

Phytochemicals and cancer

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Epidemiological studies showing a protective effect of diets rich in fruits and vegetables against cancer have focused attention on the possibility that biologically-active plant secondary metabolites exert anti-carcinogenic activity. This huge group of compounds, now collectively termed 'phytochemicals', provides much of the flavour and colour of edible plants and the beverages derived from them. Many of these compounds also exert anti-carcinogenic effects in animal models of cancer, and much progress has been made in defining their many biological activities at the molecular level. Such mechanisms include the detoxification and enhanced excretion of carcinogens, the suppression of inflammatory processes such as cyclooxygenase-2 expression, inhibition of mitosis and the induction of apoptosis at various stages in the progression and promotion of cancer. However, much of the research on phytochemicals has been conducted *in vitro*, with little regard to the bioavailability and metabolism of the compounds studied. Many phytochemicals present in plant foods are poorly absorbed by human subjects, and this fraction usually undergoes metabolism and rapid excretion. Some compounds that do exert anti-carcinogenic effects at realistic doses may contribute to the putative benefits of plant foods such as berries, brassica vegetables and tea, but further research with human subjects is required to fully confirm and quantify such benefits. Chemoprevention using pharmacological doses of isolated compounds, or the development of 'customised' vegetables, may prove valuable but such strategies require a full risk-benefit analysis based on a thorough understanding of the long-term biological effects of what are often surprisingly active compounds.

Phytochemicals: Anti-carcinogenic activity: Molecular effects: Chemoprevention

A review by the author and colleagues was published in 1994 (Johnson *et al.* 1994) that explored the potential anti-carcinogenic effects of a range of biologically-active secondary plant metabolites commonly present in human diets, and tentatively considered whether such compounds, now commonly referred to collectively as 'phytochemicals', might usefully be regarded as a new class of micronutrients. The interest in these compounds was stimulated partly by the work of pioneers in this field such as Wattenberg (1975*a,b*), who first demonstrated the principles of chemoprevention by plant constituents in animal models, and partly by the wealth of epidemiological evidence then emerging for protective effects of diets rich in fruits and vegetables against a variety of cancers (Block *et al.* 1992). A great deal of new evidence has become available in the last decade, and awareness of the importance of plant foods in the prevention of chronic disease in general has grown apace (Davignus *et al.* 2005; Houston *et al.* 2005; He *et al.* 2006). The aim of the

present paper is to review some important aspects of emerging knowledge about the anti-carcinogenic effects of plant secondary metabolites at the cellular and molecular levels, and to reappraise the overall importance of these compounds and their metabolites in human nutrition. To begin with, however, it will be useful to consider briefly the most recent epidemiological evidence for anti-carcinogenic effects of fruits and vegetables.

Cancer, plant foods and human diets

Much of the earliest evidence for protective effects of fruits and vegetables against cancer was based on case-control studies. In an important review Block and colleagues (Block *et al.* 1992) summarised the available evidence and showed that after controlling for confounding factors, individuals in the lowest population quartile for fruit and vegetable intake experience about twice the risk of cancer compared with those in the highest quartile.

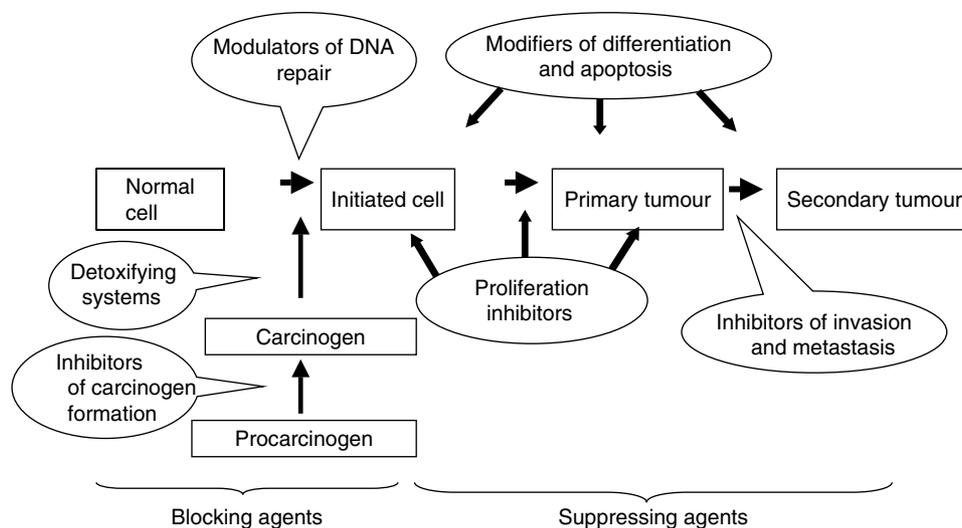


Fig. 1. A general scheme for interactions occurring between blocking agents and suppressing agents and the various stages in the sequence of events associated with the stepwise development of neoplasia. Blocking agents act immediately before or during the initiation of carcinogenesis by chemical carcinogens, and suppressing agents act after initiation, during the prolonged stages of promotion and progression. (Adapted from Johnson *et al.* 1994.)

A few years later Steinmetz & Potter (1996) came to a similar conclusion, and the World Cancer Research Fund (1997) major study, Food, Nutrition and the Prevention of Cancer, found 'convincing' evidence for protective effects of fruits and vegetables against cancers of the upper aerophagic tract, stomach and lung, and of vegetables against cancers of the colon and rectum. Since the turn of the millennium, however, there appears to have been a shift in opinion toward the view that the evidence is less strong than originally thought, mainly because several large prospective studies focusing on the main causes of cancer in Western countries have found either no protective effects (Michels *et al.* 2000; Smith-Warner *et al.* 2001) or weaker effects than those expected on the basis of previous case-control studies (Terry *et al.* 2001; Smith-Warner *et al.* 2003). The recent publication of results on breast and prostate cancer derived from the European Prospective Investigation into Cancer and Nutrition (Key *et al.* 2004; van Gils *et al.* 2005) suggests that this trend is continuing.

The most comprehensive summary of the field currently available is probably that of the International Agency for Research on Cancer (2003), which has concluded that: 'There is *limited evidence* for a cancer-preventive effect of consumption of fruit and of vegetables for cancers of the mouth and pharynx, oesophagus, stomach, colon-rectum, larynx, lung, ovary (vegetables only), bladder (fruit only) and kidney. There is *inadequate evidence* for a cancer-preventive effect of consumption of fruit and vegetables for all other sites'. Nevertheless, although both the perceived size and statistical significance of the anti-carcinogenic effects of diets rich in fruits and vegetables have diminished with the passage of time, interest in the biological effects of their constituents has grown. The emerging evidence for a variety of potentially-important anti-carcinogenic mechanisms has stimulated interest in the broad concept of chemoprevention, focused

attention on particular fruits and vegetables rich in the most active compounds and encouraged a more mechanistic approach to epidemiology. Some of these issues will be discussed after the molecular effects of particular groups of phytochemicals have been reviewed.

Blocking tumour initiation

Studies of the anti-carcinogenic effects of a variety of plants, plant constituents and synthetic chemopreventive agents led Wattenberg (1985) to define food-borne anti-carcinogens generally as either blocking agents, which act immediately before or during the initiation of carcinogenesis by chemical carcinogens, or suppressing agents, which act after initiation during the prolonged stages of promotion and progression. This framework has been valuable for the development of research on the many different types of secondary plant metabolites that show biological activity in mammals (Johnson *et al.* 1994), and it continues to provide a valuable approach to the classification of these compounds and their effects. A scheme for the interactions between blocking agents, suppressing agents and the generalised sequence for the onset of neoplasia is shown in Fig. 1.

Modulation of carcinogen metabolism

Blocking agents prevent genotoxic carcinogens from forming adducts with DNA, either by inhibiting their activation from procarcinogens or by enhancing their detoxification and excretion. These actions are thought to be achieved primarily through the modulation of phase I and phase II metabolic enzymes in the gut, liver and other tissues exposed to the environment. Phase I metabolism involves the oxidation, reduction and hydrolysis of xenobiotics, including drugs, toxins and carcinogens,

principally by the cytochrome P450 enzymes (Nelson *et al.* 1993). The products of phase I metabolism are often highly-reactive genotoxic intermediates that form substrates for phase II enzymes such as glutathione S-transferase (GST), NAD:quinone reductase and γ -glutamylcysteine synthetase, which then catalyse the formation of less-reactive water-soluble conjugates. These conjugates are then readily excreted via the kidneys or in bile. Certain anti-carcinogenic phytochemicals induce transcriptional activation of phase I and II enzymes, via the xenobiotic response element and the antioxidant response element respectively. The so-called monofunctional inducers are those that interact primarily with the antioxidant response element, and hence selectively induce phase II enzymes, without simultaneously inducing activation of carcinogens via increased phase I activity (Prochaska & Talalay, 1988). The mechanism of induction is thought to be triggered by cellular stress, which leads to activation of intracellular protein kinases favouring the translocation of the transcription factor NF-E2 p45-related factor-2 to the nucleus, where it binds to the antioxidant response element and up regulates transcription (Itoh *et al.* 1997). Recent findings have shown that a second transcription factor, the aryl hydrocarbon receptor, interacts directly with the NF-E2 p45-related factor-2 promoter, and that this functional link contributes to the coupling of phase I and II enzymes as an integrated system (Miao *et al.* 2005).

Several groups of phytochemicals have now been identified as potent inducers of phase II enzymes; two of the most actively investigated are the flavanols, including epigallocatechin gallate, which is the principal biologically-active component of green tea (Chou *et al.* 2000), and the glucosinolate breakdown products, derived from cruciferous vegetables and herbs. Glucosinolates, of which >100 have been identified, are water-soluble glycosides containing a common S moiety and a variable side chain. They are found in cruciferous plants, a large and diverse group that contains mustard (*Brassica juncea*), rocket (*Eruca sativa*), radishes (*Raphanus sativus*) and the brassica vegetables (Mithen *et al.* 2000). Intact glucosinolates are biologically inactive and remain sequestered within cells throughout the plant. However, when the raw tissues are mechanically disrupted, the glucosinolates come into contact with the endogenous enzyme myrosinase and undergo hydrolysis, yielding an unstable intermediate that can give rise to a variety of active products (Mithen *et al.* 2000). Depending on the structure of the parent glucosinolate and the ambient conditions, these active products include isothiocyanates (ITC), nitriles, cyanopitthioalkanes and thiocyanates (Lambrix *et al.* 2001). In addition, the indolyl glucosinolates give rise to the indole-3-carbinols, which also induce detoxifying enzymes and act as anti-carcinogens in animal models (McDanell *et al.* 1988). Glucosinolate breakdown products can be formed in food processing, during digestion of uncooked vegetables in the upper gastrointestinal tract (Rouzaud *et al.* 2004) and by exposure to bacterial myrosinase in the colon (Rouzaud *et al.* 2003). Fig. 2 illustrates the release of glucosinolates in the gut lumen, the uptake and metabolism of the isothiocyanates and some possible sites of action and routes of excretion. The ITC sulforaphane, derived

from broccoli (*Brassica oleracea*), acts via the NF-E2 p45-related factor-2 pathway (McWalter *et al.* 2004) and is amongst the most active food-borne monofunctional inducers yet identified (Talalay & Fahey, 2001).

A considerable amount of work has also been done to explore the importance of these effects at the whole organism level by studying carcinogen metabolism in animal models and in human subjects exposed to carcinogens from cigarette smoke. Both indole-3-carbinol and phenethyl ITC have been shown to modify the metabolism of the tobacco smoke carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rodent models and inhibit the development of lung tumours, although their mechanisms of action differ. Indole-3-carbinol induces hepatic α -hydroxylation of NNK, thus reducing the delivery of NNK to the lung (Morse *et al.* 1990), whereas phenethyl ITC inhibits α -hydroxylation, and hence adduct formation in lung tissue (Hecht *et al.* 1996). The shunting of NNK metabolism away from the lung leads to increased hepatic metabolism and higher urinary excretion of NNK metabolites. Increased urinary excretion of two such metabolites, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]- β -D-glucosiduronic acid in human smokers consuming approximately 170 g watercress (*Rorippa nasturtium-aquaticum*)/d for 3 d suggests that a similar mechanism occurs in human subjects (Hecht *et al.* 1995). Hecht and co-workers (Hecht *et al.* 2004) have recently conducted a study of NNK metabolites in Singapore Chinese smokers and have reported an inverse correlation between cruciferous vegetable consumption and excretion of NNK metabolites that appears consistent with the known metabolic effects of indole-3-carbinol. Overall, the experimental and epidemiological evidence supports the conclusion that glucosinolate breakdown products modulate phase II metabolism in man, although the contribution of this mechanism to the putative anti-carcinogenic effects of cruciferous vegetable consumption remains to be fully established.

Suppressing tumour progression

Once the critical genetic damage has occurred, the next stages in the development of neoplasia are the acquisition of further mutations and epigenetic marks that cause progressively abnormal gene expression. This progression is the promotion stage of cancer development, characterised by poorly-regulated cell proliferation and differentiation, and a reduced tendency for damaged cells to undergo apoptosis. Eventually, the cancer phenotype emerges at one or more discrete sites. There are many stages at which this complex process might, in principle, be slowed or interrupted, and a correspondingly large number of plausible mechanisms for tumour suppression. Chemoprevention, the use of drugs or natural compounds at pharmacological levels to inhibit the development of cancer, is currently attracting a great deal of interest, particularly in the USA, but many of the same mechanisms may be triggered by phytochemicals occurring in substantial quantities in human diets.

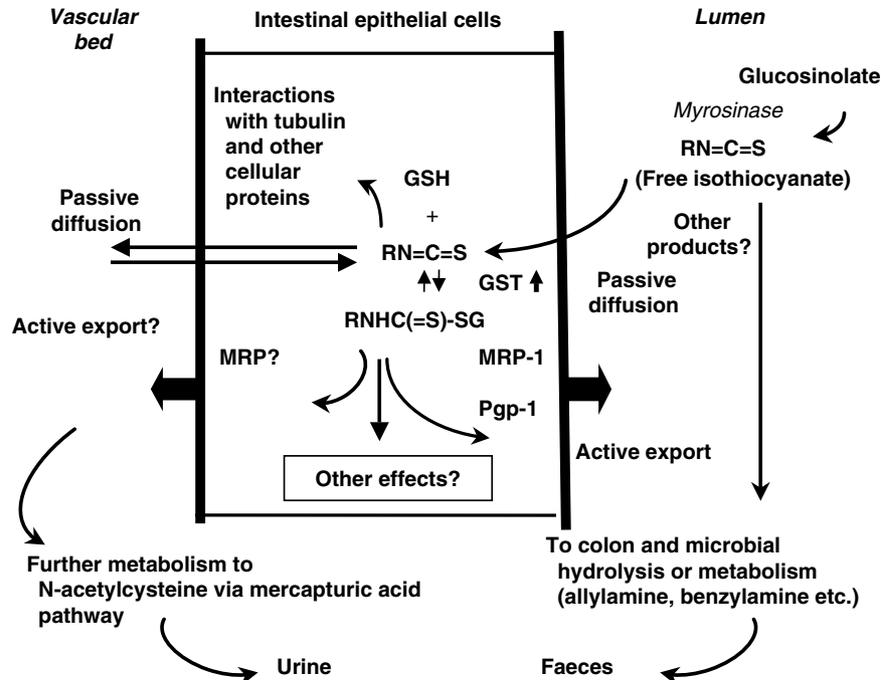


Fig. 2. Glucosinolates are broken down by myrosinase during food processing or during digestion, and isothiocyanates and other breakdown products are released into the lumen. Some remain unabsorbed and are eventually broken down by the gut microflora, but a substantial quantity of free isothiocyanates diffuse passively into the mucosal epithelial cells where they are rapidly conjugated with glutathione (GSH), and then partially secreted back into the gut lumen by P-glycoprotein (Pgp-1) and multi-drug-resistance protein (MRP-1). Some free and conjugated isothiocyanates enter the bloodstream, however, and become available to interact with target tissues before further metabolism and urinary excretion. GST, glutathione S-transferase.

Inflammation and cancer

It has long been recognised that inflammation is a risk factor for certain cancers (Balkwill & Mantovani, 2001). One obvious example is the increased risk of colo-rectal cancer associated with inflammatory bowel disease (Ekobom *et al.* 1990), but there are other examples of the involvement of inflammatory processes in the initiation and progress of cancer both within (Macarthur *et al.* 2004) and beyond the alimentary tract (Askling *et al.* 1999). There is also circumstantial evidence that chronic systemic inflammation, associated perhaps with the adverse metabolic factors now so common in prosperous Westernised societies (Johnson, 2005), may be a key driver for cancers of the alimentary tract (Helzlsouer *et al.* 2006). Furthermore, chronic use of aspirin and other anti-inflammatory drugs reduces the risk of cancers of the colon (Chan *et al.* 2005) and oesophagus (Vaughan *et al.* 2005). These various lines of evidence have focused attention on the molecular signals associated with inflammation, and on the phytochemicals that may modulate them.

NF-κB

NF-κB is a collective term for a family of dimeric proteins that act as transcription factors. In its inactive form

NF-κB resides in the cytoplasm as a complex with the regulatory protein IκB. The activation pathway for NF-κB involves phosphorylation, of IκB by IκB kinase, leading to ubiquitination and proteolysis of IκB. This step frees NF-κB to translocate to the nucleus, where it binds to the κB sequence motif in DNA and acts as a transcription factor for at least 200 genes involved in the regulation of inflammation, cell proliferation, differentiation and apoptosis. NF-κB is constitutively activated in many tumours, and its key role as an up-regulator of inflammation suggests that it plays a key role in the earliest stages of promotion and progression (Karin *et al.* 2002).

A host of phytochemicals, including resveratrol, limonene, glycyrrhizin, gingerol, indole-3-carbinol, genistein, apigenin and many others, have been shown to inhibit NF-κB activity at various different stages in its regulatory pathway. For example, curcumin, a widely-studied anti-carcinogenic plant metabolite found in the spice cumin (*Cuminum cyminum*), suppresses TNF-induced activation of IκB kinase (Singh & Aggarwal, 1995), whereas caffeic acid phenethyl ester specifically prevents binding of NF-κB to its target DNA sequence (Natarajan *et al.* 1996). The downstream effects of NF-κB inhibitors include reduced expression of the key pro-inflammatory enzymes inducible NO synthase and cyclooxygenase (COX)-2.

Cyclooxygenase inhibitors

COX (PGH synthase) exists as two distinct isoforms; COX-1, which is constitutively expressed, produces PG essential to platelet aggregation and the maintenance of gastric mucosal integrity, whereas COX-2 is induced by tumour promoters and endogenous cytokines and produces PG involved in inflammatory processes. A third enzyme (COX-3) has been described that is a splice variant of COX-1, and its physiological role in man is not yet clear. Both COX-1 and COX-2 are inhibited by aspirin, an acetylated derivative of the secondary plant metabolite salicylate, originally isolated from willow (*Salix alba*). Both aspirin and salicylate irreversibly inhibit the COX enzymes by selectively acetylating the hydroxyl group of a single serine residue, and they both also suppress NF- κ B by inhibiting I κ B kinase activity (Yin *et al.* 1998). There is abundant evidence that a number of flavonoids are COX-2 enzyme inhibitors (Baumann *et al.* 1980) and some (e.g. apigenin, chrysin and kaempferol) can suppress COX-2 transcription by mechanisms including activation of the PPAR γ transcription factor (Liang *et al.* 2001) and inhibition of NF- κ B expression (Liang *et al.* 1999). Importantly, COX-2 transcription is inhibited *in vitro* not just by quercetin aglycone, but also by its human metabolites quercetin 3-glucuronide, quercetin 3'-sulfate and 3'-methylquercetin 3-glucuronide, and both quercetin and quercetin 3'-sulfate also inhibit COX-2 enzyme activity (O'Leary *et al.* 2004).

Direct inhibition of mitosis: the Wnt signalling pathway

The Wnt proteins are extracellular signalling molecules involved in the regulation of cell proliferation via the β -catenin signal pathway (Kundu *et al.* 2006). The nineteen known *Wnt* genes code for cysteine-rich glycoproteins; these proteins are released into the extracellular milieu where they modulate β -catenin signalling in target cells, via the membrane receptors Frizzled and LDL receptor-related protein. The intracellular protein β -catenin regulates many aspects of cellular organisation, ranging from cytoskeletal structure to the regulation of cell proliferation and death (Wikramanayake *et al.* 2003). Levels of β -catenin in the cytoplasm are tightly regulated by a system of proteosomal degradation that begins when β -catenin forms a complex with the adenomatous polyposis coli protein and another scaffolding protein axin. This complex then associates with casein kinase I, which phosphorylates the N terminus of β -catenin, and glycogen synthase kinase 3 β , which phosphorylates other β -catenin residues. The phosphorylated β -catenin molecule is then recognised by cytoplasmic β -transducin repeat-containing protein, which mediates its ubiquitination and degradation.

Wnt signalling plays an important role in gut formation during mammalian embryogenesis, and contributes to the maintenance of normal gut homeostasis in the adult. Disruption of Wnt signalling is a key abnormality during the development of cancers of the alimentary tract (Gregorieff & Clevers, 2005). In normal cells there is a relatively large and stable pool of inactive β -catenin associated with the cytoskeletal protein cadherin, and a small labile pool in the cytoplasm. However, in cancer cells the degradation

pathway is often suppressed and the balance is altered in favour of the cytoplasmic pool. Active β -catenin migrates to the nucleus, where it activates the lymphoid enhancer factor/T-cell factor family of transcription factors (T-cell factors 1, 3 and 4, lymphoid enhancer factor), freeing them from repression by groucho and histone deacetylase, and leading to the transcription of a variety of effector genes including *C-myc*, *cyclin-D1*, *c-jun* and *COX-2*. It is well established that synthetic COX-2 inhibitors, including indomethacin and aspirin, suppress β -catenin/T-cell factor-mediated gene transcription in colo-rectal carcinoma cells (Dihlmann *et al.* 2001), and sulindac reduces nuclear β -catenin accumulation in the adenomatous polyps of patients with familial adenomatous polyposis (Boon *et al.* 2004). Moreover, a number of phytochemicals, including quercetin (Park *et al.* 2005), which suppresses colo-rectal crypt cell proliferation in the rat *in vivo* (Gee *et al.* 2002), also interact with the β -catenin pathway *in vitro* (Jaiswal *et al.* 2002; Joe *et al.* 2002). Dashwood *et al.* (2002) have shown that green tea, a minimally-processed 'white' tea and physiologically-relevant levels of epigallocatechin gallate all suppress nuclear β -catenin/T-cell factor 4 activity in HEK293 (kidney) cells *in vitro*. In the *APC^{min}* mouse model of colo-rectal cancer, in which the adenomatous polyposis coli protein is disabled by an *APC* mutation, treatment with white tea, green tea or the non-steroidal anti-inflammatory drug sulindac all suppress tumourigenesis (Orner *et al.* 2003). There was also evidence for suppression of β -catenin and its downstream products in the normal mucosa of mice treated with tea, and particularly in those treated with a combination of tea and sulindac.

Induction of apoptosis

Although the suppression of abnormal mitosis may slow the development of precancerous lesions and inhibit their competitive growth, the induction of programmed cell death can completely eliminate genetically-damaged cells from a tissue (Johnson, 2001). Several classes of phytochemicals, including organosulfur compounds (Xiao *et al.* 2005) from garlic (*Allium sativum*) and ITC, have been shown to induce apoptosis in cell lines. Although glucosinolate breakdown products act as powerful inducers of phase II enzymes and modulate carcinogen metabolism *in vitro* and *in vivo*, it is unlikely to be their only mode of action; there is also much interest in their ability to block mitosis and initiate apoptosis in a variety of epithelial cell lines and tissues. Oral administration of the pure glucosinolate sinigrin (Smith *et al.* 1998), the precursor of allyl ITC, or raw Brussels sprouts (*Brassica oleracea* var. *gemmifera*) rich in sinigrin (Smith *et al.* 2003) leads to an amplification of the apoptotic response occurring in colo-rectal crypts 48 h after exposure to the chemical carcinogen 1, 2 dimethylhydrazine. A high level of crypt-cell apoptosis is protective against colo-rectal neoplasia in both animal models (Chang *et al.* 1998) and human subjects (Martin *et al.* 2002). Importantly, the plasma and urinary metabolites to which the target tissues of human subjects are exposed *in vivo* may be just as effective as inducers of apoptosis as the parent ITC. Thus, the *N*-acetylcysteine conjugates of allyl ITC, benzyl ITC, phenethyl ITC and

sulforaphane all readily inhibit proliferation and induce apoptosis in human bladder cancer cells (Tang *et al.* 2006). Furthermore, raw Brussels sprout juice exerts the same anti-proliferative effects as pure allyl ITC *in vitro*, yet contains no free allyl ITC (Smith *et al.* 2005). It is not entirely clear which of the other glucosinolate breakdown products and metabolites in raw Brussels sprout juice are responsible for this biological activity, but of those identified 1-cyano-2,3-epithiopropane, the gluconapin hydrolysis product, 3-butenyl isothiocyanate and the glucobrassicin metabolite ascorbigen are all candidates for this role. The mechanism of cell cycle arrest *in vitro* appears to involve a direct inhibition of tubulin polymerisation (Smith *et al.* 2004, 2005), but the involvement of this mechanism in the induction of apoptosis *in vivo* remains to be established.

Molecular epidemiology

One effect of the discovery that certain biologically-active constituents of plants exert anti-carcinogenic effects in model systems has been to focus the attention of epidemiologists on the particular fruits, vegetables and beverages that contain them. Interest in the area has also encouraged a more hypothesis-driven approach to epidemiology and the use of Mendelian randomisation to explore the effects of genetic polymorphisms on the consequences of particular dietary exposures (Davey Smith & Ebrahim, 2005). Studies on cruciferous vegetables provide some of the best examples of this trend, although the results are still rather ambiguous.

As mentioned previously, the GST superfamily contains a group of phase II detoxifying enzymes that metabolise environmental chemicals including carcinogens, drugs and phytochemicals. Approximately half the individuals in most human populations are homozygous for the null alleles of *GSTT1* and *GSTM1*, and hence cannot express some important components of GST activity. These genotypes appear to carry little increased risk of lung cancer, but Lin *et al.* (1998) have hypothesised that the *GSTM1* null condition might lead to a slower rate of metabolism of glucosinolate breakdown products. In high consumers of brassica vegetables this reduced rate of metabolism might be expected to enhance the exposure of target tissues to ITC, and hence confer greater protection against cancer. To test this hypothesis Lin *et al.* (1998) have conducted a case-control study of middle-aged patients undergoing screening sigmoidoscopy. A total of 459 cases having a first-time diagnosis of histologically-confirmed adenomas were compared with 507 controls with no polyp detected. Broccoli intake was assessed using a 126-item semi-quantitative FFQ. Subjects with the highest quartile of broccoli intake (3.7 servings per week) had an OR of 0.47 (95% CI 0.30, 0.73) for colo-rectal adenomas compared with subjects who ate no broccoli. When stratified by *GSTM1* genotype this protective effect was observed only in patients null for *GSTM1* ($P < 0.001$ for trend; $P < 0.01$ for interaction). However, Tjhuis *et al.* (2005) have conducted a similar study in The Netherlands and have reported increased risk of adenomatous polyps amongst high consumers of cruciferous vegetables, an effect that was

shown to be amplified in those with a single nucleotide polymorphism in *GSTP1* (OR 1.94 (95% CI 1.02, 3.69)). No effects of the *GSTT1* and *GSTM1* deletion polymorphisms were observed. This unexpected finding might reflect differences in the population, the environment or the nature of the brassicas consumed, but it certainly deserves further investigation. Gasper *et al.* (2005) have suggested that some of the contradictory reports concerning the protective effects of cruciferous vegetables against cancers of the lung and other sites might reflect varietal differences in the vegetables consumed by oriental and Western populations.

Unlike colo-rectal cancer, most lung cancer can be clearly linked to well-defined environmental carcinogens. GST enzymes are involved in the metabolism of both tobacco-smoke carcinogens and glucosinolate breakdown products, and a number of studies have been focused on the possibility of interactions between *GST* polymorphisms and the putative protective effects of cruciferous vegetables. Spitz *et al.* (2000) have undertaken a case-control study in which patients newly-diagnosed with lung cancer and their controls were genotyped for *GSTM1* and *GSTT1*, and have reported on consumption of crucifers by means of a semi-quantitative FFQ. Overall, lower intake of cruciferous vegetables was reported by the cases compared with the controls ($P = 0.009$). Amongst current cigarette smokers a low intake of crucifers was found to carry an OR of 2.22 (95% CI 1.2, 4.1) when combined with the *GSTM1*-null genotype and 3.19 (95% CI 1.54, 6.62) when combined with the *GSTT1*-null genotype. In individuals carrying both null genotypes and consuming low levels of crucifers the OR rises to 5.45 (95% CI 1.72, 17.22). In complete contrast to this finding, however, Zhao *et al.* (2001) have conducted a similar study amongst female patients with lung cancer and controls in Singapore and have shown stronger protective effects of cruciferous vegetable intake in individuals with null genotypes for *GSTM1* and *GSTT1*. For example, in those null for both alleles and consuming higher levels of cruciferous vegetables, the OR is 0.47 (95% CI 0.23, 0.95) compared with low consumers. Similar effects have been reported by London *et al.* (2000), working with another Chinese population in which exposure to cruciferous vegetables was determined objectively using urinary excretion of ITC metabolites as a biomarker, and more recently by Brennan *et al.* (2005), who have studied a central European population.

Generally speaking, phytochemicals are rather poorly absorbed by human subjects, and are themselves rapidly metabolised by the phase II enzymes. Much of this metabolism occurs in the gut mucosa (Fig. 2), and a large proportion of the resulting conjugates are actively secreted back into the gut lumen (Petri *et al.* 2003). A recent study of the uptake and metabolism of the ITC sulforaphane from broccoli by human volunteers (Gasper *et al.* 2005) has demonstrated that in comparison with *GSTM1*-positive individuals those who are *GSTM1* null excrete a greater proportion of the consumed dose of sulforaphane via the urine. They consider various possible explanations for this observation, and tentatively propose that expression of *GSTM1* leads to retention of sulforaphane at some systemic site or sites, which facilitates its anti-carcinogenic

activity. An alternative explanation may be that in the *GSTM1*-null individuals the absence of *GSTM1* expression reduces the efficiency with which the ITC is transported back into the gut lumen following absorption (Petri *et al.* 2003); however, further studies will be needed to resolve this issue.

Conclusion

Whatever the final assessment of the overall contribution of diets rich in fruits and vegetables to cancer prevention turns out to be, there is no doubt that the phytochemicals they contain do exert a range of fascinating and potentially-important biological effects on human cells. One obvious weakness of the current state of research, however, is that much of it has been conducted *in vitro*, with little regard for the bioavailability of the compounds studied. In most cases the small proportion of any compound that is absorbed undergoes extensive metabolism before reaching target organs, and the products are readily excreted. Although such phytochemicals cannot be regarded in any meaningful way as nutrients, their beneficial side effects may well be exploited in chemoprevention, much as those of drugs are, either by using pharmaceutical delivery systems, or through the development of 'customised' vegetables with high levels of selected phytochemicals (Mithen *et al.* 2003). Such strategies will, however, require a full risk-benefit analysis, based on a thorough understanding of their overall biological effects.

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