REVIEW

The potential role of nutritional genomics tools in validating high health foods for cancer control: Broccoli as example

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Nutritional genomics reflects gene/nutrient interactions, utilising high-throughput genomic tools in nutrition research. The field also considers the contribution of individual genotypes to wellness and the risk of chronic disease (nutrigenetics), and how such genetic predisposition may be modified by appropriate diets. For example, high consumption of brassicaceous vegetables, including broccoli, has regularly associated with low cancer risk. Bioactive chemicals in broccoli include glucosinolates, plant pigments including kaempferol, quercetin, lutein and carotenoids, various vitamins, minerals and amino acids. Cancer prevention is hypothesised to act through various mechanisms including modulation of xenobiotic metabolising enzymes, NF-E2 p45-related factor-2 (Nrf2)-mediated stress-response mechanisms, and protection against genomic instability. Broccoli and broccoli extracts also regulate the progression of cancer through anti-inflammatory effects, effects on signal transduction, epigenetic effects and modulation of the colonic microflora. Human intervention studies with broccoli and related foods, using standard biomarker methodologies, reveal part of a complex picture. Nutrigenomic approaches, especially transcriptomics, enable simultaneous study of various signalling pathways and networks. Phenotypic, genetic and/or metabolic stratification may identify individuals most likely to respond positively to foods or diets. Jointly, these technologies can provide proof of human efficacy, and may be essential to ensure effective market transfer and uptake of broccoli and related foods.

Keywords:
Broccoli / Nutrigenetics / Nutrigenomics / Sulforaphane / Transcriptomics

1 Introduction

'At its core, Systems Biology focuses on the integrated interplay among the thousands of molecules within- and between-cells that underlie health and disease. In the genetic network that co-ordinates these processes, genes turn one another on and off in complex ways. When this process goes awry, cancer can result' [1].

How can we keep something healthy that is so complex that it cannot be completely understood with the tools of reductionist science? Can we really identify foods or food ingredients that reduce cancer risk or retard its progression? Is it possible to validate the health benefits of these, utilising the new tools that have become available? And if so, could we then market them with this message, in a way that the public will understand its significance?

It is becoming apparent that a wide array of foods, food extracts and nutrients act on the expression of the human genome. By altering the expression of specific genes, they are able to influence the risk and progression of various chronic diseases. In the specific example of cancer, there is strong evidence for various dietary

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Abbreviations: ARE, antioxidant-response elements; **CYP,** cytochrome P450; **DNA,** deoxyribonucleic acid; **GST,** glutathione *S*-transferase; **GSTM1,** glutathione *S*-transferase mu 1; **HDAC,** histone deacetylase; **ITC,** isothiocyanate; **I3C,** indole-3-carbinol; **Keap1,** Kelch-like ECH-associated protein 1; **Nrf2,** NF-E2 p45-related factor-2; **NF-κB,** nuclear factor-κ B; **PBL,** peripheral blood lymphocyte; **PhIP,** 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine; **Se,** selenium; **SF,** sulforaphane (R-1-isothiocyanate-4-methylsulfinylbutane); **SNP,** single nucleotide polymorphism; **VEGF,** vascular endothelial growth factor

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influences on cancer susceptibility, cancer progression, invasion and metastasis. Additionally, there is increasing recognition of the potential of a new field of nutrition research, 'nutritional genomics' [2, 3], that provides the tools to help understand how diseases such as cancer can be induced, up- or down-regulated, by modulating the expression of specific genes with dietary components. Nutritional genomics can be divided into two disciplines. Nutrigenomics studies the effect of nutrients on health through altering the genome, proteome or metabolome, and the resulting changes in physiology. The complementary field of nutrigenetics studies how genetic polymorphisms affect the individual response to nutrients [3]. Jointly, these approaches are highly relevant to the paradigm of cancer prevention and inhibition of cancer progression.

2 Identifying foods that prevent cancer risk – The example of brassicaceous vegetables such as broccoli

Observational studies done on populations with clear food preferences give a statistical base for the absence or prevalence of certain diseases in the presence of differing levels of certain foods. A case–control study examined the diets of 256 white male patients with cancer of the colon and of 330 white male patients with cancer of the rectum, in comparison with 1405 age- and sex-matched healthy controls [4]. Although other current hypothesis of cancer causation was not supported by their data analysis, they found a statistically significant decrease in risk associated with frequent ingestion of certain vegetables, especially brassicaceous vegetables including cabbage, Brussels sprouts, cauliflower and broccoli (all included in the genus and species *Brassica oleracea*). These data were consistent with the results from animal models, and were also confirmed in a validation study utilising female rather than male human subjects. They have been subsequently validated in more than 250 studies from various countries including the USA, China and parts of Europe [5–9]. Much of this study is critically summarised in recent reviews [10–15].

Brassica vegetables are from the plant order Brassicales, which used to be known as the cruciferous family, cruciferaceae [16]. The studies identified above associate high brassicaceous vegetable intake with a reduction in cancer risk at several sites, including the lung, breast, colon, and rectum and prostate. Some of these studies have specifically identified broccoli as most strongly associating with the reduced cancer risk. In many cases, these kinds of studies led to further mechanistic investigations that resulted in elucidation of the bioactive chemicals and also the molecular mechanisms by which the foods can provide protection against cancer development and disease progression.

3 Bioactive chemicals in broccoli

There is a considerable body of evidence that secondary plant products, and especially glucosinolates, are major active anticarcinogenic materials in cruciferous vegetables [15]. Among all, some of the most important products are shown in Figs. 1 and 2. The glucosinolate molecule is composed of a glycone moiety, common to all members of this group, and a variable aglycone side chain derived from amino acids. Many of these glucosinolates are hydrolysed to produce isothiocyanates (ITCs), with characteristic side chains. Sulforaphane (SF: R-1-isothiocyanate-4-methylsulfinylbutane) is the most studied of the ITCs obtained through the consumption of broccoli (*B. oleracea* var *italica*). It is formed only after plant cell-wall breakdown through the action of myrosinase (Fig. 1). Light cooking leads to the efficient myrosinase release from the cytoplasm, resulting in almost 100% conversion of glycosinolates to SF. Further cooking denatures myrosinase, and intact glucosinolates are ingested. These can, however, be converted to SF in the colon by microbial thioglucosidase activity [15].

There are a wide variety of glucosinolates, not all of which are hydrolysed to produce ITCs [16]. Some glucosinolates produce indoles, thiocyanates and other products. Indole-3-carbinol (I3C) is produced by the breakdown of the glucosinolate glucobrassicin, which can be found at relatively high levels in brassicaceous vegetables. It is also important to recognise that not all glucoinolates are beneficial to health. Indeed, one has been recently reported to become a potent mutagen upon activation by myrosinase [17].

There are at least two main flavonol glycosides in broccoli – quercetin 3-*O*-sophoroside and kaempferol 3-*O*-sophoroside [18] (Fig. 2). Vallejo et al. used HPLC to analyse the phenolic content of several broccoli cultivars [19]. In all of these, kaempferol 3-*O*-sophoroside represented up to 90% of the total flavonoid content.

Other phenolics are also found in broccoli, including flavonoids (as rutin), caffeic acid derivatives (as chlorogenic acid) and sinapic acid derivatives (as sinapic acid), and these vary in concentration across different cultivars. Broccoli also contains good quantities of antioxidant vitamins including

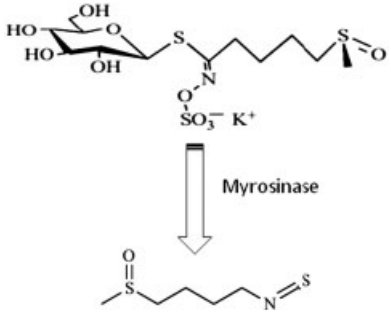

Figure 1. Glucosinolates as bioactive components of broccoli. On the breakdown of cell walls through cooking or chewing, the active sulforophane component is released from glucoraphanin through the action of myrosinase.

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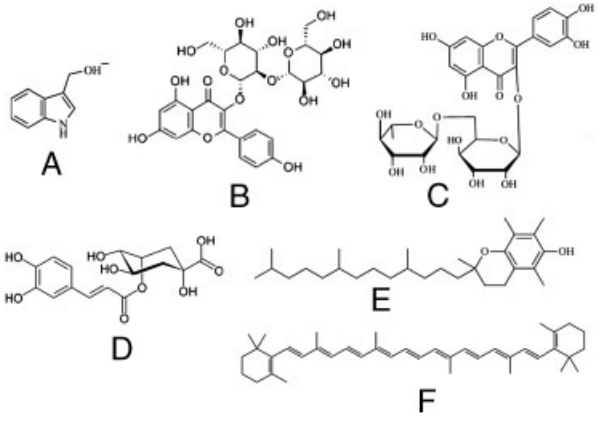

Figure 2. Examples of other bioactive compounds in broccoli. Structure diagrams of (A) I3C, (B) kaempferol 3-*O*-sophoroside, (C) rutin, (D) chlorogenic acid, (E) α-tocopherol, (F) β-carotene.

tocopherols and carotenoids (lutein and zeaxanthin) [20, 21]. In general, brassicaceous seeds and sprouts are good sources of Vitamins C, E, K, B1 and B2 and folate [12]. There has been considerable variation in the levels of the different vitamins in different studies. This variation appears due to various factors such as cultivar, environmental stress, growth conditions, storage and food processing [12, 22–24].

A high bioaccumulation capacity of broccoli leads to a high micronutrient content. Thus, broccoli is a good vegetable source of major mineral elements such as sodium, potassium, calcium, magnesium, chlorine, potassium and sulphur, and trace elements such as iron, zinc, copper, manganese and selenium (Se). Indeed, broccoli has the ability to accumulate high levels of Se with the majority of the seleno amino acids in the form of Se-methylselenocysteine, and this may also be an important factor in chemoprevention [25–27]. Health benefits of broccoli may also be enhanced due to the presence of proteins containing the essential amino acids isoleucine, leucine, lysine phenylalanine, tryptophan, methionine, valine and threonine, as well as beneficial fatty acids [28].

4 Mechanisms by which broccoli may prevent cancer

Some of the postulated mechanisms are shown in Fig. 3. More comprehensive reviews on various aspects of the topic are provided elsewhere [12, 29–32].

4.1 Antioxidant and detoxification mechanisms

Early mechanistic studies showed that high intakes of brassicaceous plants may have antioxidant effects. These effects of broccoli components may be partly related to their direct ability to scavenge reactive oxygen species (ROS) [33, 34]. Some of the broccoli components at high levels, such as vitamin C, are recognised for this property.

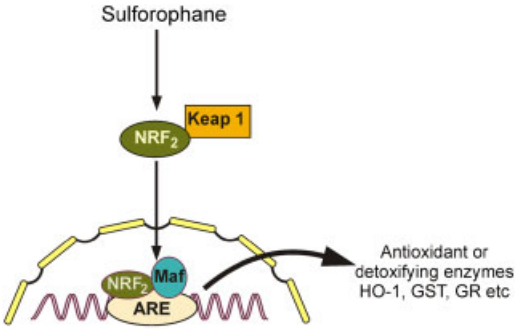

Figure 3. Antioxidant and detoxifying effects of sulforophane (SF), SF activates phase II enzymes through the transcription factor, NF-E2 p45-related factor-2 (Nrf2). In the cytoplasm, Nrf2 is inactivated because of an association with Kelch-like ECH-associated protein 1 (Keap 1). SF is thought to modify the sensor cysteines in Keap 1, resulting in the release of free Nrf2, which is then translocated to the nucleus. There, it becomes a heterodimer in association with the v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian) protein. This heterodimer then binds to the ARE present in the promoter or enhancer region of many genes encoding antioxidant and/or detoxifying enzymes, including hemoxygenase-1 (HO-1), various GST and glutathione reductase (GR). These, in turn, are responsible for multiple detoxification processes [137].

Cells can also survive chronic oxidative stress by enhancing activities of antioxidant enzymes, thereby protecting cells from deoxyribonucleic acid (DNA) damage. In common with many other ITCs, SF raises tissue glutathione (GSH) levels by stimulating the antioxidant-response elements (ARE) in the 5′-upstream region of the gene for the heavy sub-unit of g-glutamylcysteine synthetase [35–37]. This enzyme catalyses the rate-limiting step in glutathione synthesis. The genes encoding NF-E2 p45-related factor-2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1) play an important role in the induction of antioxidant enzymes against oxidative stress. Nrf2 is a major transcription factor involved in regulating antioxidant and pro-inflammatory genes (Fig. 3). It occurs in the cytoplasm in an inactive state, because of the association with Keap1. SF provides an exemplar of activation of Nrf2, possibly by modifying the sensor cysteines present in Keap1. This enables its nuclear translocation, where it interacts with a small v-maf musculoaponeurotic fibrosarcoma oncogene homolog (Maf) protein. The heterdimer then binds to the ARE, present in the promotor or enhancer region of many genes encoding antioxidant and detoxifying enzymes, leading to the induction of antioxidant or phase 2 detoxification enzymes [11]. Thereby, SF leads to the up-regulation of cellular defence capacity against oxidative or electrophilic insults.

4.2 Effects on metabolic activation

Both dietary and environmental carcinogens are typically subjected to metabolism once they enter the human body. This physiological process is primarily catalysed by the cytochrome

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P450 (CYP) enzymes. Thereby, pro-carcinogens are converted into highly reactive intermediates that can bind to critical macromolecules such as DNA. A large body of data is available, which demonstrates that either broccoli itself, or bioactives isolated from broccoli, may inhibit DNA-adduct formation and chemical carcinogenesis through alteration of the level of certain CYP isoforms [35, 38, 39]. At least in rodents, this occurs via a competitive mechanism, as well as by direct covalent modification. An example of one such study follows.

The effect of dietary broccoli on the levels of P450IA and IIB mRNA and proteins was studied in Sprague–Dawley rats fed a 10% broccoli diet for 7 days [40]. The expression of the CYP forms was altered in both the liver and the colon. Total P450IA mRNA in the liver was increased, together with the P450IA1 and IA2 proteins. Colonic P450IA1 mRNA and protein were induced although colonic P450IA2 protein and mRNA were unaffected by the broccoli diet. Liver P450IIB and IIE1 proteins, but not P450IIB mRNAs, were increased. The authors suggested that a feedback mechanism caused a decrease of P450IIB mRNA levels. They further postulated that at least part of the protective effect of the broccoli diet on chemically induced tumours in rodents may be caused by changes in P450IA- and IIB-associated enzyme activities.

5 Mechanisms by which broccoli may retard cancer growth and progression

Recent reviews [41, 42] identify the hallmarks of cancer and cancer metastases, and distinguish pathways that are currently being targeted by anti-cancer drug developers. Epigenetic targets are also of increasing interest and importance [43]. It is of some interest that many of these pathways are demonstrably affected by brassicaceous vegetable or broccoli consumption, or administration of some of the identified bioactives (Fig. 4). This may suggest that a key role of increasing ingestion of vegetables such as broccoli may be to target multiple pathways in cancer progression.

5.1 Anti-inflammatory effects

New dimensions have been opened with the discoveries of anti-inflammatory effects of bioactive compounds in broccoli and related vegetables [13, 29, 44, 45]. An acute inflammatory response is the expected reaction to environmental insults such as wounding, chemical exposures or pathogens. Where this inflammation is not resolved and becomes chronic, the probability of developing chronic diseases such as cancer is greatly increased [46]. Figure 5 shows the points in cancer initiation and progression where inflammatory responses play key roles. Inflammation also affects immune surveillance and responses to therapy [47, 48].

The ability to modulate cellular signalling pathways involved in chronic inflammation is an important mechanism not only of cancer prevention, but also in suppression or slowing of cancer growth. Elements of the cell-signalling network, especially those which act through the eukaryotic redox-sensitive transcription factor, nuclear factor-κ B (NF-κB), have been implicated in many inflammation-associated disorders. Under normal physiologic conditions, NF-κB is sequestered in the cytoplasm by binding to an inhibitory protein, nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α (IκBα) which may be degraded by proteasomes through a series of events [45]. NF-κB may itself be a direct target of anti-inflammatory chemicals such as SF [45, 49].

5.2 Enhancement of apoptosis and related events

Apoptosis, or programmed cell death, is a process that eliminates damaged cells, or cells no longer necessary to the organism, and hence they cannot continue to establish a damaged genetic line. The process plays an important role in the development and maintenance of homeostasis, and is a recognised mechanism of anti-cancer drug action [42]. One of the differences between normal cells and cancer cells is that normal cells are sensitive to, but cancer cells resistant to, apoptosis. This means that DNA damage in cancer cells may not be adequately recognised, and where DNA repair processes fail, the damaged cells may not be eliminated. This is suggested to be one of the reasons for the increase in genomic instability associated with the progression of cancer [42]. Thus, bioactive compounds which stimulate apoptosis in cancer cells, such as SF, may slow the progression of cancer.

Several interacting signalling pathways, including ERK, JNK and MAPK signalling, may be involved in the apoptosis induced by SF and related glucosinolates, as well as flavonoids such as kaempferol [50–53]. These pathways sense extracellular stresses, leading not only to the induction of Nrf2-mediated transcription (described in Fig. 3), but also to the transmission of apoptotic and cell-cycle arrest signals [31, 54, 55]. Cell-cycle arrest by such compounds has been suggested to occur through various mechanisms, including down-regulating the expression of the cyclin D1 protein, reducing levels of kinase cdk4, regulation of CDK inhibitors and disruption of microtubules by inhibition of tubulin polymerisation [11, 56].

In C57BL/6 mice, SF inhibited B16F-10 melanoma-cell-induced metastasis [57]. It has been suggested that this property of SF acts through effects on angiogenesis, an essential step in the growth of solid tumours and metastasis [42]. More generally, the administration of broccoli bioactives, especially SF, I3C, quercetin and kaempferol, leads to inhibition of angiogenesis in human endothelial cell models [36, 58–60]. These effects have been proposed to act through vascular endothelial growth factor (VEGF), a pro-angiogenic

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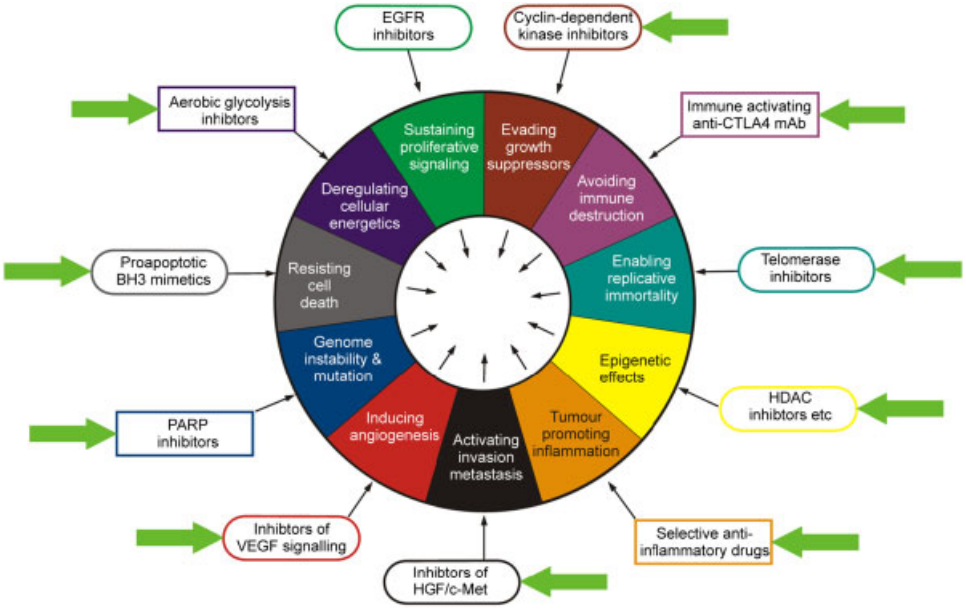

Figure 4. An illustration of points in cancer progression and metastases, showing where broccoli, broccoli extracts and various bioactives could interact (modified from Hanahan and Weinberg [42]). The central diagram shows the range of mechanisms implicated in cancer progression, whereas the first outer circle identifies pharmaceutical approaches to inhibition that have been used or suggested. Arrows indicate where broccoli sprout extract and/or broccoli bioactives are likely to have an effect. Bioactives including kaempferol, SF and I3C have potential effects on aerobic glycolysis via mitochondrial inhibition [138–141]. Broccoli and Brussels sprouts juice, kaempferol, SF and I3C have been shown to be pro-apoptotic [33, 36, 142–147]. Various broccoli flavonoids, including quercetin and kaempferol, enhance genomic stability [74]. VEGF signalling is inhibited by SF, kaempferol and vitamin E [58, 148–150]. Kaempferol has also been reported to reduce hepatocyte growth factor-induced signalling [151]. Anti-inflammatory effects have been attributed to Se, kaempferol and SF [13, 45, 152, 153]. Cyclin-dependent kinase inhibition is a common effect of many of these bioactives, including I3C and SF [36, 59, 154]. Epigenetic effects are being reported for SF [71], folate and various other bioactives.

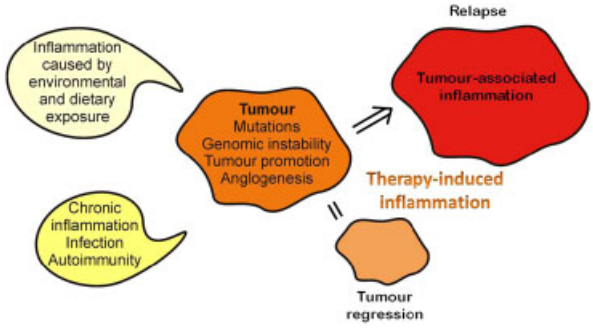

Figure 5. Role of inflammation in cancer initiation and progression. Chronic inflammation may be induced through exogenous factors, including environmental or dietary exposures. It may also be induced endogenously by pathogenic organisms or contaminants. Either mechanism may directly lead to mutation and/or to genomic instability, which may lead to the initiation of tumours and/or enhancement of their progression [46]. Tumour therapy may itself cause inflammation. Additionally, tumour-associated inflammation often occurs [42]. Several of the broccoli bioactives, including SF, I3C and Se, act to reduce inflammation. This may have the beneficial effect of slowing progression of the tumour.

molecule secreted by tumour cells [58, 61, 62]. VEGF acts as a selective mitogenic, chemotactic and morphogenesis-inducing endothelial cytokine.

Animal studies have also suggested the effects on basement membrane integrity, through the suppression of transcription of matrix metalloproteinase-2, and its associated tissue inhibitor [63].

5.3 Epigenetic effects

DNA methylation is an essential component of the epigenetic machinery of mammalian cells. It regulates gene expression and provides a mechanism for sustaining these expression patterns through mitosis. It is thus not surprising that altered DNA methylation is also a central component of the molecular nature of neoplasia, deregulating key pathways in the control of cell growth. Chemicals that interfere with the maintenance of DNA methylation may reactivate silenced genes and reverse part or most of this pathway deregulation, leading to a number of desirable characteristics, including differentiation, apoptosis and enhanced recognition by the immune system. DNA

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methylation inhibitors are used clinically as part of the standard care in certain forms of leukaemias [43, 64].

Natural food components may be as effective as pharmaceuticals in regulating DNA methylation [65]. For example, vitamins involved in one carbon metabolism are methyl donors, whose levels will regulate the levels of methyl groups available for maintenance methylation. In this respect, it is of interest that one cup of broccoli contains 25% of the daily requirements for folate, as well as significant amounts of B vitamins.

An important mechanism of gene regulation is the reversible acetylation of histones. The addition of acetyl groups to histones promotes gene expression by creating an 'open' chromatin conformation and acting as a docking site for the transcription machinery, thereby enabling access to DNA and the activation of transcription. In contrast, removal of acetyl groups by histone deacetylases (HDACs) leads to a 'closed' DNA conformation that effectively prevents transcription. Additionally, the acetylation of lysine residues on non-histone proteins such as α-tubulin and HSP90 by certain types of HDACs plays a major role in many different cellular processes. For example, changes in acetylation status of α-tubulin can lead to alterations in microtubule dynamics and stability, cell migration and autophagy [66, 67]. All of these processes are critically important in regulating cell-cycle arrest, mis-folded protein toxicity and cell death.

Specific modifications in acetylation patterns on histones are apparent during the progression of prostate and other cancers. Targeting the epigenome, including the use of HDAC inhibitors, is an important strategy for cancer treatment [43]. Ho and co-workers [68] found that SF inhibited HDAC activity in human colorectal and prostate cancer cells. HDAC inhibition by SF led to enhanced histone acetylation, de-repression of P21 and Bax, and induction of cell-cycle arrest/apoptosis [69, 70]. Importantly, Clarke et al. [71] showed that this epigenetic effect of SF selectively targeted benign hyperplasia cells and prostate cancer cells, whereas not affecting normal prostate cells. SF selectively induces cell-cycle arrest and apoptosis, highlighting the use of SF-containing foods as safe and relatively non-toxic, with considerable potential for regulating cancer cell growth.

5.4 Inhibition of genomic instability

Several of the recognised broccoli bioactive components show various properties that slow the development of, or prevent, genomic instability. Recent reviews consider folate and B vitamins [72] and also Se [73] as key regulators of this process. Various mechanisms are advanced, including epigenetic effects and actions on telomerase activity and mitochondria. Several of the polyphenols present in this class of vegetables also act to stabilise DNA [74].

5.5 Hormonal effects relevant to breast and prostate carcinogenesis

The broccoli component I3C is suggested to show protection against breast and prostate carcinogenesis, that is, hormonally regulated cancers. Wu and co-workers [75] suggest that effects on the breast may involve induction of cell division cycle 25A (Cdc25A) phosphatase degradation, leading to cell-cycle arrest. This phosphatase is over-expressed in a variety of human cancers and other diseases. These authors not only performed in vitro, but also in vivo studies of I3C inhibition of breast carcinogenesis in a mouse xenograft model. Souli et al. [76] investigated the effect of I3C in prostate cancer (PC) cell lines, and on prostate cancer tumour cells inoculated subcutaneously in C57BL/6 mice. I3C, injected intraperitoneally, significantly inhibited tumour growth and affected angiogenesis by decreasing microvessel density and complexity. Both I3C and its active metabolite appear to target multiple aspects of cancer cell-cycle regulation and survival, including Akt-NF-κB signalling, caspase activation, cyclin-dependent kinase activities, estrogen metabolism, estrogen receptor signalling, endoplasmic reticulum stress and BRCA gene expression [77].

5.6 Modulation of the gut microbiota

SF has the ability to inhibit the growth of both Gram-positive and Gram-negative bacteria such as *Escherichia coli* 0157:H7, *Salmonella* and *Shigella*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Cryptococcus neoformans* [52]. SF also acts as a potent bacteriostatic agent against 3 reference strains and 45 clinical isolates of *Helicobacter pylori*, a bacterial pathogen implicated in gastric cancers [79].

6 Proving efficacy of broccoli in cancer prevention

Association studies and animal models provide strong reason to believe that broccoli and related vegetables will prevent human cancer. However, well-designed human dietary intervention trials are necessary to prove such an effect. A conventionally designed dietary intervention trial with a manageable number of patients, i.e. $n < 100$, will, in most cases, fail to show statistically significant prevention of disease, as even the best effort of stratification may leave a high variability within the test group. The needed duration of the intervention to obtain a statistically meaningful outcome in such a small number of subjects is too long. Compared with a prescription drug, there will be a high level of remaining uncertainty for the regulator as to the fact that the intake of the product at question will indeed prevent the disease for the individual consumer. If n needs to be much greater than 100 (n>>100) and the intervention needs

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to be for much longer than 1 year, then the trial is effectively not fundable for private enterprise, or the expected returns will not justify the expenditure.

This is particularly true if there is more than one bioactive molecule contained in the food or health product at question. In many natural foods such as broccoli, there is indeed more than one bioactive, and the concentration of the main bioactive is highly variable between plant cultivars, in different climates, different growing conditions and different storage conditions [12, 80]. Furthermore, the release of the bioactive is dependent on the activation mechanism, and that activation mechanism is very dependent on food preparation and processing. All of the above must currently be seen as severe hurdles for any ambitions to affix a preventative health claim to any broccoli floret found in the supermarkets.

There are a limited number of examples of adequately powered and designed human dietary intervention studies that seek to prove cancer prevention. Three sets of studies that have been well resourced and well publicised are highly relevant to broccoli since they utilised broccoli bioactives. In each case, there were strong arguments in trial design that it was essential to utilise the purified compound, rather than a natural source with high levels. The α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study supplemented 29 133 eligible male cigarette smokers aged 50–69 years, randomly assigned to receive β-carotene (20 mg), α-tocopherol (50 mg), β-carotene and α-tocopherol, or placebo daily for 5–8 years [81]. The β-Carotene and Retinol Efficacy Trial (CARET) randomised 18 314 participants at high risk for lung cancer, because of a history of smoking or asbestos exposure, to a daily combination of 30 mg of β-carotene and 25 000 IU of retinyl palmitate, a similar randomised study design [82]. The results of both of these trials showed the reverse of the expected protective benefit, and were stopped prematurely. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) for prostate cancer prevention randomised 35 533 healthy men, >55 years old, to 200 g/day L-selenomethionine and 400 IU/day α-tocopherol, both as supplements, or placebo, for 7–12 years. This trial was also stopped before completion because of suggestive negative results [83].

It is still not clear why these trials failed. However, it is important to emphasise that none of the trial designs is completely relevant to the normal human intake of high health vegetables, such as broccoli. The trials were at doses considerably higher than found in a normal diet, and ignore the effects of the biological matrix [84], as well as potential synergies among different bioactives [51, 85]. It is also possible that the regimen was inappropriate, in being a continuous high dose rather than intermittent nutrient intake, which would more accurately reflect the natural diet situation. Furthermore, different forms of the bioactives may act differently. For example, Finley and Davis [26] showed that Se from high-Se broccoli is utilised differently to selenite, selenate or selenomethionine. Indeed, in contrast to these other isolated Se compounds, high-Se broccoli is highly effective in inhibiting colon carcinogenesis. For all these reasons, a large number of trials were designed to show cancer prevention, or retardation of cancer progression, utilise surrogate biomarkers in preference to than cancer, as an endpoint. Examples of these trials, published since 2000, are summarised in Table 1.

Small trials may be successful in looking at biomarker endpoints relevant to detoxification. For example, a small clinical trial demonstrated that the consumption of a high dose of 250 g/day of broccoli sprouts significantly increased the urinary excretion of a potential carcinogen found in well-done meat, namely 2-amino-1-methyl-6-phenylimidazo [4, 5-*b*] pyridine (PhIP) [103]. We have previously found an association between meat cooked in this manner and prostate cancer risk in New Zealand men [106]. Other studies have combined feeding of three potentially bioactive foods: crucifera, chlorophyllin and yogurt, and showed that the combination was effective in inhibiting well-done meat-induced colorectal DNA damage and systemic genotoxicity in humans [102] (Table 1). Table 1 is based on a Medline search with selection criteria as follows: [cruciferae or Brassica$ or broccoli] and [human intervention or clinical trial] and [genotype or metabo$ or proteomics or transcriptomics].

Other cancer prevention-related endpoints, also summarised in Table 1, include modulation of the activity of xenobiotic metabolising enzymes, changes in pharmacokinetics and bioavailability of nutrients and environmental toxicants, and effects on oxidative stress.

Better insights into genotypic variation may allow a better stratification for patients without genotypic outliers. Several examples of such genetic stratification are summraised in Table 1 [86–90]. A good example is provided by a recent proteomic trial, where interpretation of the protein patterns found was possible only when the results were stratified according to glutathione *S*-transferase mu 1 (GSTM1) genotypes [86]. Steck and Hebert [107] summarised the literature showing an association between genotypic variation, measured as single nucleotide polymorphisms (SNPs), in glutathione *S*-transferase (GST) genes, and excretion of both dietary carcinogens and ITC metabolites after brassicaceous vegetable consumption. They were able to identify several feeding studies in humans that examined the effects of SNPs in GSTs on glucosinolate metabolite excretion, and also show evidence for the modulation of the mutagenicity of PhIP and related carcinogens by such dietary interventions. From the mechanistic discussions above, we would suggest that genes encoding GSTs represent only some of the potential classes of genes in which variations would affect individual response to brassicaceous vegetable consumption. A considerable range of SNPs, in a considerable range of genes, are likely to interact with Brassica consumption. If individuals are to be stratified according to more than one or two genes, then bioinformatic and/or metabolomic and/or proteomic methodologies will become essential [2, 3, 108, 109].

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Table 1. Examples of human dietary intervention studies investigating various Brassica vegetables or extracts produced from them, in relation to recognised biomarkers of cancer

Hypothesis tested or question asked	Nutrient supplement or dietary change tested	Type and duration of intervention	Biomarker endpoint (and justification where appropriate)	Number of subjects, sex and age range	Effect on biomarker frequency	Reference
Nutrigenetics and proteomics						
What is the effect of GSTM1 genotypes on response to fruit and vegetable free, or Brassica-enhanced diets?	A fruit- and vegetable-free basal diet or the basal diet supplemented with Brassica vegetables. These were provided as a mixture of broccoli, cabbage and radish sprouts, at 7 g/kg body weight	Two randomised, crossover, controlled feeding studies. Both involved four diet periods of 14 days, with at least 21 days washout period between	High-throughput proteomic methods to examine how human serum peptides (the 'peptidome') change in response to Brassica vegetable feeding, in individuals of different GSTM1 genotypes	34 healthy men, 33 healthy women, age range 20–40 years, stratified according to GSTM1 genotype	Brassica vegetable intake in GSTM1+ individuals led to changes in circulating levels of several peptides/ proteins, several of which changed distribution according to genotype. These included TTR, a known marker of nutritional status, and a fragment of ZAG, an adipokine that plays a role in lipid mobilization	Brauer et al. [86]
Nutrigenetics						
Does (APAP) conjugation differ by UGT1A6 and UGT2B15 genotype, and can it be modulated by diets containing Brassica (and related) vegetables?	Diets supplemented with high levels of known dietary inducers of UGT (Brassicas, soy, and citrus), with a diet devoid of fruits and vegetables	Two randomised, crossover, controlled feeding studies, as for Brauer et al. (2011) above). Volunteers received 1000 mg of APAP orally on days 7 and 14 of each 2-wk feeding period. Saliva and urine were collected over 12 h	Urinary recovery of the APAP dose as free APAP, or as APAP glucuronide	33 healthy men, 33 healthy women, age range 20–40 years, stratified according to UGT genotype	There were highly significant differences among UGT genotypes, as well as significant male/female differences, in APAP conjugation. Selected fruits and vegetables, known to affect UGT activity, led to greater glucuronidation and less sulfation	Navorro et al. [87]
Is there a difference in SF metabolism in GSTM1-null and GSTM1-positive subjects after they consume standard broccoli and/or high-glucosinolate broccoli (super broccoli)?	Standard broccoli soup (0.7 mmol/L), high glucosinolate broccoli soup (2.3 mmol/L), or water	Randomised, 3-phase crossover dietary trial (acute exposure, 24 h)	LC linked to tandem mass spectrometry used to quantify SF and its thiol conjugates in plasma and urine	7 healthy men, 9 healthy women, age range 18–46 years	GSTM1 genotypes have a significant effect on the metabolism of SF derived from standard or high-glucosinolate broccoli. It is possible that the difference in metabolism may explain the greater protection that GSTM1-positive persons appear to gain from consuming broccoli	Gasper et al. [88]

Table 1. Continued

Hypothesis tested or question asked	Nutrient supplement or dietary change tested	Type and duration of intervention	Biomarker endpoint (and justification where appropriate)	Number of subjects, sex and age range	Effect on biomarker frequency	Reference
What is the effect of GSTM1 genotype on induction of GSTs by a high Brassica diet?	Basal (vegetable free), and basal supplemented with three botanically defined groups of vegetables including Brassica	Randomised crossover design for 6 days	Fasting blood samples, collected on the last 2 days of each feeding period, were analysed for GST-α, serum GST and peripheral-lymphocyte GST-mu activity	21 healthy men and 22 healthy women, nonsmokers, age range 20–40 years	The results demonstrate that GSTM1 genotype has a significant effect on GST responses to diet, and that Brassica vegetables are effective at inducing GST-α and GST-mu. GST induction may improve detoxification and excretion of potentially harmful compounds	Lampe et al. [89]
Transcriptomics and nutrigenetics						
Are there changes in global gene expression patterns in the human prostate gland before, during and after a 12-month dietary intervention with a broccoli-rish diet?	A broccoli-rich as compared with a pea-rich diet	Prospective single-arm pilot dietary intervention study. Six-month controlled diet in high-prostate cancer risk group	Comparison of gene expression profiles from biopsies obtained pre- and post-intervention. RNA from prostate needle biopsies; gene chip arrays from all subjects, qRT-PCR validation. DNA from whole blood, genotyped using RT-PCR	22 men, age range 57–70 years, with a previous diagnosis of high-grade prostatic intraepithelial neoplasia (HGPIN)	Regular consumption of broccoli interacts with GSTM1 genotype to result in complex changes to signalling pathways associated with inflammation and carcinogenesis in the prostate. These changes may be mediated through the chemical interaction of ITCs with signalling peptides in the plasma	Traka et al. [90]
Transcriptomics						
Does a major diet and lifestyle intervention (including increased vegetable intake) modulate prostate gene expression?	Low-fat whole foods plant-based diet, stress management, moderate aerobic exercise, 1 h group support/wk	Prospective single-arm pilot diet and lifestyle intervention study. Extensive diet and lifestyle intervention for 3 months	Comparison of gene expression profiles from biopsies obtained pre- and post-intervention. RNA from prostate needle biopsies; gene chip arrays from all subjects, qRT-PCR validation	30 men, age range 62 ± 15 years, with low-risk prostate cancer	Substantial modulation of gene expression of a number of cancer regulatory pathways. These included protein metabolism and modification, intracellular protein traffic and protein phosphorylation	Ornish et al. [91]

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Table 1. Continued

Hypothesis tested or question asked	Nutrient supplement or dietary change tested	Type and duration of intervention	Biomarker endpoint (and justification where appropriate)	Number of subjects, sex and age range	Effect on biomarker frequency	Reference
Does high glucosinolate or ordinary broccoli have effects on gene expression in the gastric mucosa?	Standard broccoli soup (0.7 mmol/L), high-glucosinolate broccoli soup (2.3 mmol/L), and water	A randomised, 3-phase crossover dietary trial (acute exposure, 24 h)	Comparison of gene expression profiles from gastric mucosal biopsies obtained pre- and post-intervention. RNA from gastric mucosal biopsies, gene chip arrays for four subjects, and in selected genes by real-time RT-PCR in the other individuals	7 healthy men, 9 healthy women, age range 18–46 years	Consumption of high-glucosinolate broccoli resulted in up-regulation of several xenobiotic metabolizing genes, including thioredoxin reductase, aldoketoreductases, and glutamate cysteine ligase modifier subunit, which have been previously reported to be induced in cell and animal models after exposure to SF. Only one such gene was significantly up-regulated after consumption of standard broccoli	Gasper et al. [92]
Proteomics						
Does regular Brussels sprouts consumption up-regulate the expression of specific proteins?	300 g Brussels sprouts per day, added to a normal diet	Prospective single-arm pilot dietary intervention study, over 5 days	PBMC samples were metabolically labelled and proteins compared using 2-DE and LC-MS/MS	3 healthy men, 2 healthy women, age range 33±7 years	Two proteins with potential anti-cancer properties were up-regulated	Hoelzl et al. [93]
Development of methods to ensure compliance with high Brassica diet	A basal diet devoid of fruits and vegetables, or a basal diet supplemented with high levels of Brassica vegetables	Prospective single-arm pilot dietary intervention study, over 7 days	MALDI-TOF spectra were generated from fasting blood samples before and after the dietary intervention	38 healthy men and women, age range 20–40 years, sex not identified	Validated the methods that could classify participants based on diet (basal versus high Brassica) with 76% accuracy	Mitchell et al. [94]
Pharmacokinetics and bioavailability						
Bioavailability and tolerability of SF from broccoli sprout-derived beverages depends on glucoraphanin content	Two broccoli sprout beverages – one glucoraphanin rich (GRR) and the other sulforaphane rich (SFR)	Short-term, cross-over clinical trial in Qidong, China. 5-Day run-in period, 7-day administration of beverages, 5-day washout period, and 7-	Bioavailability of SF from two broccoli sprout beverages, as measured by urinary excretion of SF and its metabolites (in approximately 12-h	50 healthy men, age range 25–65 years	Bioavailability was substantially greater with the SFR (mean = 70%) than with GRR (mean = 5%) beverages. Inter-individual variability in	Egner et al. [95]

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Table 1. Continued

Hypothesis tested or question asked	Nutrient supplement or dietary change tested	Type and duration of intervention	Biomarker endpoint (and justification where appropriate)	Number of subjects, sex and age range	Effect on biomarker frequency	Reference
		day administration of the opposite intervention	collections after dosing)		excretion was considerably lower with SFR than with GRR beverage	
What is the human absorption and plasma disappearance of vitamin K following kale consumption?	400 g serving of ^{13}C-labelled kale with 30 g vegetable oil. The kale provided 156 nmol of vitamin K	Prospective single-arm pilot dietary intervention study (acute exposure, 24 h)	Absorption and plasma disappearance of vitamin K from kale in a human feeding study	4 healthy men, 3 healthy women, age range 46 $\pm$ 14 years	Six of the subjects showed significant amounts of ^{13}C-labelled vitamin K in plasma. After ingestion of the labelled kale, plasma ^{13}C-vitamin K concentration increased rapidly to a peak between 6 and 10 h, and then rapidly decreased	Novotny et al. [96]
What are the plasma pharmacokinetic characteristics of SF?	Single and repeated intakes of raw broccoli	Prospective single-arm pilot dietary intervention study (acute exposure, 24 h)	Pharmacokinetic behaviour or plasma levels of SF, and evidence of accumulation	3 healthy men, 3 healthy women, age range 18–60 years	SF was rapidly absorbed, but with a long terminal elimination phase. Repeated intake of broccoli had no impact on the pharmacokinetic behaviour or plasma levels of SF, and there was no evidence of accumulation	Hanlon et al. [97]
Modulation of activity of xenobiotic metabolising enzymes						
SF may affect the expression of phase II enzymes in the upper airway of human subjects	Study subjects consumed oral SF doses contained in a standardised 200 g BSH	Placebo-controlled dose escalation trial (acute exposure, 24 h)	Expression of GSTM1, GSTP1, NQO1 and HO-1	65 non-smokers $\geq$ 18 years, sex not specified	Increased Phase II enzyme expression in nasal lavage cells occurred in a dose-dependent manner with maximal enzyme induction observed at the highest dose of 200 g broccoli sprouts prepared as BSH. Significant increases were seen in all sentinel Phase II enzymes RNA	Riedl et al. [98]

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Table 1. Continued

Hypothesis tested or question asked	Nutrient supplement or dietary change tested	Type and duration of intervention	Biomarker endpoint (and justification where appropriate)	Number of subjects, sex and age range	Effect on biomarker frequency	Reference
					expression compared with baseline. Phase II enzyme induction was not seen with ingestion of non-SF containing alfalfa sprouts	
What is the effect of supplementing the diet with broccoli on CYP1A2 and CYP2A6 activities?	Standard diet containing broccoli (500 g/d). Enzyme activities were determined after a 100 mg caffeine tablet on the 7th day	Prospective single-arm pilot dietary intervention study for 6 days	Broccoli-induced CYP1A2 activity	5 healthy men, 5 healthy women, non-smokers, 21–45 years of age	This study also demonstrated the effect of gender and broccoli consumption on CYP2A6 activity in Jordanians	Hakooz and Hamdan [99]
What are the effects of defined concentrations of glucosinolates, in altering the disposition of two known carcinogens?	Hot water infusions of 3-day-old broccoli sprouts, containing either 400 or <3 μmol glucoraphanin	Double-blind feeding trial of infusions taken nightly for 2 wk	Effects on the disposition of aflatoxin B1 and phenanthrene	200 healthy men and women, age range 25–65 years, sex not specified	An inverse association was observed for the excretion of dithiocarbamates and aflatoxin B1-DNA adducts ($p = 0.002$; $R = 0.31$) in individuals receiving broccoli sprout glucosinolates	Kensler et al. [100]
Brassica vegetables increase CYP 1A2 activity in humans	Four controlled diets: basal (vegetable free) and basal with three botanically defined vegetable groups including Brassica	Randomised crossover design, over 6 days	Effects on CYP 1A2, NAT2 and xanthine oxidase activity in humans	36 healthy men and women, non-smokers, age range 20–40 years	Mean CYP1A2 activity for 19 men and 17 women with basal, Brassica, and other vegetable diets differed significantly, irrespective of the caffeine metabolite molar ratio used to describe CYP1A2 activity. Brassica vegetables increased activity compared with the basal diets	Lampe et al. [101]
Effects on oxidative stress						
Brussels sprouts consumption may affect the ability to reduce DNA migration induced by the	Meat cooked at either low (100°C) or high (250°C) temperature, alone or in combination with three different	Pilot cross-over dietary intervention study, 1 wk for each study arm	Single-cell gel electrophoresis (COMET) assay of rectal biopsy cells and	4 healthy men, 4 healthy women, non-smokers, age	A decrease of the endogenous formation of oxidised bases was observed and DNA damage caused by	Shaughnessy et al. [102]

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Table 1. Continued

Hypothesis tested or question asked	Nutrient supplement or dietary change tested	Type and duration of intervention	Biomarker endpoint (and justification where appropriate)	Number of subjects, sex and age range	Effect on biomarker frequency	Reference
heterocyclic aromatic amine PhIP	mutagen inhibitors. The mix included 500 g Brassica vegetables, 50% cooked, 50% raw, including green and red cabbage, broccoli, cauliflower and Brussels sprouts		PBLs, before and after the intervention	range 20–40 years	hydrogen peroxide was significantly (39%) lower after the intervention. This was seen in the rectal biopsy cells, but not in the PBLs	
What are the effects of broccoli sprouts on the induction of various biochemical oxidative stress markers?	Fresh broccoli sprouts added to a normal diet (100 g/day)	Prospective single-arm pilot dietary intervention study for 1 wk	Before and after the treatment, natural killer cell activity, plasma amino acids, plasma phosphatidylcholine hydroperoxide, the serum coenzyme Q(10), urinary 8-isoprostane, and urinary 8-OHdG were measured	6 healthy men, 6 healthy women, non-smokers, age range 20–40 years	All subjects showed reduced PCOOH, 8-isoprostane and 8-OHdG, and increased CoQ(10)H(2)/CoQ(10) ratio. Only 1 wk intake of broccoli sprouts improved cholesterol metabolism and decreased oxidative stress markers	Murashima et al. [103]

APAP, acetaminophen; BSH, broccoli sprout homogenate; 8-OHdG, 8-hydroxydeoxyguanosine; GSTP1, glutathione-*S*-transferase P1; HO-1, hemoxygenase-1; NAT2, N-acetyltransferase 2; NQO1, NADPH quinone oxidoreductase; PBMC, peripheral blood mononuclear cell; TTR, transthyretin; ZAG, zinc α2-glycoprotein.

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What needs to be emphasised here is that trials such as these consider quite specific endpoints, most of which have not yet been adequately validated as surrogate biomarkers for cancer prevention although there are good mechanistic rationales for their use. Thus, while they provide evidence that at least some of the bioactive components of the brassicaceous vegetables reach human target tissues, the extent to which the effects demonstrated would play a significant role in human cancer is unclear. We suggest that a more all-inclusive endpoint such as transcriptomics or gene expression profiling, which shows effects on the multiple pathways implicated in human cancer [42], may be significantly more relevant than considering a single biomarker endpoint for cancer and other chronic diseases [110, 111].

7 Proving efficacy of broccoli in retarding cancer progression and metastasis

Tang et al. [112] found that high intake of brassicaceous vegetables modifies bladder cancer survival. They analysed questionnaire data from the Roswell Park Cancer Institute Tumour Registry alongside patient medical records, to examine potential associations between the intake of brassicaceous vegetables (raw versus cooked) and the survival among bladder cancer patients. After adjusting for other prognostic factors, they were able to show a strong and significant inverse association between bladder cancer mortality and broccoli intake, especially raw broccoli intake. They reported no other significant associations for total vegetables, total fruits or other brassicaceous vegetables.

Nutrigenomics comprises transcript-, proteome- and metabolome-profiling techniques, in which responses to diets or individual ingredients are assessed in biological samples. Transcriptomic approaches have illustrated the complexity of mechanisms of chemoprevention by SF in animals. Several Nrf2-dependent genes were shown to be SF inducible by comparing transcriptional profiles from the small intestine of Nrf2(+/+) and Nrf2 knockout female mice treated with SF [113, 114]. These studies identified the effects of SF on a wide range of genes that were classified as xenobiotic-metabolising enzymes, antioxidants, ubiquitin/proteasome systems, stress-response proteins, kinases and phosphatases, immunity proteins, cell adhesion, cell cycle and cell growth, metabolism, transport proteins and transcription factors. This approach confirmed that SF administration not only had potential for cancer prevention, but was also likely to play a role in retarding cancer progression, at least in animal models.

There are three available human studies showing the value of transcriptomic approaches in studying the effects of brassicaceous vegetable consumption in humans. Unfortunately, none of these could be considered to have used non-invasive monitoring technologies. One acute feeding study monitored gastric mucosa, and showed that high glucosinolate but not ordinary broccoli had effects on gene expression in the gastric mucosa [92]. Two other studies were longer term, and considered RNA samples from prostate biopsies in high-risk prostate cancer volunteers, before and after being fed a diet high in brassicaceous vegetables. While both studies provided evidence for modulation of gene expression in various cancer-related pathways and probable reduction of the rate of prostate cancer progression by diet and lifestyle changes, only the study by Traka and co-workers definitively associated the positive result with Brassica vegetable consumption [90].

These authors recruited high-risk prostate cancer volunteers, who were randomly assigned to either a broccoli-rich or a pea-rich diet over 6 months [90]. Comparison of biopsies obtained pre- and post-intervention revealed more changes in gene expression occurred in individuals on a broccoli-rich diet than in those on a pea-rich diet. There were significant differences pre- and post-feeding in gene expression between GSTM1-positive and null individuals on the broccoli-rich diet, associated with changes to the expression of genes in the transforming growth factor β 1 (TGFβ1) and epidermal growth factor (EGF) signalling pathways. The authors interpret the results to suggest that consuming broccoli interacts with GSTM1 genotype, leading to complex changes in signalling pathways associated with inflammation and carcinogenesis in the prostate. The authors consider the results, which provide strong support for observational studies, that diets rich in brassicaceous vegetables may reduce the risk of prostate cancer and other chronic disease.

One of the problems with human intervention studies is ensuring that there has been compliance with the required dietary regime over a significant period of time. It is of interest that proteomic technologies are coming into their own as methods to ensure compliance with high Brassica diet consumption [94].

8 Is it possible to market cancer-preventive foods such as broccoli?

The rationale behind many marketing campaigns is that individuals select foods based on health claims. Unfortunately, this appears to be untrue [115–117]. Primary drivers in food selection are taste, convenience and price [118–121]. Where quantitative research has explored the effect of health benefit information on food selection, it has repeatedly been found that only minor shifts in food purchase and consumption can be achieved, even with quite intensive campaigns [122]. Many healthy individuals, especially among the younger age groups, have neutral or negative attitudes towards functional or enriched food products. Watts and Segal [121] concluded that market failure of many of these products resulted from a reluctance of individuals to consider preventive health where the benefits are delayed. If the individuals have no reason to see immediate health benefits, they will express a

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preference for medical or pharmaceutical interventions (at a considerably later date) in the management of chronic disease.

This situation changes dramatically when people have developed the disease. Thus, further study showing effects of broccoli and related vegetables on cancer growth and progression becomes very important.

9 Taking high bioactive broccoli, or related products, to the marketplace

Cancer progression may be a more realistic target of marketing campaigns of 'high health' broccoli than cancer prevention. The illumination of the protective mechanism plus the statistical finding of the reduced prevalence of disease with elevated intake of protective foods is not sufficient to provide the regulatory assurance for a preventative health claim. Such claims require human intervention studies. In theory at least, these do not need to go as far as disease, and a recognised and validated biomarker may be adequate. Disease reduction claims for foods are regulated under article 14 of the European Food Safety Authority (EFSA) (http://www.efsa.europa.eu/en/ndaclaims/ndaclaims14.htm). It is noted that no guidance is currently provided in respect to potential claims on cancer prevention or progression. However, mechanistic studies are essential to support health claims.

A considerable literature base has shown very significant differences among uptake of high health of functional foods and/or supplements between normal healthy individuals and cancer survivors [123–132]. High cancer risk individuals and/or cancer survivors are very significantly more willing to take up high health foods or herbal supplements which, they believe, could improve their survival prospects.

The ability of SF, for example, to target aberrant acetylation patterns, in addition to a wide range of other effects relevant to cancer initiation, progression and metastases, potentially make it an effective anti-cancer agent [133–136]. If foods containing such bioactive components are fed, for example, to high-risk prostate cancer patients, this opens the possibility to enhance their survival through dietary choices incorporating easily accessible foods into their diets [69, 70].

From the study described above, it becomes apparent that one of the reasons broccoli has such a high reputation, both as a cancer preventive and a cancer-retarding agent, is not just because of one component, but the complex interactions of a range of various nutrients and bioactives, acting in a range of biochemical pathways. This makes chemical analysis of single or even multiple bioactives, on single or even multiple biomarkers, impossible to interpret for market consistency.

While gene expression profiling is now being successfully applied to show the diversity of mechanisms associated with broccoli consumption in mice and to a limited extent in high-risk prostate cancer patients, the important question is whether they are sufficiently mature for human intervention trials in the general population. Prostate cancer and gastric mucosa biopsies are far from being non-invasive sampling techniques. However, the increased sensitivity and accuracy of the most up-to-date transcriptomic methods have been successfully used to show the power of fish-oil supplementation in inducing anti-inflammatory gene expression profiles in human peripheral blood mononuclear cells (PBMCs), in an elderly Dutch population [110, 111]. Theoretically at least, they could be applied to monitoring the effects of high intakes of broccoli and related vegetables.

Wittwer et al. [128] critically evaluated the use of nutrigenomic approaches in human intervention trials, comparing the advantages and limitations of the current methodologies. The authors also emphasised the potential powers of such methods, and drew attention to a growing trend towards systemic approaches in which different technologies are combined and applied to the same sample. This allows physiological changes to be assessed very robustly throughout the various molecular layers of mRNA, protein and metabolite changes. They suggested that nutrigenomics is maturing as a branch of the life sciences, and is gaining significant recognition in the scientific community.

10 Concluding remarks

Broccoli is an excellent example of a vegetable with well-characterised properties in the maintenance of health and prevention of disease. Chemically, it has been shown to contain a whole range of potentially very useful ingredients, e.g. minerals such as sodium, potassium, calcium, magnesium, chlorine, potassium and sulphur, and trace elements such as iron, zinc, copper, manganese and Se; Vitamins C, E, K, B1 and B2 and phenolics, with two main flavonol glycosides as quercetin 3-*O*-sophoroside and kaempferol 3-*O*-sophoroside. It also contains a range of glucosinolates of which the main one can be enzymatically converted to the ITC, SF. Since there is no single bioactive, if broccoli is to be marketed as a health food, this implies that we have to control quality for a variety of parameters.

Among these different nutrients and bioactive compounds, there are a variety of protective mechanisms, including direct antioxidant effects, up-regulation of detoxifying enzymes and also down-regulation of CYPs. These effects are well validated in cancer chemoprevention. In addition, however, the various broccoli components have shown effects on various cell-signalling pathways in cancer, anti-inflammatory effects, effects on gut microbiota, and on epigenetic regulation. Taken together, this suggests that here is no one main way of protecting against cancer; and all pathways jointly contribute to protection against both cancer development and disease progression, with little ability to

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predict the individual contribution of each pathway to the overall protection.

It is unlikely that any further long-term studies will attempt to show cancer protection by individual foods or chemicals. For example, the single compound fortification approach of the α-Tocopherol, β-Carotene Cancer Prevention trial showed a detrimental acceleration of cancer progression, rather than the expected cancer slowing or prevention. The dietary questionnaire studies show quite conclusive evidence for health benefits associated with regular intake of vegetables (for some vegetables), and even more if they are associated with lifestyle changes. However, the evidence is variable with dietary intervention studies [102, 104, 105]. Thus, biomarker studies become increasingly important. We suggest that the most feasible approach, that will enable consideration of effects on multiple pathways at once, is to use transcriptomic profiling, initially in animal studies to validate individual samples of broccoli extracts or high broccoli (or related) diets, followed by gene expression analysis from RNA samples from peripheral blood lymphocytes (PBLs) taken from humans fed defined amounts of well-characterised broccoli, in well-controlled intervention trials.

Although there is need for international coordination, agreement on methods and interpretation of results, the technologies of nutritional genomics have become sufficiently robust to begin convincing the scientific community of their value. The challenge will be in conveying to the consumer that this methodology, albeit complex, has sufficient substance to reassure them that positive results in such trials will ensure that the dietary regime being marketed will positively affect their health.

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