gene disruption through the reactivation of endoparasitic sequences⁽⁵¹⁾. Furthermore, the state of histone acetylation is also important in regulating chromatin structure and gene transcription. Acetylation of histones is required to maintain chromatin in an open and transcriptionally active state. On the other hand, histone deacetylases (HDAC) act to keep histone residues deacetylated, promoting transcriptional silencing.

There is currently considerable interest in the development of effective and non-toxic inhibitors of DNA methyl transferase (DNMT) not only for therapy but also for chemoprevention. In this regard, some chemical components of edible fruits and vegetables are promising

chemotherapeutic agents that have been widely used in an attempt to reverse abnormal DNA hypermethylation patterns in cancer, and restore the expression of silenced genes.

Epigenetic modulation of gene expression by dietary isothiocyanates

Despite the fact that most work on the cellular effects of ITC relates to their influence on detoxifying enzymes, recent data support the effect of these dietary compounds on the reactivation of epigenetically silenced genes in cancer cells, particularly through the inhibition of HDAC activity⁽⁵²⁾. The first *in vitro* study reporting these changes was conducted using SFN on prostate and colon cancer cell lines, where the inhibition of HDAC activity was accompanied by global increases in histone H3 and H4 acetylation on the promoter regions of *p21* and *bax* genes, facilitating cell-cycle arrest and apoptosis in the context of cancer chemoprevention⁽⁵³⁾. Later, the same group confirmed HDAC inhibition by SFN *in vivo*, using the adenomatous polyposis coli multiple intestinal neoplasia mouse model that had ingested 443 mg SFN/kg (about 6 µmol SFN/d) for 70 d, and observed the re-expression of *p21* and *bax* genes that triggered cell-cycle arrest and apoptosis in transformed cells and microadenomas, thereby suppressing polyp formation compared with controls⁽⁵⁴⁾. Although the growing interest in the epigenetic regulation mediated by ITC has focused mainly on its HDAC inhibitory activity, their potential chemopreventive mechanisms involving DNA methylation mechanisms remain relatively unknown.

Studies carried out in a human myeloma cell line have found that phenylhexyl ITC induced histone H3 hyperacetylation and demethylation of the aberrantly methylated p16 promoter in a concentration-dependent manner, suggesting that phenylhexyl ITC has dual epigenetic modulating effects on both DNA methylation and chromatin⁽⁵⁵⁾. Comparable results were obtained in prostate cancer cell lines, where phenethyl ITC inhibited the activity and level of histone deacetylases and promoted glutathione S-transferase p1 promoter demethylation (dual action)⁽⁵⁶⁾.

More recently, the involvement of ITC on DNMT has been confirmed in a study in which SFN was found to inhibit DNMT in breast cancer cells. Meeran *et al.* showed that SFN treatment dose- and time-dependently inhibited human telomerase reverse transcriptase, the catalytic regulatory subunit of telomerase, in both MCF-7 and MDA-MB-231 human breast cancer cells and that it had insignificant effects on normal control cells. Furthermore, DNMT protein expression (particularly DNMT1 and DNMT3A), was also reduced in SFN-treated breast cancer cells. Additionally, site-specific CpG demethylation was observed primarily in the first exon of the *TERT* gene. This facilitated binding of CCCTC-binding factor, which is associated with *TERT* repression, leading to cellular apoptosis of the breast cancer cells⁽⁵⁷⁾.

Role of selenium in DNA methylation changes

Numerous studies have reported an effect of ITC on the methylation status of genes involved in the cancer process

in different cell lines and animal models. While many of these studies have focused particularly on SFN others have also focused on Se. This mineral, apart from being an important player in Se-anti-carcinogenesis by way of its intermediary Se-metabolites, or as an essential component of antioxidant enzymes that are active in the removal of reactive oxygen and nitrogen species, has been found to affect DNA methylation by interfering with DNMT activity⁽⁵⁸⁾. However, previous animal and *in vitro* studies investigating the impact of Se on DNA methylation have been inconsistent. For instance, rats fed Se-deficient diets had global DNA hypomethylation in the colon and liver^(59,60). In contrast, *in vitro* studies have shown that exposure of human carcinoma HCT116 cells to phenylenebis-(methylene)-selenocyanate (another form of Se), sodium selenite and benzyl selenocyanate for 24 h caused DNMT inhibition⁽⁶¹⁾. Also, reports from other investigators have indicated that dietary Se can inhibit DNMT1 activity *in vitro* from rat liver and Friend erythroleukaemic cells^(62,63). Furthermore, treatment of LNCaP prostate cancer cells with this trace mineral was found to cause an induction of *GSTP1*, *APC* and *CSRI* promoter demethylation (through a decrease in the protein levels of DNMT1), in addition to a reduction in HDAC activity, leading to increased acetylation together with decreased methylation levels of lysine 9 on histone H3, which promoted gene expression⁽⁶⁴⁾.

In order to clarify discrepancies between *in vitro* and *in vivo* studies reported previously Pilsner *et al.* studied the association between plasma Se concentration and genomic methylation of leucocyte DNA in a cross-sectional study of 287 Bangladesh adults to test the hypothesis that Se deficiency is linked with genomic DNA hypomethylation. The study results were consistent with earlier *in vitro* findings (i.e. Se is inversely associated with genomic DNA methylation), suggesting that high levels of Se are associated with a decrease in DNMT expression and/or activity⁽⁶⁵⁾.

However, the efficacy of different forms of Se, particularly SeMSC, in relation to cancer prevention through DNA methylation mechanisms has not been investigated in sufficient depth. To further expand this knowledge, we examined the effect of SeMSC or selenite (ranging from 0.2 to 5 µM) either individually or in combination with the ITC SFN or iberin (ranging from 6 to 8 µM) for up to 12 d on factors modulating gene-specific (*p16^{INK4A}*, *ESR1*), global (long interspersed nuclear element-1) methylation (a surrogate marker of genome-wide methylation), and DNMT expression, in two colorectal cancer cell lines namely Caco-2 and HCT116. However, none of the compounds assessed influenced the methylation status of the genes studied, nor did we find changes in the 5-methylcytosine content of the genome⁽⁴⁰⁾.

Deregulation of methylation patterns is a common characteristic in tumour cells observed in almost all types of cancer. Several previous studies suggest that diet-derived factors offer potential for the prevention and therapy of a wide variety of cancers by altering various epigenetic modifications. However, the literature and our results suggest that, while some tissues may respond effectively to particular food compounds by impacting on levels of gene-specific methylation, others do not respond.

Also, even within the same gene, exon-specific DNA methylation patterns have been found to be affected differently by dietary compounds. For instance, folate has been found to modify the level of methylation within exons 6 and 7 of the colonic *p53* but not that of exon 8⁽⁶⁶⁾. Although for some genes it has been suggested that methylation occurs in an 'all-or-none' manner⁽⁶⁷⁾, it is also apparent that particular cytosines within a CpG island can have a distinctly greater likelihood of being methylated^(68,69) than others. Thus, despite significant progress in understanding cancer chemoprevention through dietary agents, much remains to be elucidated about the effects of ITC and Se on epigenetic alterations and antioxidant enzyme expression at different stages of colorectal carcinogenesis, and about their importance as mechanisms by which diets may modulate gene expression and attenuate (or in some cases exacerbate?) cancer progression.

Conclusion

We have provided insights into potential mechanisms of cancer chemoprevention by ITC and Se, both individually and synergistically, through the regulation of key selenoproteins responsible for the removal of damaging reactive molecules, which are implicated in the progression and development of cancer. However, future studies should address both the potential cancer prevention activity of antioxidant enzymes such as TrxR1 and GPx2, in addition to their contrasting role in the promotion of cancer. Results from these studies will undoubtedly help in defining the optimal intakes of Se and cruciferous vegetables to prevent cancer development in the future. In addition, the precise role of these food constituents on aberrant epigenetic modifications that are implicated in tumorigenesis needs to be further addressed, particularly in light of the fact that these dietary components appear to modulate distinct regions of the epigenome. Moreover, further investigation concerning the influence of ITC and Se on factors affecting the complete epigenetic setting of the transformed cell, including DNA methylation, chromatin remodelling factors, histone modifications and CpG-binding proteins seems warranted in order to decipher their impact on DNA methylation patterns in cancer development.

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