PASSCLAIM[1] – Diet-related cancer

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■ **Summary** *Background* The role of dietary factors in the aetiology of human cancer is an area, which has attracted intense interest in recent years. The suggestion that approximately one third of all cancers may be caused by an 'inappropriate' balance of food components has led to the attractive contention that we can significantly decrease cancer incidence through dietary recommendations and a change in dietary habits in populations. Thus, a key issue must be to establish clear criteria, which must be met in order to be able to make 'cancer risk reduction' claims for food components. In this area, the one true marker is the malignant human tumour, which for practical reasons is usually not accessible to claims. In its absence, we must rely on alternative markers – biomarkers/surrogate endpoints. This paper mainly deals with the link of these biomarkers to the endpoint tumour and their usefulness for making claims. Some claims have been made based on epidemiological studies. *Aim* Can we identify targets/biomarkers in the chain of events from initial 'exposure' to overt malignant tumour, whose modification can be used to make 'anticancer' claims for food components? *Results* We identified 18 targets/markers in the above chain of events whose modification 'have the potential' to be used for 'reduction of cancer risk' claims for food components. These targets/markers fall under 5 broad headings: tumours & preneoplastic changes; cellular targets/markers; gut luminal markers; angiogenesis & metastasis; carcinogen metabolising enzymes; genetic events. *Conclusions* The strongest markers presently available are precancerous lesions (e. g. polyps or aberrant crypt foci) in humans and precancerous lesions and tumours in animal models. The only marker that presently can be used for a 'reduction of disease risk' claim (type B) for food components is 'polyp recurrence'. Type B claims cannot be made on the basis of results in animal models. All of the other biomarkers examined presently lack validation against the 'true endpoint', the tumour, and thus cannot be used for type B claims. 'Reduction of disease risk' claims in the area of 'diet-related cancer' should be based primarily on human intervention studies using relevant/acceptable endpoints. An important area for future research will be the validation of these surrogate endpoints.

■ **Key words** functional foods – diet-related cancer – colon cancer – biomarkers – surrogate endpoints

General introduction

Epidemiological studies indicate that the processes of carcinogenesis and tumourigenesis are mainly induced by environmental factors. An extensive review of the link between nutrition and cancer on a global scale has been recently published by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR). Some 35 % of all cases of death by cancer are caused by high risk diet (e. g. high saturated fat/low fibre/low consumption of fruit and vegetables), 30 % is due to tobacco smoking, together with reproductive behaviour (7 %) and alcohol abuse (3 %), thus about 75 % of death by cancer is due to diet and lifestyle. Another 20 % are due to an unhealthy environment (7 %), infection (9 %) and pollution (4 %). Finally there are about 5 % of death by cancer which are caused by hereditary, genetic factors.

Cancer incidence in Europe is highest for lung cancer in man and breast cancer in women, followed by colorectal cancer for both sexes and then prostate cancer in man and lung cancer in women. Smoking is the major cause for lung cancer and diet, but particularly a diet high in antioxidant-rich foods (fruits and vegetables) reduces the risk of disease even in heavy smokers.

The development of breast and prostate cancer seems to be linked to the hormonal status and both cancers are promoted by endogenous estrogens, respectively. However, phytooestrogens from dietary sources appear to decrease the risk of these hormone-dependent cancers as has been indicated by epidemiological studies.

Carcinogenesis for most cancers is a process developing for decades (10–30 years). Several stages in the process can be discriminated, e. g. initiation, promotion and progression. At these various stages characteristic molecular and cellular changes occur (see Fig. 1). Many of these differe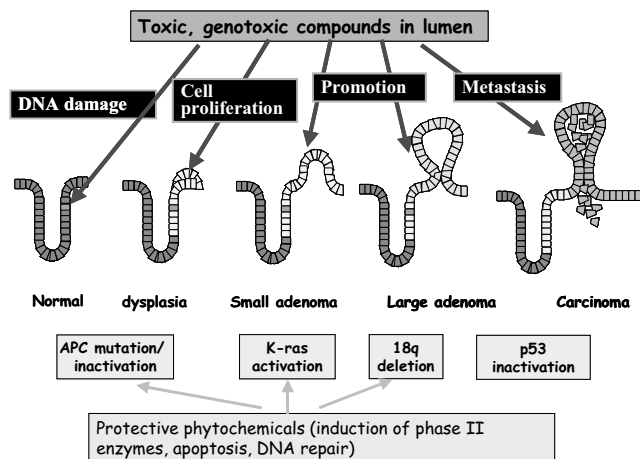nt stages can be modulated by dietary factors (food components and ingredients) either by direct interaction with gene expression or through the modulation of key enzyme activities involved in cell proliferation and differentiation, respectively.

Their ability to undergo extensive proliferation is the main characteristic of cancer cells. This finds its origin in the typical overproduction of growth factors and/or in the overexpression of receptors for growth factors by these cells. In this context the action of insulin and insulin-like growth factors appears to be especially important. Obesity and diabetes seems to be a predisposing condition for the development of some types of cancers.

Cellular proliferation begins when cell-surface receptors recognise their appropriate growth factors. Next, a cascade of reactions mediated by cytoplasmatic protein kinases culminates in transcriptional activation, cell-cycle progression and cell division. This growth factor induced mitogenic signalling can be blocked by certain exogenic factors which prevent receptor activation or which prevent binding of a growth factor to its receptor.

Next comes cell-cycle progression. In the cell cycle, 4 distinct phases can be distinguished. In the M-phase there is active mitosis; the S-phase is the state of DNA synthesis; these two phases are separated by two G or gap phases (G1 and G2). The progression of one phase to the other is mediated by cyclins (proteins whose concentration rises and falls during the cell cycle) cyclin-dependent kinases (CDKs, which are activated by specific cyclins) and inhibitors of the assembly and activity of cyclin-CDK complexes (CDKIs). If cells are inhibited at any point in the cell cycle, their progression through the cell cycle is disturbed, which in its turn prevents mitosis and cell division.

Malignant cells are immortalised cells. This is mainly due to the action of telomerases, which preserve the integrity of the telomeres. These are regions at the end of chromosomes, which are replicated by special processes, counteracting the tendency of the chromosomes to become shorter during a round of replication. Consequently, inhibiting telomerase decreases the potential of cancer cells to become immortal. It has been demonstrated in cell systems that certain flavonoids potently affect telomerase activity.

Besides being immortalised, tumour cells have infinite replicative potential. DNA replication takes place in the S-phase of the cell cycle. The process of replication essentially duplicates the genetic material with the help of the replication machinery (DNA polymerases, DNA ligases, topoisomerases). Inhibiting replication ensures that malignant cells do not progress in the cell cycle.

Healthy cells undergo apoptosis, programmed cell death. This process is initiated by means of death sensors and effectors. Evading apoptosis as tumours cells do, requires mechanisms that inhibit transmission of the death signals. Potentially any mechanisms which

Fig. 1 Molecular changes related to carcinogenesis as exemplified by the adenoma-carcinoma sequence in colorectal carcinogenesis

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specifically would increase the rate of apoptosis in tumour cells could be used in cancer therapy.

Fast growing tumours need oxygen and nutrients, which need to be provided by new blood vessels. Malignant cells can induce angiogenesis by secreting vascular endothelial growth factors (VEGFs) and fibroblast growth factors (FGF1/2). The signalling pathways leading to angiogenesis are activated when these growth factors bind to their receptors on the endothelial cell. The signalling leading to angiogenesis also can occur via integrin receptors (integrins are transmembrane proteins promoting adhesion of cells).

When primary cancer cells come loose out of the tumour mass, they can metastasise and invade healthy tissue anywhere in the body, where they can further proliferate, and destroy surrounding tissue, or hinder it in its physiological functioning. The loosening of cells is possibly due to altered expression of adhesion molecules, into less 'sticky' ones. Another retained possibility is through the activation of matrix metalloproteases (MMPs). These deteriorate extracellular matrix proteins (laminin and collagen). It was also observed that cell surface integrin receptors, which were isolated from metastatic cancer cells more efficiently bind to degraded matrix proteins.

The clinical therapy of existing cancers is still primarily based on surgery, radiation treatment and chemotherapy. However, for example advanced colon carcinoma are very refractive to any of these therapies. It is put forward that cancer more efficiently can be controlled by reducing the risk to get it, by preventing it. In order to make a healthy cell cancerous, several errors need to occur at the same time. Many of the external factors, which seem to cause death by cancer can be controlled. Nutrition entails some of the external factors which can cause errors in a cell to occur, and as such certainly has a potential to reduce the risk for cancer. The enzymes involved in the biotransformation of xenobiotics, e. g. phase I and II enzymes, are involved in toxification of chemical carcinogens like the aflatoxins; however, they also are involved in the metabolism of certain phytochemicals occurring in fruits and vegetables. The expression and activity of these enzymes has been shown to be affected by various phytochemicals. This may be a mechanistic link to the cancer risk reduction observed in groups with a high consumption of fruits and vegetables.

Nutritional studies have demonstrated effects in experimental models. Some mechanisms of interaction between nutrition and one or several steps described above have been postulated, and have to some extent been demonstrated in experimental models. Animal models, particularly rats and mice have been used extensively to study the processes of carcinogenesis. In these animal studies often times carcinoma are induced by chemical carcinogens. Recently gene knock-out mice have been used to investigate the involvement of certain specific genes, like APC, in the carcinogenic process. Certainly these model systems are important to understand the mechanisms involved in carcinogenesis; however, a direct link to the human situation is particularly questionable for the association between nutrition and cancer development.

In the present paper, it will be discussed which conditions should be met, in order to claim that food ingredients, or foods, have a potential to reduce the risk for cancer. The paper will focus on the numerically most important cancers. These are colon cancer, lung cancer, breast cancer, and prostate cancer. The following chapter deals with sporadic tumour formation, cancers with a strong genetic disposition will not be discussed in detail. Colon cancer receives much attention as evidence indicates that this is a tumour particularly amenable to dietary influence.

Prospective, dietary intervention studies are considered the gold standard in evidence-based nutrition for evaluating the relation between a disease and nutritional factors. However, especially for carcinogenesis this is exceedingly difficult, time-consuming and expensive to perform and also creates ethical problems if a 'control' group is included. For this reason, much of the chapter deals with early/intermediate 'markers' for cancer, their link to the endpoint tumour and their usefulness for making claims. It can also be mentioned that to date some claims have been made based on epidemiological studies.

Inflammatory bowel disease increases the risk of colorectal carcinomas; however, this issue will be dealt with in the ITG on Gut Health and Immunity.

Tumours and preneoplastic changes

Carcinoma incidence is the ultimate endpoint of cancer prevention intervention studies. In rodents, using a chemical carcinogen, this can mostly be achieved within a year. However, human trials with carcinoma as the primary endpoint will be looking at many years (> 10 yr) to prove a chemopreventive effect of a certain agent. Therefore, there is a strong need for sensitive and specific intermediate biomarkers that accurately reflect the multiple stages of carcinogenesis. The most promising biomarkers are discussed below.

■ Tumours and mortality in animal models

■ **Definition of target.** Macroscopically visible tumours in rodents can be induced by chemical carcinogens. Examples are 1,2-dimethylhydrazine (or its metabolite azoxymethane) for colon cancer, dimethylbenz(a)anthracene for breast cancer, and diethylnitrosamine for

lung cancer. In long-term studies, also the incidence of mortality, resulting from the progression of these tumours, can be analysed as a marker.

■ **Relationship of target with cancer.** The presence of adenomas and adenocarcinomas, their size and multiplicity are directly linked to cancer. Mortality is a hard endpoint resulting from tumour progression.

■ **Modulation of target by diet.** Macroscopic tumours can be modulated by diet in several ways: the size of tumours can vary, as well as the number of tumours per animal, and the number of tumour-bearing animals per group. Also, the incidence of mortality per group can be modulated by diet. As an example for colon cancer, several types of dietary fibre have been frequently shown to reduce the number of tumours in rodent models [reviewed in 1]. Antioxidants appear to have a high potential for inhibiting lung cancer. NSAIDs are effective in bladder cancer, and retinoids are efficacious in breast cancer models [reviewed in 2].

■ **Methodological considerations.** The incidence of macroscopic tumours has been the 'gold standard' endpoint of chemoprevention studies in rodents for many years. There are however several practical drawbacks of these types of studies: the large groups of rodents required; the long time scale (up to 8 months) needed for tumours to develop; and the time-consuming histological procedures for confirmation of the tumours. For colon cancer, an alternative marker has been developed and validated: aberrant crypt foci (see below, section Precancerous lesions (aberrant crypt foci)). Therefore, the rodent tumour model for colon cancer has gradually lost attractiveness, due to the above-mentioned drawbacks.

More recently, other models have been introduced for cancer research, such as tumour-transplantation animal models, available for breast and prostate cancer, and mouse transgenic models. Indeed in 1990, the mutant Min mouse was found with multiple intestinal neoplasia. It was shown to have a mutated *Apc* gene, similar to that in patients with familial adenomatous polyposis, and in many sporadic cancers. Then other mice have been genetically modified (with truncated *Apc* at different positions or mutated *Msh2* or *Mlh1*). They have been used as models to evaluate the effect of diets and chemopreventive agents in more than 70 studies [3]. Compared with the carcinogen-treated rat model, the use of mutated mice avoids the hazard of carcinogen handling, and leads to shorter studies. Dietary treatments are initiated in mice by the age of 4–5 weeks, when tumours may be already present. This timing mimics human clinical trials, where dietary treatments are given to adults, likely to bear minute polyps, the visible ones having been removed before randomisation.

■ **Conclusions.** Chemical carcinogen-induced rodent models are well accepted and widely used for the study of potential chemopreventive agents. New transgenic and implantation rodent models are interesting from a mechanistic point of view, but should be interpreted with great care. For instance, in the APC Min mouse, adenomas develop mainly in the small intestine and in the first weeks of life, which is very much unlike the human situation.

■ **Precancerous lesions (aberrant crypt foci)**

■ **Definition of target.** Aberrant crypt foci (ACF), first described in 1987 [4], are precancerous lesions of the colonic epithelium, which consist of a number of crypts that are larger than normal crypts, slightly elevated above the mucosal surface, with a thick epithelial lining.

■ **Relationship of target with cancer.** Several lines of evidence strongly suggest that ACF with certain morphological, histological, cell kinetic and genetic features are precursor lesions of colon cancer in both rodents and humans [reviewed by 5]:
- ACF are induced by all colon carcinogens in a dose-dependent manner [6];
- In ACF, a shift of proliferation towards the luminal surface of the epithelium occurs, like in adenomas [7]. Apoptosis is far less defined in experimental ACF.
- The number of ACF, as well as the number of crypts per ACF (so called crypt multiplicity), are affected by chemopreventive agents, and predict tumour incidence. A recent review by Corpet [1] reported a significant correlation between the potencies against ACF and tumours for 57 agents studied for both biomarkers. Using two different protocols, the interference of chemopreventive agents with the initiating phase and the post-initiative phase of carcinogenesis can be discriminated [8].
- ACF correlate with colon cancer risk and adenoma size and number in humans. For instance, the number of ACF was higher in patients with Familial Adenomatous Polyposis, and dysplasia was shown in most cases [9]. Also, the number of ACF in resected sections of the normal mucosa of cancer patients was higher in regions with higher colon cancer mortality [10].

■ **Modulation of target by diet.** The following parameters can be modified by diet: number of ACF in colon; number of crypts per ACF; proliferation rate in ACF. For instance, lycopene [8, 11] and fish oil [12–14] have been shown to reduce the number of ACF in rats. See also the review by Corpet [1].

■ **Methodological considerations.** ACF can be easily visualised with methylene blue staining and scored with a

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low-magnitude microscope in resected colon samples of rodents and humans, without tissue sectioning. Several studies have described ACF visualisation *in vivo* during endoscopy in cancer patients, with variable results [15–17]. This technique is technically difficult, but promising, and has to be further developed and validated.

Although most data support the use of ACF as a surrogate end point for the screening of agents for chemoprevention, there is still some discussion whether ACF are true preneoplastic lesions. The azoxymethane dose that yields one cancer per rat, yield 100–200 ACF per colon, indicating that most ACF regress and never become cancers. In some ACF, hyperplastic or carcinoma tissue has been observed [18]. Also, crypt multiplicity in ACF was a predictor of tumour incidence in several studies [e. g. 19]. Most probably only larger foci, with altered morphology, dysplastic, with altered cell kinetics, and with mutations in some genes involved in carcinogenesis, will progress toward cancer.

Since, a few agents have shown opposite effects on ACF and on tumours (for example, dietary cholic acid, a known colon tumour promoter decreases ACF) other types of micro-lesions have been studied (that are enhanced by cholic acid).

β-Catenin-Accumulated Crypts (BCAC), were first described by Yamada et al. (2003) [20]. As they are only identified in the histological sections of *en face* preparations, BCAC are not yet ready to be used for routine chemoprevention studies.

Mucin Depleted Foci (MDF), first described by Caderni et al. (2003) [21]. Fifteen weeks after injections of azoxymethane to rats, six to ten MDF could be scored on the unsectioned colon stained with High-Iron Diamine Alcian Blue (HID-AB). Thus, in contrast with BCAC, MDF might be used for chemoprevention studies.

It is possible that MDF and BCAC are two views of the same lesion. Indeed, mucin production is usually absent in BCAC, and it is likely that MDF cells accumulate beta-catenin.

■ **Conclusions.** From the above it can be concluded that ACF are a valuable biomarker in rodents, providing a quantitative assessment of the development of colon cancer. To judge the relevance in humans of ACF and *a fortiori* of MDF, not enough data are available at the moment.

■ **Adenomatous polyps**

■ **Definition of target.** Adenomatous polyps are well demarcated, circumscribed lumps of epithelial dysplasia. Adenomas are present in all segments of the large bowel, but their distribution tends to parallel that of colorectal

malignancies, so that about 70 % of adenomas are localised in the left colon and rectum. Endoscopic examination for the detection of adenomatous polyps is a common diagnostic procedure, in which single or multiple polyps can be visualised and removed surgically. Common clinical practice is that all polyps detected during endoscopy are resected.

■ **Relationship of target with cancer.** Adenomatous polyps in the colon and rectum are generally considered to represent the most likely precursor lesions for colorectal cancer in humans. They can vary in the number, size, histological type and degree of dysplasia [22]; all are important determinants of potential malignancy. Adenoma size and number have been shown to be associated with adenoma recurrence [23], and removal of polyps decreases the risk of colon cancer [24]. Furthermore, it was shown that invasive carcinomas developed at sites where large polyps (> 1 cm) were not excised [25]. FAP patients, who have hundreds of polyps in their large bowel, will almost all develop cancer if left untreated [26]. In addition, adenomatous polyps and cancer share the same aetiological factors as well as molecular genetic alterations.

■ **Modulation of target by diet.** The following parameters can be modified by diet: size, number, growth and recurrence of polyps; histological features. As an example, calcium has been shown to reduce the recurrence of colorectal polyps in a few studies [27, 28]. On the other hand, a beneficial effect of fibre supplementation on polyp recurrence could repeatedly not be demonstrated [28–30].

■ **Methodological considerations.** The major drawback for using polyp recurrence as a biomarker in human chemoprevention trials is the long time scale required to allow recurrence of the polyps: at least 3–5 years is needed, which makes these studies complex and very expensive. In addition, the cohort of patients studied should be quite large. An alternative is found in studying the growth (or regression) of non-resected polyps over time. This allows results to be obtained earlier. However the awareness that detected polyps are deliberately not being removed, sometimes encounters ethical objections, as it will increase the risk of developing malignant lesions.

A further limitation of the use of polyp recurrence as a biomarker of colon cancer risk is the fact that polyps occur only in part of the general population [31]. Therefore, these persons might already reflect a certain susceptibility to colon cancer and might not be representative of the general public.

Finally, the majority of adenomatous polyps do not progress into invasive cancer. Therefore, although many studies have indicated that polyp recurrence may be

modulated by chemopreventive agents, it remains to be determined whether dietary or pharmacological modulation of polyp recurrence leads to a reduction in the incidence of colon cancer.

■ **Conclusions.** However, even taking into account the above-mentioned reservations, the recurrence of adenomatous polyps is still probably the most reliable surrogate end-point in human chemoprevention studies, since it measures the occurrence of a lesion in the pathway leading from normal mucosa to cancer. In addition, polyp size and their histological features (tubular/villous) are important biomarkers too.

However, caution should be taken in interpreting the results of studies which show no or even a negative effect, such as the large fibre intervention trials, mentioned above. Because of the overwhelming amount of available scientific evidence that dietary fibre is protective against colon cancer, it becomes clear that also polyp recurrence is a biomarker that might be hindered by methodological issues.

■ **Tumour markers**

Tumour markers are molecules that indicate either the presence or the progress of malignancy. Tumour markers are directly secreted or exfoliated by tumour tissue. They are useful for diagnosis, progression of disease and efficacy of cancer therapy, rather than for screening of healthy subjects.

Widely used tumour markers are PSA (prostate-specific antigen) for prostate cancer, and oestrogen and progesterone receptors for breast cancer [32]. A change in concentration can easily be detected in a blood test. As an example of dietary modulation, PSA levels in blood can be decreased by supplementation with vitamin D [33].

For colon cancer, no such marker is widely used. Several biochemical markers have been identified as risk indicators of colonic neoplasia, such as insulin and insulin-like growth factor [34], and plasminogen activators and inhibitors [35]. Epidermal growth factor receptor has been detected in lung and colon cancer patients [36]. Very promising are recently developed molecular markers. These detectable mutations include 8 p, 18 q, DCC, p1, p53 and K-*ras* [reviewed by McLeod, 37].

In general, the currently available tumour markers lack sensitivity for early cancer diagnosis, and lack specificity for scoring malignancy. However, improvements in molecular and genetic techniques have permitted the detection of more sensitive and more specific markers, such as tumour derived DNA and RNA circulating in plasma, so that genetic mutations that occur during tumour progression can be detected in a blood sample. For colon cancer, although a large number of studies directed at molecular markers have been reported to provide prognostic or predictive indicators, none of the potential markers has yet come to a widespread clinical application [37]. For developing health claims, only markers that have diagnostic value on the level of dietary intervention, such as PSA for prostate cancer, are important.

Cellular targets/markers

This section presents the main targets, at the cellular level, which have consequences for carcinogenesis during the post-initiation phase and can be modulated by food components.

These targets are known to have common regulatory pathways. Indeed, various endogenous or exogenous agents, including dietary compounds, may affect several of these targets simultaneously.

Cellular targets have two main limitations:
- Very often, these targets are studied using cellular models (spontaneously transformed rodent cell lines, or human tumour cell lines) that do not completely reflect the in vivo situation in a normal tissue.
- The choice of the cell type is also critical, since cell specificity of regulatory mechanisms could influence the outcome of the study.

So, for cellular targets, results that are mostly obtained from in vitro and animal studies are not sufficient to base a claim on but they could be used as supportive evidence.

■ **Cell proliferation**

■ **Definition of target.** In adults, most cell types (especially epithelia) undergo a number of cell divisions, before differentiating and dying. In each tissue, cell proliferation is induced and finely regulated by the binding of endogenous hormones and growth factors to their specific receptors expressed in the tissue.

Cell division itself is regulated by a complex interplay of many genes that control the cell cycle, with DNA replication and mitosis as major checkpoints. The cell cycle is tightly regulated to minimise transmission of genetic changes to subsequent cell generations. Progression through the cell cycle is under the control of different types of proteins: cyclins (A, B, D, E), cyclin-dependent kinases (CDKs: cdk2, cdk3, cdk4, cdk6, cdc2…) and inhibitors of cyclin-CDK complexes of the WAF1/CIP1 family (p21), the KIP family (p27, p57) and the INK4 family (p16, p15, p18). Tens of molecules have been identified as components of the signalling cascades which couple regulation of the cell cycle and detection of DNA damage. The product of the tumour

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suppressor gene p53 is one of these key regulatory proteins [38].

■ **Relationship of target with cancer.** Cell proliferation plays a crucial role at different stages of multistage carcinogenesis [39]:

- Throughout the successive cell cycles, errors in DNA replication due to genetic instability, which are not or incorrectly repaired, can result in DNA sequence changes (gene mutations). Also, DNA replication converts DNA alterations (DNA adducts) due to stress (irradiation, exposure to carcinogens) to DNA sequence changes. Thus, in proliferating tissues, various genetic changes can progressively accumulate.
- Clonal proliferation of the genetically damaged cells leads to the formation of foci, nodules and then to tumours which can escape from the control of neighbouring cells.

Perturbation of cell proliferation is observed in all malignant tissues. For example, in colon, the mucosal cell proliferation, which is normally restricted to the stem cell zone, extends to the entire crypt during carcinogenesis and leads to the formation of aberrant crypt foci, polyps, adenomas or carcinomas. Patients with a high risk of colorectal cancer (e. g. FAP) have a corresponding high mucosal proliferation [40]. Refer also to section on 'short chain fatty acids' under Metabolites below.

■ **Modulation of target by diet.** Cell proliferation is inhibited by many chemopreventive agents, including retinoids, vitamin D, polyphenols, phytooestrogens, fatty acids, sodium butyrate, indole-3-carbinol. They act in different ways on the regulation of cell proliferation: at the level of growth hormones and growth factors or their receptors or on the genes and proteins regulating the cell cycle [for review, see 41, 42].

■ **Methodological consideration.** To assess cell proliferation, different methods are available:

- Cell growth curves are done with cell cultures in vitro.
- DNA synthesis, determined by incorporation of precursors: tritiated thymidine or bromodeoxyuridine (BrDU) into cells or tumour fragments *in vitro*.
- Distribution of cells in different cell cycle phases, using flow cytometry.
- Measurement of proliferation biomarkers, detected using immunohistochemistry. Among them, Ki-67 and PCNA (proliferating cell nuclear antigen) are nuclear proteins whose level varies as a function of the cell cycle. Ki-67, whose function is unknown, is expressed throughout the cell cycle except G0 [43]. PCNA, which is an auxilliary protein to polymerase delta, is expressed during the G1 phase of the cell cycle [44]. Recently a monoclonal antibody to recombi-

nant parts of Ki-67 antigen, named Mib-1, has been developed. It appears as a more reliable tool for measuring proliferation activity in human tissues [45].

In clinical trials, cell proliferation is frequently evaluated by the BrdU index or PCNA content. These methods have both advantages and disadvantages, which have been recently discussed by Renehan et al. [46].

■ **Conclusion.** Inhibition of cell proliferation could be a valuable surrogate marker. The possibility to use it as a basis for a claim may depend on the methodological approach (dynamic or static assessment, measurement in cellular or animal models, preferably in humans).

A nutritional inhibition of cell proliferation could be beneficial in the case of tumour cells (therapy), whereas it remains questionable in the case of normal cells (prevention).

■ **Apoptosis**

■ **Definition of target.** Apoptosis is a cell suicide mechanism that enables multi-cellular organisms to regulate cell number in tissues and to eliminate unneeded or ageing cells. Apoptosis is a programmed cell death. In can be distinguished both morphologically and functionally from necrosis, which is a pathological cell death, resulting from gross insults such as prolonged ischaemia that affects many adjacent cells simultaneously. In contrast, apoptosis typically occurs in single cells. Normally, it is initiated by endogenous stimuli, such as the absence of vital growth factors or hormones and the action of cytokines, like tumour necrosis factor α or Fas ligand [47, 48].

Apoptosis is characterised by membrane blebbing, cell shrinkage, chromatin condensation and DNA fragmentation followed by rapid engulfment by neighbouring cells or macrophages [49].

It involves a series of death sensors and effectors. Cellular death receptors (e. g. tumour necrosis factor receptor TNFR, death receptor CD95 Fas) are sensors that initiate the death pathway. Death signals typically are focused on the mitochondria where the release of cytochrome C (haeme-containing protein that transfers protons during cellular respiration) catalyses apoptosis. Caspases (death effectors) finally transmit the death signal: these proteases specifically cleave vital proteins of the nuclear lamina, such as poly (ADP-ribose) polymerase, and the cytoskeleton (actin, fodrin, lamin), which results in cell disassembly.

Endogenous molecules, which have other activities in the cell, regulate apoptosis. Some regulatory transmembrane proteins of the Bcl-2 family, such as Bax, Bak and Bad, are pro-apoptotic effectors. The tumour suppressor gene p53 (of which the corresponding p53 pro-

tein functions in the checkpoint control that arrests human cells with damaged DNA in G1) can induce expression of Bax, as well as death receptors. Antiapoptotic effectors in the Bcl-2 family include Bcl-2 and Bcl-XL. These are central in inhibiting signals addressed to the mitochondria, which leads to cell survival. Other genes preventing programmed cell death are for instance NF-κB, which expresses cell survival genes, which induce IAPs, which bind to and inhibit certain caspases.

■ **Relationship of target with cancer.** Apoptosis provides a protective mechanism against neoplasia by removing genetically damaged stem cells from the epithelium before they can undergo clonal expansion [50]. Loss of apoptosis may result in disordered cell growth. It contributes to tumour development but also to tumour initiation, progression and metastasis. Defects in apoptosis allow neoplastic cells to survive beyond senescence, thereby providing protection from hypoxia and oxidative stress as the tumour mass expands.

Evading apoptosis as tumour cells do, requires mechanisms that inhibit transmission of the death signals. Genetic alterations of regulators of apoptosis in tumours have been described: for example, mutations of p53 gene in more than 50 % of tumours, overexpression of Bcl-2 due to chromosomal translocation in lymphomas [for review, see 51].

■ **Modulation of target by diet.** Many chemopreventive agents have been found to induce cell death. They include retinoic acid, perillyl alcohol, curcumin, EGCG, apigenin, quercetin, chrysin, resveratrol and genistein [41].

In the case of the colorectal mucosa, different kinds of dietary components have been shown to modulate apoptosis both *in vitro* and *in vivo*: they include polyunsaturated fatty acids (PUFA), the short-chain fatty acid butyrate and some flavonoids and glucosinolate breakdown products [for review see: 50].

■ **Methodological consideration.** Various complementary techniques have been developed to monitor apoptosis. Since the common methods have their own set of limitations, it is recommended to confirm the results by using a combination of the following techniques [52]:
- Morphological analysis of apoptotic cells (electron microscopy) and counting of apoptotic bodies (membrane-bound structures containing nuclear fragments and condensed cell contents).
- Detection of DNA fragmentation in the nuclei of apoptotic cells in tissue sections, by the TUNEL (TdT-mediated dUTP nick end labelling) method [53]. One important weakness of the TUNEL method is that it may detect necrotic cells containing damaged DNA.
- Annexin V binding: annexin V binds to membrane-

bound phosphatidyl serine, a constitutive anionic membrane phospholipid that is normally restricted to the inner leaflet of the plasma membrane lipid bilayer but is selectively exposed on the surfaces of cells as they undergo apoptosis. In vivo uptake of ^{99m}Tc-radiolabeled annexin V, assessed by SPECT imaging, allows non invasive monitoring of cell death dynamics [54, 55].
- Immunodetection of caspase activity, using antibodies directed against caspase-cleaved substrates, has been recently developed. Such a method would be very useful in future clinical trials [56].

■ **Conclusion.** Induction of apoptosis could be a valuable surrogate marker. The possibility to use it as a basis for a claim will depend mainly on the methodological approach (use of a combination of complementary assays to overcome artefacts and difficulties related to measuring apoptosis in humans and interpretation).

As previously mentioned for cell proliferation, a nutritional induction of apoptosis could be beneficial in the case of tumour cells (therapy), whereas it remains questionable in the case of normal cells (prevention). It must be kept in mind that it is the balance between proliferation and apoptosis that is important.

■ **Differentiation**

■ **Definition of target.** Normal cells do not live indefinitely due to senescence. Senescent cells cannot be stimulated to divide further, become resistant to programmed cell death and acquire differentiated functions. Differentiation is obviously associated with slowing of the cell cycle.

Differentiation can be described as the process by which, during development or tissue maturation, cells acquire functions that are specific of the tissue or cell type. It results from the induction of a differential expression of genes, some becoming activated and others repressed.

■ **Relationship of target with cancer.** It is generally agreed that the malignant neoplastic cell is less differentiated than the normal adult cell in the organ from which the cancer originates. However, for a long time, there has been a controversy on the link between transformation and de-differentiation. Nevertheless, malignant neoplastic transformation occurs only in cells that are capable of dividing. In general, it appears that the commitment to a given pathway of cellular differentiation is irreversible, but the steps of terminal differentiation may be reversible.

■ **Modulation of target by diet.** There are a number of examples of animal malignant tumours or human can-

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cer cells in culture that can be induced to lose their malignant phenotype by treatment with certain differentiation-inducing agents: differentiation of murine embryonal carcinoma cells by exposure to retinoic acid or sodium butyrate; differentiation of human acute promyelocytic (HL-60) cells in culture by sodium butyrate, vitamin D_3 and retinoic acid analogues. Among dietary compounds, curcumin and phytic acid have also been shown to induce differentiation of various tumour cell lines [57, 58].

The best examples of cancer treatment through induction of cellular differentiation is the treatment of acute promyelocytic leukaemia and oral leukoplakia with retinoids [59]. Since the 1980 s, vitamin D_3 has been recognised as a potent antiproliferative and prodifferentiation agent (more recently, it has also been shown to induce apoptosis and to inhibit tumour invasion and angiogenesis). Several epidemiological studies support the hypothesis that vitamin D_3 is an important endogenous cancer protective agent. Trials of systemic 1,25 $(OH)_2D_3$ and vitamin D_3 analogues are now under way in patients with various malignant diseases [for review, see 60].

■ **Methodological consideration.** Various morphological signs of differentiation are studied. Some of them are specific to the cell type taken into consideration:

- Increase of the nuclear/cytoplasmic ratio (for mouse embryonal carcinoma PCC4 cells, for example).
- Morphological differentiation of human teratocarcinoma NT2 cells in neurons, of epidermal precursors in keratinocytes, of myelopoietic progenitors/stem cells in monocytes-macrophages, and mononuclear precursors in osteoclasts [60, 61].
- Induction of tissue-specific molecular markers: for example in breast cancer cells, expression of milk components (lipid droplets, β-casein mRNA) and down-regulation of the Her2/neu membrane receptor [62].
- Expression of components of the cytoskeleton, like laminin A, actin, cytokeratin, which are considered as differentiation-specific markers [57].

■ **Conclusion.** Induction of cell differentiation could be a valuable surrogate marker. The possibility to use it as a basis for a claim may depend on the use of a set of reliable and complementary markers. The science base would be more solid if the results from different methods are consistent.

■ **Cyclo-oxygenase 2 (COX-2)**

■ **Definition of target.** Cyclo-oxygenase (COX) is an important modulator protein in the eicosanoid pathway. It catalyses the conversion of arachidonic acid to prostaglandins. It is a bifunctional enzyme that has both cyclo-oxygenase and peroxidase activities. There are two COX isoforms that differ both in their tissue distribution and their regulation. COX-1 is a constitutively expressed "housekeeping" gene involved in processes like gastric acid secretion, vascular homeostasis and water reabsorption in the kidney. COX-2 is inducible and thought to be involved in different processes such as inflammation and ovulation [63].

■ **Relationship of target with cancer.** COX-2 is overexpressed in many cancers including oesophagus, stomach, colon, lung, pancreas, head and neck, and prostate [for review, see 64]. Recent studies using animal models provide evidence that COX-2 is mechanistically linked to cancer development: 1) over-expression of COX-2 in transgenic mice induces mammary gland tumours [65]; 2) knocking out of the COX-2 gene in the Apc$^{\Delta 716}$ mouse reduces the number and size of intestinal polyps and of skin papillomas [66, 67].

Both COX-2 and its substrates, the prostaglandins, play an active role in carcinogenesis, since they affect xenobiotic metabolism, angiogenesis, apoptosis, inflammation, immuno-suppression and invasiveness [for review, see 64]. Several population-based studies have shown that regular use of non-steroidal anti-inflammatory drugs (NSAIDs), which are COX inhibitors, decrease the relative risk of colorectal and prostate cancer [68, 69].

■ **Modulation of target by diet.** Inhibitors of the enzyme both reduce the formation of tumours in many tissues and suppress the growth of established tumours [64]. Many studies are currently performed with celecoxib, aspirin, curcumin, and genistein, for example.

It has been demonstrated that lipid components of the human faecal fraction in intimate contact with the epithelium (faecal water) and specific bile acids can alter the transcription of COX-2. Available data support an influence of diet on the levels of the faecal water components. In view of the knowledge that the amount of COX-2 is important, since there is a correlation between its level of expression and the size of the colorectal tumours and their propensity to invade underlying tissue, even small effects over time on the transcriptional regulation of this enzyme by dietary components may be important for tumour development in the colon.

■ **Methodological consideration.** COX-2 expression in cells or frozen sections of animal or human tissues is generally studied by immunohistochemistry or immunoblotting [for a detailed description of the techniques, see ref 70].

■ **Conclusion.** Inhibition of COX-2 activity is a valuable surrogate marker, since convincing data obtained in humans with NSAIDs are available. However the effect of diet is less explored.

The possibility to use it as a basis for a claim may depend on the availability of clinical data for the tissue taken into consideration: COX-2 data are mainly arising from colon (and prostate and breast) cancer, and it is not sure if it is also valid for other types of cancers. Definition of target groups may be particularly relevant for this marker.

■ Potential targets

Several other potential targets (intercellular communication, gene expression, signal transduction, etc), for which numerous but incomplete data are available, are listed in Table 1. However at present, they are further away from the true endpoint and as such can only be used for mechanistic supporting evidence. This field will particularly benefit from a development of research and the use of high scale technologies for genomics and proteomics.

Gut luminal markers

The gut microflora has been implicated in the aetiology of colorectal cancer by a number of studies [reviewed by Mallet and Rowland 71] and these observations form the theoretical basis for use of gut flora biomarkers (faecal biomarkers). They are composed of two main categories: those examining the activity of bacterial enzymes or bacterial metabolites and those based on bioassays on faecal water. A number of these have been used to investigate gut bacterial function [72, 73] and some have potential mechanistic links to colorectal cancer (CRC) aetiology.

■ Bacterial enzymes

A wide range of enzyme activities capable of generating potentially carcinogenic metabolites in the colon is associated with the gut microflora, including β-glucuronidase, β-glucosidase, nitrate reductase and nitroreductase (Table 2). These are usually assayed in faecal suspensions and appear to be present in many bacterial types [74]. Of these enzymes, β-glucuronidase has been the most extensively investigated as a biomarker of CRC risk. It should be noted that these factors are associated with the generation of carcinogens and promoters and do not have a direct link with tumours.

β-Glucuronidase

■ **Definition of target.** Many carcinogenic compounds are metabolised in the liver and then conjugated to glucuronic acid before being excreted via the bile into the small intestine. In the colon bacterial β-glucuronidase can hydrolyse the conjugates, releasing the parent compound or its activated, hepatic metabolite.

■ **Relationship with cancer.** The activity of β-glucuronidase in the colon can alter the likelihood of tumour induction in animal models of CRC. The use of a β-glucuronidase inhibitor administered in conjunction with the carcinogen azoxymethane (which undergoes activation and conjugation in the liver) significantly reduces the number of tumours formed in the rat colon, indicating that microflora β-glucuronidase has a role in tumour induction [75]. Metabolic epidemiological studies have shown that populations at high risk of CRC have high levels of faecal β-glucuronidase activity. Furthermore, faecal β-glucuronidase activity in colon cancer patients is significantly higher than in healthy controls [76].

■ **Modulation by diet.** The activity of β-glucuronidase is influenced by diet. High-risk diets for colorectal cancer have consistently been shown to increase β-glucuronidase activity relative to low risk diets [77]. Furthermore, various types of fibre decrease the activity of β-glucuronidase in rats [78–80].

■ **Methodological considerations.** β-Glucuronidase can be assayed in fresh or frozen faecal samples by a simple reproducible colorimetric procedure. It is usually expressed as mmol p-nitrophenol released/min/g wet weight faeces. There is considerable interindividual variation in activity.

■ **Conclusions.** Although it represents a simple reproducible marker, evidence for a role for β-glucuronidase in human CRC is indirect and is remote from the final endpoint (tumours).

■ Metabolites

A wide range of metabolites with potential genotoxic, tumour promoting and anti-carcinogenic activities have been identified in faeces. These are summarised in Table 3.

N-nitroso compounds

■ **Definition of target.** Nitrate, ingested via diet and drinking water, is reduced by gut bacterial nitrate reductase to its more reactive and toxic reduction product, nitrite. Nitrite reacts with nitrogenous compounds in the body to produce N-nitroso compounds (NOC). The reaction can occur chemically in the acidic conditions prevalent in the human stomach and can also be catalysed at neutral pH by gut bacteria in the colon [81].

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Table 1 Cellular effects: other potential targets

a. Miscellaneous					
Target	Definition	Relationship with cancer	Modulation by diet	Methods	References
Gap junctional intercellular communication (GJIC)	• Gap junctions: channels between adjacent cells permitting direct cell-cell communication. • Important role of GJIC in the control of tissue homeostasis and regulation of cell proliferation, differentiation and apoptosis. • Modulation of GJIC at different levels of regulation: specific connexin gene expression, protein phosphorylation, connexon assembly, cell adhesion and channel gating.	• Dysruption of GJIC involved in carcinogenesis. • Reduced level of GJIC in most human and animal tumours. • Transformed cells (in vitro and in vivo) do not communicate with neighbouring normal cells. • Many tumour promotors inhibit GJIC. • Transfection of connexin genes into communication-deficient malignant cells reduces or eliminates the tumourigenicity of recipient cells.	• Retinoids, carotenoids, and flavonoids (apigenin, tangeretin) enhance GJIC in vitro.	• Dye transfer after microinjection in cell monolayers and tissues. • Expression of tissue-specific connexins by immunoblotting or immunostaining. • Electrophysiology. • Metabolic cooperation. • Photobleaching. • Scrape loading.	[240–244]
ODC	• Ornithine decarboxylase (ODC) -catalysing formation of putrescine from ornithine: a critical step in polyamine biosynthesis. • Role of polyamines in cell proliferation, differentiation and malignant transformation.	• ODC participates in carcinogenesis. • Blocking ODC in vitro inhibits cell transformation. • ODC is now considered a putative proto-oncogene.	• Retinoids, green-tea polyphenols and flavonoids inhibit ODC activity in vitro.	• ODC activity. • Spermidine/spermine ratio.	[245–247]
Telomerase	• Senescence of human cells (after 50–70 divisions) due to telomere attrition. • Human telomeres consist of repeated TTAGGG sequences on the 3'end of single-strand-DNA. They cap and protect the ends of chromosomes against degradation, inappropriate recombination and interchromosomal fusions. • During the successive rounds of cellular division driven by DNA polymerases, incomplete replication of the telomere results in a loss of telomeric repeats.	• Human telomerase prevents telomere erosion and is able to synthesise new telomeric repeats. • Telomerase is detected in more than 85 % of cancers, and is usually undetectable in benign tumours and in normal tissues surrounding tumours. • Telomerase inhibition leads to an immediate induction of apoptosis.	• Dietary restriction inhibits liver telomerase activity in vivo.	• Enzyme activity. • Fiber optical biosensor for enzyme activity detection in tumours.	[186, 248–250]
Lipoxygenases	• Lipoxygenases (5-LOX, 8-LOX, 12-LOX, 15-LOX) catalyse the conversion of arachidonic acid to hydroperoxyeicosatetraenoic acid (PHETE) and leukotrienes.	• LOX products involved in development and progression of human tumours. • Abnormal expression and activities of LOX in pancreatic cancer. • LOX inhibitors reduce chemically-induced carcinogenesis, and induce apoptosis in human cancer cells.	• Inhibition of LOX by curcumin, phenolic compounds of olive oil.	• Enzyme activity. • LOH products.	[251–254]

Table 1 *Continued*

b. Gene expression					
Target	Definition	Relationship with cancer	Modulation by diet	Methods	References
Chromatin remodeling — DNA methylation	• In normal mammalian genome, DNA methylation only on cytosines 5′ to guanosines at CpG dinucleotides. • Catalysed by methyltransferases. • It regulates gene expression and chromosomal stability.	• Abnormal DNA methylation in most cancers: global DNA methylation accompanied by gene-specific hypermethylation. • Hypermethylation of CpG islands associated with inactivation of DNA repair, cell cycle regulation, inflammatory/stress response and apoptosis.	• Selenium, folate, vitamin B12, choline, methionine, retinoic acid and isoflavones affect DNA methylation status.	• Comet assay. • Microarrays. • HPLC.	[255–258]
Chromatin remodeling — Histone acetylation	• Nucleosomes (histone octamere and 147 base pairs of DNA) repress transcription by blocking the binding of transcription factors and basal transcription machinary to promoter regulatory sequences of genes. • Hypoacetylation of lysine residues in the flexible N-terminal histone tails is correlated with transcriptional repression, whereas hyperacetylation is correlated with activation.	• Misregulation of chromatin structure can cause incorrect gene activation or improper gene silencing. • Certain oncogenic transcription factors promote oncogenesis by misregulating chromatin structure. Conversely, tumour suppressors (Rb, P53 …) utilise chromatin remodeling as part of their normal function.	• Sodium butyrate and diallyl disulfide are known to induce histone hyperacetylation via the inhibition of histone de-acetylase.	• Immunoblotting of nuclear proteins using antibodies directed against total or specific acetylated lysines of different histones. • Histone acetylase and deacetylase assays.	[259, 260]
NF-κB	• The Rel/NF-κB family: a group of structurally-related and tightly-regulated transcription factors that control the expression of a multitude of genes. • They exist in cytoplasmic complexes with inhibitory proteins of the IκB family, and translocate to the nucleus to act as transcription factors when activated.	• Misregulation of the Rel/NF-κB signal transduction pathway in human cancers. • Inhibition of Rel/NF-κB activity reverses all or part of the malignant state. • Tumour promotors induce AP1 activity, in vitro.	• Curcumin, green tea polyphenols, genistein, resveratrol and apigenin inhibit the induction of NF-κB.	• Western blotting.	[261, 262]
AP-1	• Activator protein-1 (AP-1) transcription factor, a heterodimeric protein complex composed of the products of members of the *fos* and *jun* gene family, binds to DNA sequences and induces the expression of various genes.	• Many of the genes of the fos and jun family are expressed in transformed rapidly-growing cells.	• Resveratrol inhibits TPA and UVC- induced AP-1 activity. • Human faecal water components can induce AP-1 DNA-binding activity and augment AP-1-responsive gene expression in human cultured colonocytes.	• DNA binding.	[263, 264]

■ **Relationship to cancer.** The term NOC covers a wide range of compounds including N-nitrosamines, N-nitrosamides, N-nitrosoguanidines and N-nitrosoureas, the majority of which are highly carcinogenic, DNA alkylating agents. However, the genotoxic or carcinogenic activity of the NOC produced by the bacterial N-nitrosation process in the large intestine has not yet been established.

■ **Modulation by diet.** Faecal apparent total NOC (ATNC) excretion is increased by red meat consumption [82–84]. In conjunction with high meat intakes, wheat

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Table 1 *Continued*

c. Signal transduction					
Target	Definition	Relationship with cancer	Modulation by diet	Methods	References
Farnesyl protein transferase	• Prenylation: a type of lipid modification involving covalent addition of either farnesyl (15-carbon) or more commonly geranylgeranyl (20-carbon) isoprenoids via thioether linkages to cysteine residues at or near the C terminus of intracellular proteins.	• Prenylation is catalysed by the farnesyl protein transferase (FPT): the first step in the processing of the oncogenic Ras proteins. • Ras is often overexpressed in tumours.	• Several monoterpenes, including limonene and perillyl alcohol inhibit farnesyl protein transferase.	• Measure of the enzymatic activity.	[265, 266]
Protein kinases	• Many signal transduction events involve phosphorylation steps. Activation of protein kinases is a key mechanism in regulating signals for cell proliferation. • The mitogen-activated protein kinases (MAPKs) convert various exogenous signals into intracellular responses through serial phosphorylation cascades. Three distinct and parallel MAP kinases cascades (ERK, JNK and p38) have been identified.	• The substrates of protein kinases include transcription regulatory factors, for example, the proteins encoded by the jun, fos, myb, rel and ets proto-oncogenes.	• Various polyphenolic compounds (EGCC, flavonoids, resveratrol) inhibit MAPK pathways.	• Enzyme activities. • Western blotting.	[267]
P27 Tumour suppressor function	• Central role in proliferation, differentiation and cell death. It inhibits cyclin/cyclin-dependent kinase complexes, thereby blocking cell cycle progression. • Regulated post-transcriptionally, at the level of both protein translation and stability, through phosphorylations and ubiquitination involving the ubiquitin/proteasome pathway.	• In contrast to most tumour suppressor genes (Rb, p53), which are recessive at the cellular level, p27 is happloinsufficient for tumour suppression. • Skp2, the specific recognition factor for p27 ubiquitination has oncogenic properties.	• Apigenin (and NSAIDs) upregulates p27 in vitro.	• Western blotting.	[268–270]

bran, resistant starch and vegetable consumption had no effect on faecal ATNC excretion or concentration [82, 84, 85].

■ **Methodological considerations.** N-nitroso compounds in faeces can be monitored using a group-selective method that determines ATNC in faeces and biological fluids by a procedure employing a thermal energy analyser [81, 86].

Secondary bile acids

■ **Definition of target.** The primary bile acids, chenodeoxycholic acid and cholic acid, are subject to extensive metabolism, predominantly 7-α-dehydroxylation, by the intestinal microflora [87], which converts cholic to deoxycholic acid (DCA) and chenodeoxycholic to lithocholic acid (LCA). These are termed secondary bile acids.

■ **Relationship to cancer.** The secondary bile acids exert a range of biological and metabolic effects *in vitro* and in animal models including cell necrosis, hyperplasia

Table 2 Faecal bacterial enzymes

Target	Definition of target	Relationship with cancer	Modulation by diet	Methodology	Reference
β-Glucuronidase (GN)*	Hydrolyzes biliary conjugates in the colon releasing potential carcinogens.	Populations at high risk of CRC have high GN activity.	High risk diets for CRC increase GN in human and animal studies.	Analysed by chromogenic substrate. Simple, rapid. Can be assayed in frozen samples.	[77, 271]
β-Glycosidase	Hydrolyzes glycoside conjugates of plant compounds.	Some plant aglycones have mutagenic and carcinogenic activity. Others act as anti-genotoxins.	Poor relationship to high risk diets.	Analysed by chromogenic substrate. Simple, rapid. Can be assayed in frozen samples.	[77, 271]
Nitrate reductase	Reduces nitrate to more reactive nitrite.	Nitrite is precursor of faecal NOC.	No consistent response to dietary change.	Spectrophotometric analysis, needs fresh faecal sample and strict anaerobic conditions.	[272]
Nitroreductase	Reduces nitro compounds to amines.	Some amine products carcinogenic.	No consistent response to dietary change.	Spectrophotometric analysis, needs fresh faecal sample and strict anaerobic conditions.	[272, 273]
IQ oxidoreductase	Converts IQ to 7-OHIQ.	IQ is a known dietary carcinogen requiring activation. The bacterial enzyme converts IQ to a direct acting genotoxin.	Increased by high risk CRC diets in rats.	Radiolabel chromatography assay, anaerobic conditions needed.	[274]

* For further details see main text

Table 3 Faecal metabolites

Target	Definition of target	Relationship with cancer	Modulation by diet	Methodology	Reference
Ammonia	Formed by bacterial catabolism of protein and urea in colon.	Tumour promoter in rats; induces colon cell proliferation.	High-risk diets for CRC increase faecal ammonia in rats.	Simple chromogenic assay.	[271, 275]
N-Nitroso compounds (NOC)*	Formed by bacterial action in colon from nitrite and amino compounds.	Many NOC are potent carcinogens (but activity of faecal NOC not known).	Increased by high red meat diets.	Difficult, expensive analysis (Thermal Energy Analyser).	[83, 86]
Diacylglycerols (DAG)	Produced by gut bacteria from phospholipids and triglycerides.	Activate protein kinase C. Humans with high fat content in faeces have high DAG in gut and epithelial hyperproliferation.	Faecal DAG increased by high fat diets in rats and mice. DAG decreased by wheat bran in humans.	Lipid extraction, then commercial kit.	[88, 276, 277]
Fecapentaenes	Glycerol ethers containing a pentaene moiety. Produced by gut bacteria *in vitro*. Detected in faeces in some subjects.	Direct acting mutagens in Ames test. Associated with increased cell proliferation, tumour promotion in some but not all studies. Inconsistent associations with CRC.	Faecal excretion of fecapentaenes is lower in vegetarians.	Difficult, hplc method	[88, 278–280]
Secondary Bile acids*	Formed by bacterial metabolism of cholic and chenodeoxycholic acids.	Tumour promoters, inducers of cell proliferation, genotoxic. Concentration of faecal bile acids higher in populations and individuals at high CRC risk.	High fat intake increases faecal bile acid concentrations.	Analysis by GLC or HPLC, time consuming extraction required.	[88, 89, 96]
Short chain fatty acids (SCFA)*	Major SCFA are acetic, propionic and n-butyric acids. Produced by fermentation of carbohydrates in colon.	Butyric acid has trophic effect on colonic epithelium. *In vitro* butyric acid induces apoptosis and differentiation of colon cancer cell lines, but increases cell proliferation in normal colon *in vivo*.	SCFA increased (usually) in subjects consuming diets high in fibre, resistant starch or non-digestible oligosaccharides.	Analysis by GLC. Simple, rapid.	[281]

* For further details see main text

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and tumour promoting activity in the colon, induction of DNA damage, and apoptosis [reviewed by 88]. It has also been suggested that secondary bile acids influence colorectal cancer by selecting for apoptosis-resistant cells or by interacting with various secondary messenger signalling systems. Epidemiological studies indicate that concentrations of secondary bile acids are higher in populations at high risk of CRC and in case control studies 7-α-dehydroxylase activity is higher in cases than controls. A number of human observational studies in patients with adenomas or CRC have reported a correlation between faecal bile acid (FBA) concentrations and CRC risk [89–92]. Some studies [93, 94] have also suggested that high DCA concentration and the DCA to LCA ratio are associated with increased CRC risk. It should be noted however that not all studies have confirmed these relationships between bile acids and cancer risk [95].

■ **Modulation by diet.** In human studies, high fat intake, which correlates with CRC risk, increases FBA concentrations [96], whereas increased consumption of wheat bran (negatively correlated with CRC risk) reduces FBA concentration.

■ **Methodological considerations.** Measurement of FBA is usually performed by soxhlet extraction of faecal samples followed by gas chromatographic analysis.

Short chain fatty acids

■ **Definition of target.** The short chain fatty acids (SCFA) acetate, propionate and butyrate are the principal end products of carbohydrate fermentation. These are absorbed from the colonic lumen and metabolised by various body tissues [97]. Butyrate is preferentially metabolised by colonocytes [98].

■ **Relationship to cancer.** Butyrate is the most intensively studied SFCA. Interest in butyrate's role as a possible antineoplastic agent has arisen from its potential to inhibit proliferation of cells *in vitro*, including colon tumour cell lines [99]. Moreover, at physiological concentrations, sodium butyrate induces apoptosis in human colon carcinoma cell lines [100].

In addition, reduced pH as a consequence of SCFA production, has also been implicated as a protective factor against colon cancer [101, 102]. This may be as a result of altering the solubility of bile acids, or altering the metabolic activity of colonic microflora.

■ **Modulation by diet.** There is evidence from in vitro studies and animal models (where caecal SCFA concentrations can be measured) that the type of carbohydrate has an important influence on the amount and proportions of SCFA produced, with starch and wheat bran being particularly associated with elevated butyrate production [103]. In human studies, inulin has been shown to enhance excretion of total SCFA in human faeces, whereas wheat bran increased absolute or relative proportions of butyrate in faeces [104]. Where the butyrate is produced relative to proximal and distal regions of the colon is important and should be a methodological consideration.

■ **Methodological considerations.** Measurement of SCFA in faeces is by a simple gas chromatographic method, but the value obtained represents the balance of bacterial production and the extensive colonic absorption, so may not reflect accurately the concentrations of SCFA in the colonic lumen [97].

■ **Conclusions for sections on Bacterial enzymes and Metabolites.** Gut bacterial enzymes and faecal metabolites are relatively simple to measure routinely and in general may be of use in assessing effects of diet on modulating exposure of the colon to potential carcinogens, rather than reflecting cancer risk.

■ Faecal water activities

Faecal water cytotoxicity

■ **Target.** There is considerable evidence that colon tumours are a result of gut luminal factors damaging the mucosa. Furthermore, free reactive and soluble factors are more likely to affect the epithelium than substances bound to the insoluble matrix such as fibre. Therefore, an alternative approach to assaying enzymes or metabolites in faeces is to assess toxicological activity of fractions using short-term tests for toxicity, genotoxicity and mutagenicity. Usually the aqueous phase of the human faeces (faecal water) is used [105, 106], since this will contain most of the free reactive species. For assessment of faecal water cytotoxicity, the effect on proliferation of human colon carcinoma cells in culture is used.

■ **Relationship to cancer.** Proliferative zone expansion in the colonic crypts and an increased rate of epithelial proliferation are considered to be an early step in carcinogenesis. Stimulation of proliferative activity in colonic epithelium may in part be mediated via cytotoxic mechanisms, resulting in increased cell loss at the epithelial surface and a compensatory rise in mitotic activity of the crypts. Such considerations led to the development of assays to assess cytotoxic activity in faecal water towards colon cells *in vitro* [105]. It is thought that bile acids, especially secondary bile acids, make a major contribution to faecal water cytotoxicity [105]. In a comparison of faecal water cytotoxicity in patients at low (no colon adenomas), medium (small colorectal adenomas)

and high (large tubular adenomas) risk of CRC, no significant differences between the groups were observed [95].

■ **Modulation by diet.** Interventions using dietary regimes associated with increased or decreased CRC risk have been shown to modulate appropriately faecal water cytotoxicity. For example, dietary calcium has frequently been shown to reduce the cytotoxicity of faecal water presumably by precipitating soluble bile acids [106, 107]. Faecal water cytotoxicity was higher in subjects on a high fat, low calcium, low fibre diet compared with those on a low fat, high calcium, high fibre regime [105]. In rats, high red meat consumption increases the cytotoxicity of faecal water. This effect was independent of the fat and bile acid content of the faecal water and may be related to dietary haeme [108, 109].

■ **Methodological considerations.** Human colon cells (e.g Caco-2 or HT29) are exposed to faecal water fractions (prepared by high speed centrifugation of fresh faecal samples) and inhibition of cell proliferation is measured usually using a dye such as MTT [110].

Faecal water genotoxicity

■ **Definition of target.** Venturi et al [111] demonstrated the presence of DNA damaging activity towards human cultured colon cells in samples of faecal water from healthy human subjects. A wide variation was found ranging from negligible to high activity. The presence of genotoxic activity in faecal water can be considered to reflect exposure of the colonic mucosa to carcinogens.

■ **Relationship to cancer.** There is now convincing evidence that CRC is induced by a series of mutational events in a number of critical genes [112]. Sporadic colorectal tumours have been shown to contain mutations and deletions in oncogenes and tumour suppressor genes such as Apc, K-ras and p53. DNA damage has been detected in biopsies of colon tissue derived from laboratory animals and human subjects. Thus, the presence in the colonic lumen of DNA damaging agents could represent an important risk factor for CRC. There are as yet no reports of validation studies for the endpoint in patients at different risk of colorectal cancer.

■ **Modulation by diet.** In healthy subjects, a diet high in fat and meat, but low in dietary fibre (hence considered to be of high CRC risk) was associated with a significantly increased faecal water genotoxicity by comparison to a diet low in fat and meat [113].

■ **Methodological considerations.** Human colon carcinoma cell lines (e. g.Caco-2 and HT29) are exposed to faecal water fractions and genotoxicity is usually mea-

sured using the single cell gel electrophoresis (Comet) assay, which provides a simple, rapid and sensitive method for measuring single strand breaks in DNA. The intra- and interindividual variation in faecal water genotoxicity has been studied in healthy individuals [114].

■ **Conclusions.** Cytotoxicity and particularly genotoxicity of faecal water have a good mechanistic link with colon carcinogenesis and hence provide potentially valuable, non-invasive methods for assessing colorectal cancer risk in human subjects. However, there is a need for more extensive validation of these endpoints.

■ **Diagnostic markers in faeces**

There are several tumour markers that can be detected in faeces. Haemoglobin is currently used for mass screening for colon cancer (faecal occult blood test). Haemoglobin is not continuously, but intermittently leaking through the intestinal wall, thereby compromising the sensitivity of the test. Other stool tumour markers, such as abnormally glycosylated mucins, are continuously secreted. However immuno-chemical methods to detect these mucins in stool samples have failed to accurately detect neoplasia, probably due to luminal instability [115]. Most promising are exfoliated markers, such as whole colonocytes and mutant DNA. Tumours exfoliate large amounts of epithelial cells that can be recovered from stool. Using RT-PCR, genes that are overexpressed in tumours can be demonstrated in these cells. Even more promising are neoplasm-specific DNA alterations, as DNA appears to be stable in the gut lumen. Several investigators have recovered mutant DNA in stools from colon cancer patients, mostly using K-ras mutations as marker [reviewed by 116]. More recently, the specificity of this test was improved by targeting multiple DNA mutations (including K-*ras*, APC and p53), to minimalise false-positives and false-negatives [117]. Long DNA (> 200 base pairs) appeared to be the most informative stool marker in this study. Faecal DNA testing holds a promise for the future as a non-invasive tumour marker, as a single stool specimen will be sufficient to accurately detect both precursor adenomas and carcinomas.

■ **Conclusions.** Of the considerable number of potential prognostic or predictive indicators of colon cancer based on faecal markers, only faecal occult blood has achieved widespread clinical application. However, it has limited usefulness as an indicator of cancer risk for assessment of functional foods. Validation of the newer techniques should be the goal for future research.

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Angiogenesis and metastasis

■ Inhibition of angiogenesis

■ **Definition of target.** Tumours require blood supply to develop and grow. They take over existing blood vessels and stimulate production of new vessels from these – a process termed angiogenesis.

■ **Relationship to cancer.** Angiogenesis is a crucial early event in tumour progression, beginning in premalignant lesions [see review by Ruoslahti 118]. The key initiators of endothelial cell growth are the vascular endothelial growth factors (VEGFs), which act through a family of tyrosine kinase receptors (VEGFRs) expressed by angiogenic vessels. Angiopoietins and platelet-derived growth factors are also involved and subsequently the vessels acquire supporting cells such as pericytes, smooth muscle cells and extracellular matrix.

Another group of receptors, integrins, expressed by endothelial cells in angiogenic vessels, also play an important role in the process of angiogenesis by mediating cell adhesion and transmitting signals from the extracellular matrix to cell types that regulate cell growth and survival. It has been found in neuroblastoma that the level of expression of the integrins reflects the grade of malignancy.

Given that in the absence of a blood supply, a tumour will become hypoxic and die, angiogenesis provides a logical target for therapeutic and preventative agents. A number of potential anti-angiogenic agents are currently undergoing clinical trials. These substances include drugs, peptides and antibodies that target vascular growth factors and integrins and also compounds such as endostatin, identified in activity based assays.

■ **Modulation by diet.** In vitro assays have been used to demonstrate potential anti-angiogenic effects of NSAIDs [119] and of various dietary components such as genistein [120] and conjugated linoleic acids (CLA [121]). In vivo, dietary CLA was shown to suppress angiogenesis in a mouse matrigel model [121]. In the CLA fed mice, the injected gel pellets contained fewer infiltrating cells forming only limited branching networks. Dietary CLA also decreased serum levels of VEGF. In a study of a novel oestrogen metabolite, 2-methoxy-oestradiol, Fotsis et al. [122] demonstrated that, when administered orally in mice, it strongly inhibited the neovascularisation of solid tumours and suppressed their growth.

■ **Methodological considerations.** A range of *in vitro* assays for assessing angiogenesis inhibition have been used including
- Reduction in proliferation of endothelial cells stimulated by bFGF or VEGF.

- Inhibition of endothelial differentiation into capillary like structures on Matrigel.
- Suppression of bFGF or VEGF-stimulated invasion of collagen by endothelial cells.

There are a number of *in vivo* mouse models based on modulation of angiogenesis after stimulation with various growth factors such as fibroblast growth factor –2 [119] or sub-cutaneous injection with Matrigel followed by measuring infiltration of the gel with blood vessels [121].

Potential biomarkers of angiogenesis for human studies: There is considerable interest in the use of plasma VEGF and bFGF as indicators for prognosis in cancer patients with a range of tumour types, including colorectal, myeloma and lung. If substantiated, this could provide a biomarker for intervention studies, although severe ethical problems could arise. At present the results are variable, with some studies indicating a good correlation with other prognostic indicators and others showing poor associations [123–125].

■ **Conclusions.** Inhibition of angiogenesis represents a potentially useful target for dietary compounds and there is a wide range of in vitro and in vivo assays that can be used to screen potential candidates. However, the efficacy of such substances in patients may be questioned on the basis of current experience with drugs. Furthermore, given the importance of angiogenesis for normal tissues, there is likely to be little or no use for functional foods with anti-angiogenic activity for the general population. In other words, target groups must be carefully specified in order to avoid anti-angiogenesis diets being deleterious.

■ Inhibition of metastasis

■ **Definition of target.** The metastatic escape and spread of cells from a tumour to distant organs is a great barrier to cancer cure. The process involves a complex series of interactions between the cancer and its environment.

■ **Relationship to cancer.** Epithelial cells normally lie between thin sheets of basement membranes and are surrounded by an extracellular matrix, which they secrete themselves. In order to become successfully invasive, tumour cells must adhere to the basement membrane (BM), produce and secrete proteolytic enzymes that are capable of degrading the extracellular matrix (ECM) and BM, adhere to and degrade the ECM components of host cells and regulate genes encoding for proteolytic enzymes for ECM degradation and adhesion. Degradation is achieved by enzymes termed Matrix Metalloproteinases (MMPs). MMP activity is inhibited by natural tissue inhibitors of MMPs (TIMPS) and the

balance between MMP and TIMP activity is a strong indicator of metastasis. MMP's are also involved in some of the later events in the metastatic process, namely intravasation (in which the cancer cells enter the blood or lymphatic vessels), extravasation (passage of the cell from the vessels into tissues) and proliferation of the cells at the secondary tissue [126].

■ **Modulation by diet.** N-3 fatty acids have been shown to inhibit invasion of kidney tumour cells using the Matrigel invasion assay [127]. Animal models have been used to evaluate effects of food components on metastasis. For example retinoids suppress the development of bone metastasis of prostate cancer cells [128] and modified pectin inhibited metastasis of human colorectal and breast carcinoma cells [129]. Thus they provide a method for assessing functional foods for potential anti-metastatic activity, although the relevance to the human situation remains to be established. There is some evidence from less specialised animal models that dietary change can modulate late events in cancer. For example, probiotic treatment of rats given dimethylhydrazine to induce colon tumours, resulted in a reduction in tumour size and a lower proportion of malignant tumours [130].

■ **Methodological considerations.** The Matrigel Invasion assay (MIA) is an established *in vitro* assay to assess cell invasion. Matrigel contains components of the basement membrane and only cells with invasive properties will pass through this layer. In this assay, cells from a metastatic tumour line are seeded onto matrigel-coated tissue culture inserts containing an 8-micron pore size membrane. The matrigel occludes the pores of the membrane blocking non-invasive cells from migrating through the membrane. In contrast, invasive cells are able to detach themselves from and migrate through the matrigel matrix treated membrane in response to a chemoattractant present in the underlying medium.

There are also a number of animal models (usually based on athymic mice) that are used to study the whole process of metastasis, in which the tumour cells are inoculated subcutaneously or orthotopically and the number of spontaneous metastases to secondary sites such as lung liver and bone are assessed [131]. A wide range of tumour cell lines have been used as the source of the inoculum including colorectal tumour cells, melanoma, prostate cell lines, T cell lymphomas and mammary adenocarcinomas [126, 132–135].

In humans, detection of circulating tumour cells in bone marrow and lymph nodes by immunocytochemistry is a well-defined prognostic marker for colorectal cancer progression, especially metastasis [136]. The presence of micrometastases in bone marrow appeared as an independent indicator of an aggressive tumour with a poor outcome [137]. More recently, the detection of tumour cells in the blood circulation is proving a promising biomarker of tumour presence and progression in humans. Longitudinal studies have shown that the levels of circulating epithelial cells in peripheral blood are directly linked to the tumour burden and response to therapy in e. g. prostate cancer [138]. In animals, clusters of tumour cells were found to be a more relevant prognostic marker than single cells [139]. A new technique, called immunomagnetic cell separation, was recently used to show that cell clusters were also present in peripheral blood samples of colon carcinoma patients [140]. It is believed that the vast majority of these cells originate from the primary tumour [141].

■ **Conclusions.** Inhibition of metastasis represents a potentially useful target for dietary intervention and there is a wide range of in vitro and in vivo assays that can be used to screen potential candidates.

■ **Tumour cells in circulation**

As blood samples can be obtained easily and repeatedly, this makes the detection of tumour cells in blood circulation a very promising biomarker of tumour presence and progression.

Carcinogen metabolising enzymes

Metabolism is often the major process responsible for the elimination of a chemical, and hence is of key importance as a target of functional effects. Metabolism includes phases of biotransformation and of toxicokinetics. Biotransformation is the process whereby a substance is changed from one chemical to another *(transformed)* by a chemical reaction within the body [142]. During this process a large number of phase I and phase II enzymes metabolically convert lipophilic xenobiotics to hydrophilic metabolites. This facilitates their cellular elimination (phase III) and excretion, thus minimising exposure to them.

■ **Biotransformation of carcinogens**

■ **Definition of target.** Phase I enzymes (e. g. cytochrome P450, flavin-dependent monooxygenases, oxidoreductases) convert hydrophobic compounds to reactive electrophiles by oxidation, hydroxylation and reduction reactions to prepare them for reaction with water-soluble moieties. Phase II enzymes (e. g., glutathione S-transferases [GST], UDP- glucuronosyltransferases [UGT], sulphotransferases, aryl amine acetyltransferases) primarily catalyse conjugation reactions.

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■ **Relationship with cancer.** Biotransformation enzymes play a major role in regulating the toxic, mutagenic and neoplastic effects of chemical carcinogens (Table 4). Phase I enzymes, particularly the cytochrome P450 s, have the propensity to result in bioactivation, compared with the inactivation that often results from Phase II reactions. This has led to the concept that selective induction of phase II enzymes over phase I will contribute to cancer chemoprevention. An example is the agent [4-methyl-5-(2-pyrazinyl)–3H–1, 2-dithiole-3-thione] (oltipraz) which may protect against liver cancer caused by high aflatoxin B1 exposure in Qidong, People's Republic of China [143, 144]. The studies have shown that intermittent, high-dose oltipraz inhibited phase I activation of aflatoxins, whereas sustained low-dose oltipraz increased phase II conjugation of aflatoxin B1, yielding higher urinary levels of aflatoxin-mercapturic acid [145]. There are numerous other reports indicating that the balance of metabolising enzymes are directly associated with the magnitude of toxic response for selected chemicals [146]. Genetic enzyme polymorphisms for genes encoding phase I and phase II metabolism are

Table 4 Carcinogen metabolism

Target	Relationship with cancer	Modulation by diet	Methodology	Reference
Phase I				
Cytochrome P450 (CYP) CYP1A1 CYP1A2 CYP2A6 CYP2B6 CYP2D6 CYP2E2 CYP3A4	Activate carcinogens polycyclic aromatic hydrocarbons heterocyclic amines, nitrosamines cyclophosphamide tobacco specific nitroamine vinylchloride, aflatoxin	Broccoli induces CYPs in rat tissues; dietary fibers differently modulate CYPs in rat tissues; oltipraz inhibits CYPs that activate aflatoxin; flavonoids modulate CYPs, Ffied meat induces CYP1A2	CYP540 can activate and deactivate reactive xenobiotics, therefore possible inhibition effects on their own are not considered to be beneficial. Several polymorphisms are known	[147, 282–292]
Flavin-containing monooxygenases (FMO) EC 1.14.13.8 FMO3 FMO2 FMO1	Oxidise xenobiotics with broad substrate specificity. Metabolic conversion may result in detoxication. Substrates are tertiary, secondary and primary amines.	Gaps in knowledge, reviewed in [293]	Bioactivation of substrates may not be excluded. Polymorphism leading to gene product with higher rates of metabolic conversion has been reported.	[293–296]
NAD(P)H Quinone oxidoreductases (NQO, QR) EC 1.6.99.2.	Can either bioactivate or detoxify quinones; contributes to reducing superoxide radical formation. Lower NQO1 activity may make individuals vulnerable to leukemia secondary to benzene exposure	Numerous plant food extracts or ingredients induce QR in cell models	Both bioactivation and detoxification of substrates is possible.	[297–304]
Phase II				
Glutathione S-transferases (GST) EC 2.5.1.18. GSTA GSTM GSTP GSTT	Catalyse conjugation of reduced glutathione to activated substrates. Metabolic conversion usually results in detoxication	Induction of GST has been shown for a high number of food ingredients, metabolites in animal experiments, cell cultures and in humans.	Species differences in gene regulation. Bioactivation of selected xenobiotics by GSTs is possible. Genetic polymorphisms (deletions, sequence alterations) are common	[169, 178, 191, 305–308]
UDP-Glucuronosyltransferases (UGTs) EC 2.4.1.17. UGT1A UGT2A UGT2B	Glucuronidation of endo-, xenobiotics usually results in termination or reduction of biological activity. Induction of UGTs is associated with decreased cancer related activities in some animal experiments	Green tea induces UGTs and enhances glucuronidation of 2-amino-1-methyl-6-phenyl imidazo [4.5b]pyridine (PhIP) . Feeding green tea lowers incidence of preneoplastic lesions in animal experiments.	Glucuronide formation can also result in bioactivation. Allelic variants of UGT1A1 may cause specific clinical conditions. Putative phytoprotectants (e. g. isoflavones) are UGT substrates	[309–314]
Arylamine acetyltransferases (NAT) EC 2.3.1.5. NAT2 NAT1	Catalyse three reactions involving the transfer of an acetyl moiety, resulting in N-acetylation (NAT), O-acetylation (OAT), N,O-transacetylation (AHAT). Substrates include carcinogenic aromatic amines and heterocyclic amines (food contaminants)	Gaps of knowledge: Ethanol has been shown to increase acetylation rate [Lester 1964, reviewed in [315]]	Depending on structure of the substrate, the reactions can lead to detoxification or bioactivation, e. g. of heterocyclic amines. Polymorphisms affect toxicological responses under specific exposure situations	[181, 184, 290, 315, 316]

well recognised susceptibility factors for sporadic tumours, depending on the exposure situation and functional consequences of the polymorphisms [147–150]. The induction of specific enzymes involved in the metabolism of known risk factors can result in an enhancement or reduction of exposure and accordingly will modulate cancer related endpoints in model or animal systems [151–154] and in humans [155],

■ **Modulation of target by diet.** A claim by which phytochemicals from plant foods could be of benefit is that they modulate biotransformation in such a manner that carcinogens are less active and thus exert less harm in the target cells of cancer, This is considered to be one of the mechanisms by which fruits and vegetables act chemoprotectively [156], One example is chemoprotection conferred by cruciferous vegetables, due to their high glucosinolate content and the capacity of glucosinolate metabolites to modulate biotransformation enzyme systems (e. g. cytochrome P450 and conjugating enzymes) [157]. Glucosinolates (β-thioglycoside-N-hydroxysulfates) are hydrolysed by the plant enzyme myrosinase releasing the biologically active isothiocyanates (ITC). ITCs induce expression of phase I and phase II enzymes and also directly inhibit CYP450 [120]. Broccoli and Brussels sprouts increase metabolism of cooked meat-derived heterocyclic aromatic amines, which implies that they induce both CYP1A2 and phase II enzymes involved in heterocyclic amine metabolism [158]. The results of six cohort studies have shown inverse associations between the consumption of Brassica vegetables and risk of lung cancer, stomach cancer, and all cancers taken together (reviewed in [159]). Two thirds of 74 case-control studies have also shown an inverse association for risks of several cancer sites including the colon (reviewed in [159]). In a recent study [160] dietary ITC intake from cruciferous vegetables was assessed for 213 incident cases of colorectal cancer and compared to 1194 controls. The additional analysis of GSTM1, T1 or P1 genotypes revealed that there were no overall associations between genotype and colorectal cancer risk. However, among individuals with both GSTM1 and T1 null genotypes, a 57 % reduction in risk among high versus low consumers of ITC was observed for colon cancer. The results are compatible with the hypothesis that ITCs from cruciferous vegetables modify risk of colorectal cancer in individuals with low GST activity. In the gut lumen the glucosinolate breakdown product sulforaphane can inhibit DNA adduct formation induced by a heterocyclic amine in a dose-dependent manner, possibly acting through the induction of phase II detoxification enzymes such as glutathione transferases and UDP-glucuronosyltransferases [161].

■ **Methodological considerations.** Some plant food ingredients (ITCs) induce phase I enzymes, others induce only phase II enzymes, and some induce both [158–162]. The situation is uniquely complex because each plant food contains a variety of glucosinolates and other phytochemicals [163], which modulate biotransformation systems in multiple ways. Therefore it is not easily predictable, in which manner modulated biotransformation results in risk reduction. More accurate knowledge is needed on whether carcinogens cause specific types of tumours and how these carcinogens are metabolically transformed in the relevant human tissues. It is also necessary to increase our understanding on how diet can modulate the biotransformation in a favourable manner, and how individual susceptibilities (genetic polymorphisms, regulation of the target genes, baseline expression levels) affect biotransformation and exposure. The elucidation of these complex situations will require experimental approaches of equal complexity, e. g. to elucidate multiple genes and pathways of induction arising from the impact of diet. Promising approaches to achieve the analysis are the employment of new techniques of genomics to reveal patterns of gene expression (transcriptomics), revealing complete metabolic pathways (metabonomics) and assessing the functional consequences on cellular and organism levels. The criteria for this approach, which are being developed in the field of toxicogenomics [164] and are already applied in pharmacogenetics [165] will also be an important scientific basis for better understanding dietary chemoprevention.

■ **Conclusion.** It is very feasible, from a theoretical point of view, to provide humans with foods, which enhance the capacity of tissues to metabolise chemical carcinogens in such a way that these are eliminated from the body without being transformed to reactive intermediates. The selective induction of Phase II over Phase I could mediate such protection, provided that the resulting conjugates are not more reactive than the substrates. Taken together carcinogen metabolising enzymes could be considered as potential "susceptibility markers".

Glutathione S-transferases

The glutathione S-transferases (GSTs) are dealt with in more detail below as they represent one of the more interesting specific targets within the general target "biotransformation of carcinogens".

■ **Definition of target.** The glutathione S-transferases (GSTs) are a multigene family of enzymes largely involved in the detoxification of chemicals. They catalyse the conjugation of the tripeptide glutathione via its sulphur atom to many toxins containing an electrophilic functional group, allowing these compounds to be excreted from the body [166]. The best-characterised GST isoenzymes in mammals have been grouped into four

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major cytosolic classes, termed Alpha (A), Pi (P), Mu (M), Theta (T), and three membrane-bound GSTs [167].

■ **Relationship with cancer.** Oxidative molecular damage in cells is linked to degenerative diseases including cancer [168]. Glutathione S-transferases contribute to resistance against oxidative stress [169] by inactivating toxic and mutagenic alkene products of oxidative processes and thus protect cells and tissues [170]. An important factor is that GSTs are inducible and that in rat and mouse induction appears to be mediated through the antioxidant response element (ARE), a transcription enhancer [171, 172]. It contains sequences required for basal expression of, for example rGSTA2, and its induction is mediated by phenolic antioxidants, for example green tea phenols [173]. These compounds also activate genes via the AP-1 family of transcription factors, which include Jun, fos, Maf, Nrl and Fra proteins [174]. AP-1-binding sites have been identified in the promotor regions of the hGSTA1, hGSTA4 and hGSTP1 genes [175, 176]. Other transcription factor binding sites (NF-κB, SP-1, AP-2, GRE) have been described for GSTP1 and/or GSTA1. Whether or not an enhanced expression of GSTs contributes to cancer prevention has not been demonstrated in humans conclusively. But there are several lines of evidence which support this assumption: 1) A large number of animal experiments have shown that cancer reducing activities of chemicals can be associated with increases in phase II metabolism [177]; 2) In human cells an enhanced GST-level is associated with less damage by 4-hydroxynonenal [178] whereas lower GST-levels are associated with more damage [179]; 3) Individuals with null polymorphisms [180] for GSTM1, GSTT1 or other types of genetic predispositions [181] are at higher risks for developing tumours, especially in the case of specific exposure situations [182–184]. Alternatively, sequence polymorphisms for GSTP1 protect from benzo(a)pyrene mediated genotoxic damage [185].

■ **Modulation by diet.** Numerous diets, food ingredients and related endogenous metabolites induce GSTs in animal experiments [166, 186–190]. In humans, evidence is also available on dietary or intervention mediated regulation of GSTs [191–193]. Both inducing and suppressing activities have been reported. One example is the effects in human colon cells of butyrate, an abundant fermentation product present in the human gut [178, 194]. Also, genetic polymorphisms alter the preventive effects of cruciferous vegetables, which are inducers of GST [157].

■ **Methodological considerations.**
– *Species differences in gene regulation:* Different mechanisms of transcriptional and posttranscriptional events are important in controlling levels of ex-

pression and these are to be expected in different genes, cell types and tissues. For instance most of our knowledge on the regulation of GSTs has been derived from studies in rodents. However, the sequences of the 5'-flanking regions of the human genes are different from the rodents', suggesting species-differences in the regulation [195].
– *Activation by phase II reactions:* Not all phase II reactions yield products that are less reactive than the substrate prior to conjugation. For certain haloalka(e)nes (ethylene dibromide, methylene chloride) the conjugate is instable and rapidly decomposes to reactive intermediates with mutagenic potential [196].
– *Shift in organotrophy:* The modulation of biotransformation does not necessarily provide the same protection in all tissues. In some cases there may only be a shift of metabolism, which can result in a shift of tumour localisation. For example, in animal experiments I-3-C decreased formation of aberrant crypt foci in the colon, but enhanced the formation of GST-P foci in the liver [197].
– *Genetic polymorphisms of GSTs:* Confounding situations may be encountered on the basis of individual susceptibilities. Null genotypes for GSTM1 and GSTT1 occur in frequencies of approximately 50% and 20% of the population and result in absence of the respective enzymes. The primary hypothesis has been that individuals with the GST-null genotypes are at higher risk for cancer because of a reduced capacity to eliminate activated carcinogens. Therefore epidemiological studies have focused on interactions between these polymorphisms and carcinogen exposure, whereas only few human dietary interventions have tested the effects of diet on biotransformation enzymes in relation to the genetic polymorphisms of the individuals. One was a controlled feeding study to test if GSTM1genotype affects response to a diet high in cruciferous vegetables [163]. Serum GSTα concentration, an enzyme induced by isothiocyanates, increased significantly in response to cruciferous vegetable feeding, but only in GSTM1-null individuals, indicating that the relationships between cruciferous vegetable intake and cancer risk are influenced by genetic polymorphisms of GSTs [157].

■ **Conclusion.** It seems likely, that an induced expression of the GSTs will result in the protection of cells from genotoxic insult by specific chemicals, especially since these particular enzymes are more involved in deactivation rather than activation and they are inducible. Moreover, many putative carcinogens are relevant substrates for GSTs. However, the limitations in claiming such functions from foods are based on the need for the experimental verification of the hypothesis. To date there are

no data available showing that in humans the final consequence of an induction of GST coupled to a favourable overall modulation of biotransformation is the prevention of tumours.

Genetic events

Genetic events, that is mainly damage to the genome of a cell, constitute various types of lesions such as DNA damage, DNA adducts, gene mutations, and cytogenetic alterations. Associated with these is a deficiency of DNA repair leading to genetic instability and to an even higher incidence of genetic lesions (see Table 5).

There is substantial evidence that genetic events are involved in the initiation, promotion and progression phases of carcinogenesis. The selected endpoints described in the following sections are usually measured in surrogate tissues (e. g. peripheral lymphocytes). The endpoints largely serve as biomarkers of exposure, but there are some probable lines of evidence showing that causative associations are involved as well. Therefore, several larger scale validation studies are presently underway to assess causal relationships for individual endpoints of genetic damage.

Guidelines considering several genetic endpoints are available to provide concise guidance on the planning, performing and interpretation of studies to monitor groups or individuals exposed to genotoxic agents [198]. The guidelines contain important methodological considerations, including ethical aspects, details on sampling procedures, size and characteristics of studied populations, how to assess individual exposures, and potential confounders or effect modifiers, aspects of performing the statistical analysis, as well as procedures for publishing and archiving data, and ensuring sufficient high quality of data. They provide a basis for studies to monitor subjects of dietary intervention trials. Requirements for reporting antioxidant, antimutagenic or anticarcinogenic potential of test substances in in vitro experiments and animal studies in vivo have been identified as well [199].

Optimising vitamin and mineral intake by encouraging dietary change, multivitamin and mineral supplements, and fortifying foods might prevent genetic lesions and therefore cancer and other chronic diseases [200].

■ DNA strand breaks

■ Definition of target. Breaks of the DNA backbones (single strand breaks). If two breaks occur on opposite strands in close vicinity, these are called double strand breaks.

■ Relationship of target with cancer. Many cancer-causing compounds are genotoxic and some of these have the potential to induce DNA strand breaks. However strand breaks can also result from the enzymatic activity of endonucleases in apoptosis or during DNA repair. So, the significance of strand breaks is complex and reflects DNA damage as well as DNA repair.

■ Modulation of target by diet. The first studies showing that dietary intervention in humans alters (lowers DNA strand breaks) in lymphocytes were performed with vegetable [201] and fruit juices [202]. Meanwhile several other studies are showing that other types of dietary intervention have similar effects. Faecal water obtained from humans consuming a diet high in fibre causes fewer DNA strand breaks in cultured human colon cells than a diet high in meat and animal fat, but low in dietary fibre [113]. Moderate wine consumption protects against hydrogen peroxide-induced DNA strand breaks [203]. Instead of using only the surrogate lymphocytes, studies using target tissues of cancer occurrence are considered to be more relevant. To date, DNA strand breaks in cells of potential target tissues have been shown in animal studies, e. g. in the colon of rats after oral ingestion of probiotics [204, 205], but methods are now available to investigate effects of diet also in colon cells of humans [206–209].

■ Methodological considerations. Guidelines considering this endpoint are available to provide concise guidance on the planning, performing and interpretation of studies to monitor groups or individuals exposed to genotoxic agents. They provide a basis for studies to monitor subjects of dietary intervention trials [198]. The most frequently applied assay to detect strand breaks and related events is the alkaline single cell gel electrophoresis (Comet) assay [209] which has largely replaced the technique of alkaline elution [210, 211]. Recent recommendations for performing the in vivo Comet assay are available [212]. For a review of the clinical applications of the Comet assay in different cell types (lymphocytes, buccal and nasal cells, and sperm) see also reference [213].

■ Conclusion. The determination of DNA damage, as is now being performed with the comet assay, provides a useful tool to investigate the effects of diet in a panel of different human tissues, including tissues relevant for diet-associated tumour occurrence. The modulation of DNA damage reasonably well reflects also the modulated exposure to genotoxic compounds that cause the damage. Therefore the reduction of DNA damage will indicate a reduced exposure, which in turn is related to decrease of risk.

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Table 5 Genetic events

Target	Relationship with cancer	Modulation by diet	Methodology	Reference
DNA strand breaks (e. g. Comet assay)	Reduction of damage indicates a reduction of exposure to genotoxic compounds. Since a high proportion of cancers is most likely due to exogenous compounds, reduced exposure is equivalent to reducing risks.	Vegetable and fruit juices decrease damage in peripheral lymphocytes of humans. In animals DNA damage is decreased in colon cells of rats consuming probiotics. Faecal water genotoxicity can be modulated by diet.	Can be measured in any tissue and in exfoliated cells; body fluids can be monitored for damaging capacity, reflecting exposure; multiple classes of damage can be detected; analysis proceeds on level of single cell	[201, 202, 205, 317]
DNA adducts Bulky DNA adducts from polycyclic aromatic hydrocarbons	A recent meta-analysis has shown that current smokers with high levels of bulky adducts have an increased risk of lung and bladder cancers, which also suggested that similar (aromatic) compounds may be involved in the aetiology of both types of cancer.	Specific inverse associations between DNA adduct levels and antioxidant concentrations among GSTM1 null subjects and smokers have been reported. A six-month vitamin intervention did not reduce DNA damage (bulky adducts) in oral cells of heavy smokers.	A number of different EU projects are being performed to associate DNA adducts, exposure and cancer in molecular epidemiological studies. Methodological limitations are that the level of measurement error for bulky adducts is high. More standardised measurements are needed in future investigations.	[155, 214, 318, 319]
Oxidised DNA bases (and oxidative stress)	Oxidised DNA bases cause mutations that are commonly observed in mutated oncogenes and tumour suppressor genes. Reduction indicates a reduction of exposure. An EU-funded Concerted Action (QLK1−1999−00568; ESCODD) has been set up to establish a European standards committee on oxidative DNA damage. The project aims ultimately to establish the level of DNA damage in white blood cells from representative groups of men and women. One objective is to resolve the problems in measuring this marker and to reach a consensus on the level of damage in normal cells. This is important from the point of view of estimating cancer risk and cancer-preventive effects of dietary antioxidants.	Carotenoid-rich juices and supplementation with antioxidants have reduced oxidised bases measured with the comet assay. Oxidative DNA-damage in humans is reduced by Brussels sprouts consumption. In a recent epidemiological study, individual dietary and lifestyle habits only modestly affected lymphocyte DNA oxidation (measured in the modified comet assay) and suggested that specific dietary patterns, rich in fresh fruit and vegetables, are not clearly related to decreased oxidative damage in peripheral lymphocytes in a Mediterranean population.	Different methods are available to measure oxidised DNA bases, namely oxidised pyrimidine and purine bases, using the comet assay with repair specific enzymes, 8-oxy-7- hydroxy-guanosine, using HPLC with electrochemical detection (HPLC/ECD), GC/MS methods, enzyme-linked immunosorbent assay (ELISA) techniques and 5-hydroxymethyl-29-deoxyuridine (HMdU), a product of thymine oxidation, using serum auto antibodies. Recent studies are showing that the comet assay and HPLC are equally efficient for detecting induced damage. Standards are being evaluated in the EU-funded Concerted Action (QLK1−1999−00568; ESCODD).	[318, 201, 202, 220, 221, 320−322]
Exocyclic DNA adducts (etheno- and propano-adducts)	Oxygen radicals attack lipids to generate reactive intermediates that can couple to DNA resulting in exocyclic bases. The promutagenic lesions can be related to increased probability of cancer risks if induced in oncogenes and tumour suppressor genes. For example the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma.	High dietary omega-6 polyun-saturated fatty acids drastically increase the formation of etheno-DNA base adducts in white blood cells.	Presently an EU-funded project is being performed (QLK4CT-2000−00286) with the objective to analyse the repair functions, the persistence and the mutational specificity of e-DNA adducts at the gene level, so to better define the mutagenic and carcinogenic potential of these miscoding lesions in humans. An immunohisto- chemical method has been developed to detect malondialdehyde (MDA)-modified DNA adducts in single human oral mucosa cells.	[320, 323−325]

■ DNA adducts

■ **Definition of target.** Modified DNA bases resulting from the addition of DNA with an electrophile or free radical are called DNA adducts. Adducts can also be gen-erated by the effect of ultraviolet radiation creating thymine dimers. Depending on the type of chemical in-volved, alkylated bases, oxidised bases, bulky adducts, etheno- and propano-adducts can result as adducts. Sometimes, the effect of an adduct can result in the

Table 5 *Continued*

Target	Relationship with cancer	Modulation by diet	Methodology	Reference
Mutations				
Gene mutations	The accumulation of multiple mutations, especially in protooncogens, tumour suppressor genes and DNA repair genes is associated with cancer initiation, promotion and progression. In fact tumour progression selects for cells with specific mutations including those which maintain genetic stability.	Using the HPRT (hypoxan-thine – guanine phosphoribosyl transferase) gene assay with T-lymphocytes from 312 individuals, it was found that HPRT mutant frequency (MF) was significantly decreased in relation to intake of vegetables, citrus fruits and berries, respectively, as well as calculated vitamin C intake from diet. Only a borderline significant association was observed for beta-carotene	The tissue-specific mutagenicity of IQ was studied at the lacI locus in the liver, colon and kidney of Big Blue(R) transgenic rats. This is probably the most effeicient system to analyse mutations in vivo. However, the mutations identified were different to the mutations identified in IQ-induced tumours, which indicates the responses to be non-specific in relation to the effects in humans.	[326, 327]
Chromosomal aberrations	Reduction in occurrence should indicate a reduction of exposure, and possibly of cancer risk. A present EU-funded concerted action (BIOMED 2) of 13 European institutes, is characterising cytogenetic lesions, occupational exposure and cancer incidence in large cohorts, with the aim to analyse the predictivity of chromosome changes as indicators of cancer risk	Food mutagens can induce cytogenetic lesions. Probiotics have been shown to reduce CA in animal experiments, but otherwise there are gaps of knowledge on how diet may reduce CA in humans	Putative anticarcinogens, namely the phytoestrogens coumoestrol and genistein induce structural chromosomal aberrations in cultured human peripheral blood lymphocytes	[328–331]
Micronuclei	Reduction of occurrence indicates a reduction of exposure, and possible of risk. "The International Collaborative Project on Micronucleus Frequency in Human populations (HUMN)" is collecting an international data base to determine associations with cancer and ageing http://ehs.sph.berkeley.edu/holland/humn/	Different levels of micronuclei incidence were reported with age, gender and vegetarian, versus non-vegetarian diet. Moderate wine consumption protects against hydrogen peroxide-induced micronuclei. Micronuclei are modulated in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. Cigarette smoking, intracellular vitamin deficiency, are associated with micronuclei in epithelial cells of the buccal mucosa	Micronuclei are easier to score (less time consuming) than chromosomal aberrations. Different types of staining methods can reveal the nature of origin of micronuclei. Determinations of micronuclei in exfoliated cells of buccal and bladder mucosa are a promising alternative to lymphocytes in human studies. Presently larger scale international validation and endpoint assessments are taking place.	[203, 234, 332–337]
DNA Repair				
BER	Significantly increased risk for orolaryngeal cancer was observed for polymorph hOGG1 genotypes, in smokers but not in non-smokers, suggesting that hOGG1 may play an important role in the repair of 8-OH-dG adducts in the aerodigestive tract and that the hOGG1 (Ser326Cys) polymorphism plays an important role in risk for smoking- and alcohol-related orolaryangeal cancer	Significantly increased risk for orolaryngeal cancer was observed for polymorph hOGG1 genotypes, in alcohol drinkers but not in never drinkers smokers.	Until recently, only two mammalian DNA glycosylases specific for oxidised bases had been characterised, the *E.coli* endonuclease III (Nth) homolog NTH1, and 8-oxoguanine-DNA glycosylase (OGG1), patho-physiological effects. However, mouse mutants lacking either NTH1 or OGG1 have no obvious phenotype, in spite of accumulation of mutagenic and toxic base lesions in their genomes. An explanation is that repair is mediated by additional DNA glycosylases.	[338, 339]

breaking of a base into small residues or its removal from DNA creating an abasic site.

■ **Relationship of target with cancer.** A recent meta-analysis testing the hypothesis that the presence of a

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Table 5 *Continued*

Target	Relationship with cancer	Modulation by diet	Methodology	Reference
NER	Humans with a hereditary defect in NER suffer from xeroderma pigmentosum and are predisposed to skin cancer caused by sunlight exposure. Cancer susceptibility is associated with defective DNA repair. Genetic instability is associated with increased risk of bladder cancer.	Gaps in knowledge	DNA repair inhibitors hydroxyurea and cytosine arabinoside enhance the sensitivity of the alkaline single-cell gel/electrophoresis 'Comet' assay. The Comet assay is more and more being used to determine genetic instability.	[216, 224, 340–342]
MMR	Defects in MMR are associated in the hereditary nonpolyposis colorectal cancer (HNPCC) and with a variety of other sporadic cancers.	Gaps in knowledge	MMR removes nucleotides mispaired by DNA polymerases. Defects result in 'microsatellite instability', which are measured to identify MMR deficiencies.	[225, 343, 344]

high level of bulky DNA adducts in tissues is associated with an increased risk of cancer in humans reports that current smokers with cancer have statistically significant higher levels of adducts than controls, whereas results were negative or contradictory in ex-smokers and non-smokers. The authors conclude that current smokers with high levels of adducts have an increased risk of lung and bladder cancers [214].

■ **Modulation of target by diet.** DNA adducts of acetaldehyde can be detected in peripheral white blood cells of alcohol abusers [215]. Plasma levels of a combination of antioxidant vitamins (vitamins C and E and β-carotene) rose significantly during a randomised clinical trial among heavy smokers, but the measurement of DNA damage (immunological determination of polycyclic aromatic hydrocarbon-DNA adducts) and oxidative DNA damage (8-oxo- or hydroxydeoxyguanosine) in mononuclear and oral cells was not statistically significantly different in the group with antioxidant vitamin intake than in the controls, thus providing no evidence that vitamins prevent this type of DNA damage in smokers [216]. The studies confirm earlier studies measuring oxidative DNA damage in male smokers receiving β-carotene supplementation [217]. In another study, antioxidant supplementation decreases oxidative DNA damage in human lymphocytes [218]. Oxidised DNA bases are reduced in lymphocyte-DNA of healthy, non-smoking males after consumption of vegetable juices [201] and of a soy milk supplement [219]. However when considering epidemiological studies instead of intervention trials the associations are not as apparent. In a recent epidemiological study [220], comparing dietary and lifestyle determinants of oxidative DNA damage (modified comet assay) basal levels of DNA oxidation were positively association with coffee ($P = 0.01$) and tomato consumption ($P = 0.05$). Instead, the consumption of cruciferous vegetables tended to be negatively as-

sociated with oxidative damage. A positive non-significant association between the consumption of total vegetables and fresh fruit and DNA damage was noted ($P = 0.08$ and $P = 0.10$, respectively). In contrast, the estimated intake of simple sugars was positively associated with oxidative DNA damage, while vitamin E showed a borderline positive association. The plasma levels of several micronutrients did not appear to influence DNA damage. Thus, the results indicated that individual dietary and lifestyle habits only modestly affected the levels of lymphocyte DNA oxidation and suggested that specific dietary patterns, rich in fresh fruit and vegetables, are not clearly related to decreased oxidative damage in peripheral lymphocytes in a Mediterranean population [221].

■ **Methodological considerations.** Guidelines considering this endpoint are available to provide concise guidance on the planning, performing and interpretation of studies to monitor groups or individuals exposed to genotoxic agents. They provide a basis for studies to monitor subjects of dietary intervention trials [198]. The best established methods to detect DNA adducts are sensitive chemical analytical detection methods, involving ^{32}P post labelling of the DNA [222, 223]. Biological determinations (e. g. using modifications of the comet assay) are increasingly becoming available, which may facilitate larger scale monitoring studies in the future [220].

■ **Conclusion.** Adducts are specific indicators of DNA damage caused by individual chemical groups (e. g. "Bulky" DNA adducts by polycyclic aromatic hydrocarbons, etheno-adducts by reactive aldehydes, oxidised bases by reactive oxygen species). They therefore represent an integrated marker of exposure to the specific compounds, and of the ability of the individual to metabolically activate carcinogens and to repair DNA dam-

age. A reduction of adducts therefore reflects a lower level of exposure and with that a lower risk. A number of international studies are being performed to determine in which manner specific types of DNA adducts are causally related to cancer risks. The outcome of these studies will be of high value for using the parameters as a basis for possible claims.

■ DNA repair

■ **Definition of target.** Base excision repair (BER) is primarily responsible for repairing small base modifications and abasic sites caused by endogenous damage and environmental insult. Nucleotide excision repair (NER) is the repair process which incorporates the excision of damaged and altered bases (e. g. bulky adducts, cyclobutane pyrimidine dimers) as part of an oligonucleotide fragment. Mismatch repair (MMR) is the DNA repair process by which mispaired bases are excised as single nucleotides [224]. Interstrand cross-links and DNA double strand breaks are repaired by homologous recombination and/or end-joining repair. O6-methyl-guanine methyltransferase, a single repair protein, reverts damage by removing the non-native methyl group from O6-methyl guanine [225].

■ **Relationship of target with cancer.** Inherited defects in any of these pathways, in general, predisposes to malignancy [225]. Examples are defective NER, which is the cause for Xeroderma pigmentosum (XP), a syndrome characterised by a severe predisposition to skin cancers of various types (squamous cell carcinomas and basal cell carcinomas). Defects in MMR in humans predispose to cancer of the colon, but also to uterine, ovarian and gastric cancer (reviewed in [224]). No human disorders caused by inherited BER deficiencies have been identified, but according to mouse models, the knockout of BER enzymes (glycosylases) show only mild phenotypes, although mutagenesis and cancer susceptibility are probably increased [225]. X-ray-sensitive persons afflicted with ataxia telangiectasia have a defective ataxia telangiectasia (ATM) protein kinase, which predisposes to lymphoma [reviewed in 224]. Latent genetic instability has been associated with an increased risk for several cancers. Genetic instability, as measured by the comet assay is associated with an increased estimated relative risk of bladder cancer [216].

■ **Modulation of target by diet.** Repair enzymes have not been well characterised for regulation via transcriptional activity. There are few data available related to exogenous factors involved in regulation of these different proteins, with perhaps the exception of alkyl methyltransferase albeit the physiological consequences are not well elucidated [226]. There are large gaps in knowl-

edge pertaining to the potential regulation of repair enzymes by diet, and hardly any information is available.

■ **Methodological considerations.** DNA repair deficiency is related not only to cancer, but also probably to ageing and exposure to oxidative stress. New knock-out mouse models reflecting specific repair deficiencies are becoming more and more available to study these associations. There are hardly any studies on modulation of transcriptional activity of DNA repair genes (e. g. potential induction by dietary factors), although they would be highly needed as a tool to study the possibility of repair induction.

■ **Conclusion.** Large gaps in knowledge exist. Whether or not it would be possible to make the potential claim that DNA repair can be activated by enhanced transcription of the respective genes has hardly been investigated so far.

■ Cytogenetic effects

■ **Definition of target.** Chromosomal structural aberrations are deletions, insertions, translocations, inversions, ring structures and abnormalities of chromosome number. Micronuclei originate from chromosome deletions and chromosome loss. Nucleoplasmic bridges originate from dicentric chromatids and chromosomes.

■ **Relationship of target with cancer.** A large number of studies are available which demonstrate that deletions in tumour suppressor genes and DNA repair genes [227], resulting in the loss of a functional gene product, are involved in the transformation of normal, dividing cells into tumour cells. The alterations and the sequences by which these alterations occur as well as the subsequent functional responses have been reasonably well investigated in, for example colon cancer [228]. Chromosomal aberrations and micronuclei in peripheral lymphocytes are used as surrogate markers, although the long-term effects of an increased frequency of chromosomal aberrations in individuals are still uncertain. In a recent study, new support for the associations between cytogenetic damage and cancer was obtained [229]. An increased risk of cancer in healthy individuals with high levels of chromosomal aberrations (CAs) in peripheral blood lymphocytes has been described in recent epidemiological studies [230]. This association did not appear to be modified by sex, age, country, or time. The study evaluated whether CAs predicted cancer because they were the result of past exposure to carcinogens or because they were an intermediate endpoint in the pathway leading to disease. A nested case-control study was performed on 93 incident cancer cases and 62 deceased cancer cases coming from two

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prospective cohort studies performed in Nordic countries (Denmark, Finland, Norway, and Sweden) and Italy. For each case, four controls matched by country, sex, year of birth, and year of CA test were randomly selected. Occupational exposure and smoking habit were assessed by a collaborative group of occupational hygienists. Logistic regression models indicated a statistically significant increase in risk for subjects with a high level of CAs compared to those with a low level in the Nordic cohort (odds ratio, 2.35; 95 % confidence interval, 1.31–4.23) and in the Italian cohort (odds ratio, 2.66; 95 % confidence interval, 1.26–5.62). These estimates were not affected by the inclusion of occupational exposure level and smoking habit in the regression model. The risk for high versus low levels of CAs was similar in subjects heavily exposed to carcinogens and in those who had never, to their knowledge, been exposed to any major carcinogenic agent during their lifetime, supporting the idea that chromosome damage itself is involved in the pathway to cancer. The results have important ramifications for the understanding of the role played by sporadic chromosome damage for the origin of neoplasia-associated CAs.

■ **Modulation of target by diet.** One study has addressed the effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting, and has found that stable chromosome aberrations show a greater accumulation with age than do unstable aberrations, suggesting that lifestyle factors contribute to the accumulation of cytogenetic damage [231]. Hardly any studies in humans exist showing that diet may contribute to reducing the occurrence of chromosomal aberrations. Micronuclei have been used in this context more frequently, for example intervention with vitamin E had no significant impact on spontaneous genetic damage in human lymphocytes [232]. Moderate wine consumption protects against hydrogen peroxide-induced DNA damage, measured as micronuclei [203]. The micronuclei index is enhanced in older men, deficient in homocysteine, folate and vitamin B12 [233]. A highly significant (p = 0.001) positive association between plasma levels of vitamin B12 and frequencies of SCE was reported in smokers [234]. After correction for age, gender and GSTM1 genotype, a significant association (p = 0.026) between the MTRR 66GG variant genotype and higher micronucleus rates was observed [234], whereas another study has shown that folate and vitamin B12 supplementation reduces genome damage in young adults [235]. In vitro studies have shown that genomic instability in human cells is minimised when folic acid concentration in culture medium is > 227 nmol/l. Intervention studies in humans show among other effects that micronucleus formation is minimised when plasma concentration of vitamin B12 is > 300pmol/l and plasma homocysteine is < 7.5micro-

mol/l. These concentrations are achievable at intake levels in excess of current recommended dietary intakes which may be particularly important in those with extreme defects in the absorption and metabolism of these vitamins, for which ageing is a contributing factor [236].

■ **Methodological considerations.** Cytogenetic lesions are determined mainly in surrogate tissues such as peripheral blood lymphocytes, and rarely in the target tissues of dietary related cancers, mainly since it is technically not easy to isolate dividing cells from the tissues [237]. Alternative approaches include the determinations of micronuclei in exfoliated epithelial cells of the buccal cavity [238] or of the urinary bladder excreted in the urine. Guidelines considering this end point are available to provide concise guidance on the planning, performing and interpretation of studies to monitor groups or individuals exposed to genotoxic agents. They provide a basis for studies to monitor subjects of dietary intervention trials [198].

■ **Conclusion.** Cytogenetic lesions are indicators of DNA damage caused by exposure. Also, they occur as the consequence of genetic instability in cancer patients. They therefore represent an integrated marker of exposure and of disease. A reduction of cytogenetic lesions reflects a lower level of exposure and with that a lower risk, although systematic studies for these associations are not available. A reduction of cytogenetic lesions as a consequence of retardation of disease has also not been reported. A number of international studies are being performed to determine in which manner specific types of cytogenetic lesions are causally related to cancer risks. The outcome of these studies will be of high value for using the parameters as a basis for possible claims.

Overall concluding remarks

This working group has identified 18 targets/markers in the chain of events from initial exposure to overt malignant tumour whose modification 'have the potential' to be used for 'anticancer' claims for food components (Table 6). In this area, the one true marker is the malignant human tumour, which for practical reasons is usually not accessible to claims. In its absence, we must rely on alternative markers (late and intermediate markers of disease). At present, the strongest markers available are precancerous lesions (e. g. polyps) in humans and precancerous lesions and tumours in animal models. The only marker that presently can be used for a type B claim for food components is 'polyp recurrence'. However, even this marker has several limitations (e. g. not all polyps progress to cancer) and the claim should specify only 'reduces risk of polyps'. Type B claims cannot be made on the basis of results in animal models.

Table 6 Concluding remarks

Possible claim	Markers/targets	Supportive data from animal/in vitro studies	Evidence from human studies	Target group	Potential application of marker for claims
Reduction in exposure to carcinogens	Gut enzymes Gut metabolites Faecal cytotoxicity Faecal genotoxicity DNA adducts (DNA strand breaks) (Cytogenetic effects)	+++	++	Healthy population High risk persons	A
Inactivation of carcinogens	Biotransformation of carcinogens (e.g. GSTs)	++	+	Healthy population	A
Reduction of DNA damage	DNA strand breaks DNA repair Cytogenetic effects (Apoptosis)	+	+	Healthy population	A
Modulation of cellular events	Proliferation Differentiation Apoptosis COX-2	+++	++	Healthy population Patients	A
Prevention of precancerous lesions	Aberrant crypt foci Polyps	+++	++	Healthy population High risk persons Patients	B
Prevention of tumours	Tumours in animals	+++	–	Healthy population High risk persons	B*
Inhibition of tumour progression	Angiogenesis Metastasis	++	+	Patients	A

* Animal basis for claim but must have additional data linking animal data to human

All of the other markers (intermediate markers) presently lack validation against the 'true endpoint', the tumour, and thus cannot be used for type B claims. However, the majority of these intermediate markers, which reflect events earlier in the chain of events leading to the tumour and are mechanistically well linked to the disease process, have varying degrees of support from in vitro/animal studies. They may, thus, be used for type A claims, such as 'reduces carcinogen exposure', 'inactivates carcinogens', 'reduces DNA damage', 'modulates cellular events important for cancer prevention', 'inhibits COX-2'.

'Reduction of risk of disease' claims in the area of 'diet-related cancer' should be based primarily on human intervention studies using relevant/acceptable endpoints. In the absence of the true endpoint, the tumour, which is not always practical to use, precancerous lesions (e. g. polyps, aberrant crypt foci) are to be preferred. However, in this field, the availability of additional intermediate markers is particularly important in order to make dietary intervention studies more attractive/accessible to those wishing to make claims. An important area for future research will be the validation of these surrogate markers [239]. It should also be mentioned that in certain instances, claims might be made based on results from large epidemiological studies. For further information, the reader is referred to the "set of interim criteria for the scientific substantiation of health claims on foods and food components" [see 345].

The working group emphasises that in the area of 'diet-related cancer' when a claim is made, it should be specified who may benefit from the effect, e. g. the healthy population, high-risk persons or patients and evidence should be presented from the target group. Susceptibility to the claimed effect is of particular importance in this area. We believe that a scientifically substantiated mechanism is essential for any claim, especially if evidence is presented using intermediate markers as endpoints. We would also emphasise that in human dietary intervention trials, an appropriate duration and sample size to demonstrate the intended effect be employed.

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References

1. Corpet DE, Tache S (2002) Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumour data, ranked by potency. Nutr Cancer 43:1–21

2. Steele VE, Moon RC, Lubet RA, Grubbs CJ, Reddy BS, Wargovich M, McCormick DL, Pereira MA, Crowell JA, Bagheri D (1994) Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program. J Cell Biochem (Suppl) 20:32–54

3. Corpet DE, Pierre D (2003) Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in minmice and choice of the model system. Cancer Epidemiol Biomarkers Prev 12:391–400

4. Bird RP (1987) Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett 37:147–151

5. Roncucci L, Pedroni M, Vaccina F, Benatti P, Marzona L, De Pol A (2000) Aberrant crypt foci in colorectal carcinogenesis. Cell and crypt dynamics. Cell Prolif 33:1–18

6. Bird RP, McLellan EA, Bruce WR (1989) Aberrant crypts, putative precancerous lesions, in the study of the role of diet in the aetiology of colon cancer. Cancer Surv 8:189–200

7. Corpet DE, Tache S, Peiffer G (1997) Colon tumour promotion: is it a selective process? Effects of cholate, phytate, and food restriction in rats on proliferation and apoptosis in normal and aberrant crypts. Cancer Lett 114:135–138

8. Wargovich MJ, Jimenez A, McKee K, Steele VE, Velasco M, Woods J, Price R, Gray K, Kelloff GJ (2000) Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression. Carcinogenesis 21:1149–1155

9. Roncucci L, Stamp D, Medline A, Cullen JB, Bruce WR (1991) Identification and quantification of aberrant crypt foci and microadenomas in the human colon. Hum Pathol 22:287–294

10. Modica S, Roncucci L, Benatti P, Gafa L, Tamassia MG, Dardanoni L, Ponz dL (1995) Familial aggregation of tumours and detection of hereditary non-polyposis colorectal cancer in 3-year experience of 2 population-based colorectal-cancer registries. Int J Cancer 62:685–690

11. Narisawa T, Fukaura Y, Hasebe M, Ito M, Aizawa R, Murakoshi M, Uemura S, Khachik F, Nishino H (1996) Inhibitory effects of natural carotenoids, alpha-carotene, beta-carotene, lycopene and lutein, on colonic aberrant crypt foci formation in rats. Cancer Lett 107:137–142

12. Latham P, Lund EK, Johnson IT (1999) Dietary n-3 PUFA increases the apoptotic response to 1.2- dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon. Carcinogenesis 20:645–650

13. Rao CV, Hirose Y, Indranie C, Reddy BS (2001) Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. Cancer Res 61:1927–1933

14. Verghese M, Rao DR, Chawan CB (2001) Dietary inulin suppresses azoxymethane-induced preneoplastic aberrant crypt foci and colon tumours in Fisher 344 rats. J Nutr 132 (9):2809–2813

15. Dolara P, Caderni G, Lancioni L, Giannini A, Anastasi A, Fazi M, Castiglione G (1997) Aberrant crypt foci in human colon carcinogenesis. Cancer Detect Prev 21:141–147

16. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, Kato J, Kogawa K, Miyake H, Niitsu Y (1998) Aberrant crypt foci of the colon as precursors of adenoma and cancer. N Engl J Med 339:1277–1284

17. Adler DG, Gostout CJ, Sorbi D, Burgart LJ, Wang L, Harmsen WS (2002) Endoscopic identification and quantification of aberrant crypt foci in the human colon. Gastrointest Endosc 56:657–662

18. Konstantakos AK, Siu IM, Pretlow TG, Stellato TA, Pretlow TP (1996) Human aberrant crypt foci with carcinoma in situ from a patient with sporadic colon cancer. Gastroenterology 111:772–777

19. Magnuson BA, Carr I, Bird RP (1993) Ability of aberrant crypt foci characteristics to predict colonic tumour incidence in rats fed cholic acid. Cancer Res 53:4499–4504

20. Yamada Y, Mori H (2003) Pre-cancerous lesions for colon colorectal cancers in rodents: a new concept. Carcinogenesis 24:1015–1019

21. Caderni G, Femia AP, Giannini A, Favuzza A, Luceri C, Salvadori M, Dolara P (2003) Identification of mucin-depleted foci in the unsectioned colon of azoxymethane-treated rats: correlation with carcinogenesis. Cancer Res 63:2388–2392

22. Moroson BC, Busey HJR, Day DW, Hill MJ (1983) Adenomas of the large bowel. Cancer Surv 2:451–477

23. Neugut AI, Jacobson JS, De VI (1993) Epidemiology of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 2:159–176

24. Gilbertsen VA, Nelms JM (1978) The prevention of invasive cancer of the rectum. Cancer 41:1137–1139

25. Stryker SJ, Wolff BG, Culp CE, Libbe SD, Ilstrup DM, MacCarty RL (1987) Natural history of untreated colonic polyps. Gastroenterology 93:1009–1013

26. Radice P, Cama A, Mariani-Constantini R (1996) Molecular genetics of polyposis and herediatry colorectal cancer. Forum Trends Exp Clin Med 6:275–291

27. Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, Rothstein R, Summers RW, Snover DC, Beck GJ, Frankl H, Pearson L, Bond JH, Greenberg ER (1999) Calcium supplements and colorectal adenomas. Polyp Prevention Study Group. Ann N Y Acad Sci 889:138–145

28. Bonithon-Kopp C, Kronborg O, Giacosa A, Rath U, Faivre J (2000) Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. Lancet 356:1300–1306

29. Alberts DS, Martinez ME, Roe DJ, Guillen-Rodriguez JM, Marshall JR, van Leeuwen JB, Reid ME, Ritenbaugh C, Vargas PA, Bhattacharyya AB, Earnest DL, Sampliner RE (2000) Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. Phoenix Colon Cancer Prevention Physicians' Network. N Engl J Med 342:1156–1162

30. Schatzkin A, Lanza E, Corle D, Lance P, Iber F, Caan B, Shike M, Weissfeld J, Burt R, Cooper MR, Kikendall JW, Cahill J (2000) Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. N Engl J Med 342:1149–1155

31. Arminskitc TC, McLean DW (1964) Incidence and distribution of adenomatous polyps of the colon and rectum based on 1,000 autopsy examinations. Dis Colon Rectum 7:249–261

32. Duffy MJ (2001) Clinical uses of tumour markers: a critical review. Crit Rev Clin Lab Sci 38:225–262

33. Konety BR, Getzenberg RH (2002) Vitamin D and prostate cancer. Urol Clin North Am 29:95–106, ix

34. Giovannucci E (2001) Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr 131:3109S–3120S

35. Berger DH (2002) Plasmin/plasminogen system in colorectal cancer. World. J Surg 26:767–771

36. Clarke LE, Leitzel K, Smith J, Ali SM, Lipton A (2003) Epidermal growth factor receptor mRNA in peripheral blood of patients with pancreatic, lung, and colon carcinomas detected by RT-PCR. Int J Oncol 22:425–430

37. McLeod HL, Murray GI (1999) Tumour markers of prognosis in colorectal cancer. Br J Cancer 79:191–203

38. Hainaut P, Hollstein M (2000) p53 and human cancer: the first ten thousand mutations. Adv Cancer Res 77:81–137

39. Hartwell LH, Kastan MB (1994) Cell cycle control and cancer. Science 266:1821–1828

40. Bostick RM, Fosdick L, Grandits GA, Lillemoe TJ, Wood JR, Grambsch P, Louis TA, Potter JD (1997) Colorectal epithelial cell proliferative kinetics and risk factors for colon cancer in sporadic adenoma patients. Cancer Epidemiol Biomarkers Prev 6:1011–1019

41. Kelloff GJ, Boone CW, Steele VE, Crowell JA, Lubet RA, Greenwald P, Hawk ET, Fay JR, Sigman CC (1996) Mechanistic considerations in the evaluation of chemopreventive data. IARC Sci Publ 139:203–219

42. Milner JA, McDonald SS, Anderson DE, Greenwald P (2001) Molecular targets for nutrients involved with cancer prevention. Nutr Cancer 41:1–16

43. Duchrow M, Gerdes J, Schluter C (1994) The proliferation-associated Ki-67 protein: definition in molecular terms. Cell Prolif 27:235–242

44. Bravo R, Frank R, Blundell PA, Macdonald-Bravo H (1987) Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. Nature 326:515–517

45. Liu SC, Klein-Szanto AJ (2000) Markers of proliferation in normal and leukoplakic oral epithelia. Oral Oncol 36:145–151

46. Renehan AG, O'Dwyer ST, Haboubi NJ, Potten CS (2002) Early cellular events in colorectal carcinogenesis. Colorectal disease 4:76–89

47. Kerr JF, Winterford CM, Harmon BV (1994) Apoptosis. Its significance in cancer and cancer therapy. Cancer 73:2013–2026

48. Baker SJ, Reddy EP (1996) Transducers of life and death: TNF receptor superfamily and associated proteins. Oncogene 12:1–9

49. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257

50. Johnson IT (2002) Anticarcinogenic effects of diet-related apoptosis in the colorectal mucosa. Food Chem Toxicol 40:1171–1178

51. Lowe SW, Lin AW (2000) Apoptosis in cancer. Carcinogenesis 21:485–495

52. Butler LM, Hewett PJ, Fitridge RA, Cowled PA (1999) Deregulation of apoptosis in colorectal carcinoma: theoretical and therapeutic implications. Aust N Z J Surg 69:88–94

53. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493–501

54. van Engeland M, Nieland LJ, Ramaekers FC, Schutte B, Reutelingsperger CP (1998) Annexin V-affinity assay: a review on an apoptosis detection system based on phosphatidylserine exposure. Cytometry 31:1–9

55. Hofstra L, Liem IH, Dumont EA, Boersma HH, van Heerde WL, Doevendans PA, De Muinck E, Wellens HJ, Kemerink GJ, Reutelingsperger CP, Heidendal GA (2000) Visualisation of cell death in vivo in patients with acute myocardial infarction. Lancet 356:209–212

56. Riss TL (2001) Apoptosis as a biomarker in chemoprevention trials. Urology 57:141–142

57. Batth BK, Tripathi R, Srinivas UK (2001) Curcumin-induced differentiation of mouse embryonal carcinoma PCC4 cells. Differentiation 68:133–140

58. Shamsuddin (2002) Anti-cancer function of phytic acid. Int J Food Sci Technol 37:769–782

59. Sankaranarayanan R, Mathew B (1996) Retinoids as cancer-preventive agents. IARC Sci Publ 139:47–59

60. Osborne JE, Hutchinson PE (2002) Vitamin D and systemic cancer: is this relevant to malignant melanoma? Br J Dermatol 147:197–213

61. Borghi R, Vene R, Arena G, Schubert D, Albini A, Tosetti F (2003) Transient modulation of cytoplasmic and nuclear retinoid receptors expression in differentiating human teratocarcinoma NT2 cells. J Neurochem 84:94–104

62. You H, Yu W, Sanders BG, Kline K (2001) RRR-alpha-tocopheryl succinate induces MDA-MB-435 and MCF-7 human breast cancer cells to undergo differentiation. Cell Growth Differ 12:471–480

63. Smith WL, Dewitt DL (1996) Prostaglandin endoperoxide H synthases-1 and –2. Adv Immunol 62:167–215

64. Dannenberg AJ, Altorki NK, Boyle JO, Dang C, Howe LR, Weksler BB, Subbaramaiah K (2001) Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. Lancet Oncol 2:544–551

65. Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, Haudenschild C, Lane TF, Hla T (2001) Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 276:18563–18569

66. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87:803–809

67. Tiano HF, Loftin CD, Akunda J, Lee CA, Spalding J, Sessoms A, Dunson DB, Rogan EG, Morham SG, Smart RC, Langenbach R (2002) Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Res 62:3395–3401

68. Thun MJ (1996) NSAID use and decreased risk of gastrointestinal cancers. Gastroenterol Clin North Am 25:333–348

69. Norrish AE, Jackson RT, McRae CU (1998) Non-steroidal anti-inflammatory drugs and prostate cancer progression. Int J Cancer 77:511–515

70. Madaan S, Abel PD, Chaudhary KS, Hewitt R, Stott MA, Stamp GW, Lalani EN (2000) Cytoplasmic induction and over-expression of cyclooxygenase-2 in human prostate cancer: implications for prevention and treatment. BJU Int 86:736–741

71. Mallett AK, Rowland IR (1990) Bacterial enzymes: their role in the formation of mutagens and carcinogens in the intestine. Digestive Diseases 8:71–79

72. Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL (1980) Effect of diet and Lactobacillus acidophilus supplements on human fecal bacterial enzymes. Journal of the National Cancer Institute 64:255–261

73. Rowland IR, Rumney CJ, Coutts JT, Lievense LC (1998) Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis 19:281–285

74. Saito Y, Takano T, Rowland IR (1992) Effects of soybean oligosaccharides on the human gut microflora in in vitro culture. Microbial Ecology in Health and Disease 5:105–110

75. Takada H, Hirooka T, Hiramatsu Y, Yamamoto M (1982) Effect of beta-glucuronidase inhibitor on azoxymethane-induced colonic carcinogenesis in rats. Cancer Res 42:331–334

76. Kim DH, Jin YH (2001) Intestinal bacterial beta-glucuronidase activity of patients with colon cancer. Arch Pharm Res 24:564–567

77. Reddy BS, Mangat S, Weisburger JH, Wynder EL (1977) Effect of high-risk diets for colon carcinogenesis on intestinal mucosal and bacterial beta-glucuronidase activity in F344 rats. Cancer Research 37:3533–3536

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78. Gestel G, Besancon P, Rouanet JM (1994) Comparative evaluation of the effects of two different forms of dietary fibre (rice bran vs. wheat bran) on rat colonic mucosa and faecal microflora. Ann Nutr and Metabolism 38:249–256

79. Maziere S, Meflah K, Tavan E, Champ M, Narbonne JF, Cassand P (1998) Effect of resistant starch and/or fat-soluble vitamins A and E on the initiation stage of aberrant crypts in rat colon. Nutrition and Cancer 31:168–177

80. Rao CV, Newmark HL, Reddy BS (1998) Chemopreventive effect of squalene on colon cancer. Carcinogenesis 19: 287–290

81. Massey RC, Key PE, Mallett AK, Rowland IR (1988) An investigation of the endogenous formation of apparent total N-nitroso compounds in conventional microflora and germ free rats. Food Chemistry and Toxicology 26: 595–600

82. Hughes R (1999) The effects of diet on colonic N-nitrosation and biomarkers of DNA damage. University of Cambridge

83. Hughes R, Cross AJ, Pollock JR, Bingham S (2001) Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. Carcinogenesis 22:199–202

84. Silvester KR, Bingham SA, Pollock JRA, Cummings JH, ONeill IK (1997) Effect of meat and resistant starch on fecal extraction of apparent N-nitroso compounds and ammonia from the human large bowel. Nutrition and Cancer 29: 13–23

85. Cummings JH, Beatty ER, Kingman SM, Bingham SA, Englyst HN (1996) Digestion and physiological properties of resistant starch in the human large bowel. Br J Nutr 75:733–747

86. Rowland IR, Granli T, Bockman OC, Key PE, Massey RC (1991) Endogenous N-nitrosation in man assessed by measurement of apparent total N-nitroso compounds in faeces. Carcinogenesis 12:1395–1401

87. MacDonald IA, Bokkenheuser VD, Winter J (1993) Degradation of steriods in the human gut. J Lipid Res 24:675–700

88. Gill CIR, Rowland IR (2002) Diet and cancer, assessing the risk. Br J Nutr 88:S73–S87

89. Imray CH, Radley S, Davis A, Barker G, Hendrickse CW, Donovan IA, Lawson AM, Baker PR, Neoptolemos JP (1992) Faecal unconjugated bile acids in patients with colorectal cancer or polyps. Gut 33:1239–1245

90. Stadler J, Yeung KS, Furrer R, Marcon N, Himal HS, Bruce WR (1988) Proliferative activity of rectal mucosa and soluble fecal bile acids in patients with normal colons and in patients with colonic polyps or cancer. Cancer Lett 38:315–320

91. Breuer NF, Dommes P, Jaekel S, Goebell H (1985) Fecal bile acid excretion pattern in colonic cancer patients. Dig Dis Sci 30:852–859

92. Breuer NF, Jaekel S, Dommes P, Goebell H (1986) Fecal bile acids in patients with adenomatous polyps of the colon. Case-control study. Digestion 34:87–92

93. Owen RW, Dodo M, Thompson MH, Hill MJ (1987) Fecal steroids and colorectal cancer. Nutr Canc 9:73–80

94. Kamano T, Mikami Y, Kurasawa T, Tsurumaru M, Matsumoto M, Kano M, Motegi K (1999) Ratio of primary and secondary bile acids in feces: possible marker for colorectal cancer? Diseases of the Colon and Rectum 42:668–672

95. de Kok T, Van Faassen A, Glinghammar B, Pachen D, Rafter JJ, Baeten C, Engels L, Kleinjans JCS (1999) Bile acid concentrations, cytotoxicity, and pH of fecal water from patients with colorectal adenomas. Digestive Diseases and Sciences 44:2218–2225

96. Thompson MH, Owen RW, Hill MJ, Cummings JH (1985) Factors affecting faecal bile acid concentrations: effect of fat and fibre. Biochemical Soc Trans 13:392

97. Cummings JH, Pomare EW, Branch WJ, Naylor CPE, Macfarlane GT (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28:1221–1227

98. Roediger WEW (1982) Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 83: 424–429

99. Salminen S, Bouley C, Boutron-Ruault M-C, Cummings JH, Franck A, Gibson GG, Isolauri E, Moreau MC, Roberfroid MB, Rowland IR (1998) Functional food science and gastrointestinal physiology and function. Br J Nutr 80:147–171

100. Hague A, Paraskeva C (1995) The short-chain fatty acid butyrate induces apoptosis in colorectal tumour cell lines. Eur J Cancer Prevention 4:359–364

101. Thornton JR (1981) High colonic pH promotes colorectal cancer. Lancet 1:1081–1083

102. Van Dokkum W, De Boer BC, Van Faassen A, Pikaar NA, Hermus RJ (1983) Diet, faecal pH and colorectal cancer. British Journal of Cancer 48: 109–110

103. Edwards CA, Rowland IR (1991) Bacterial fermentation in the colon and its measurement In: Dietary Fibre. A component of Food, Nutritional Function in Health and Disease. ILSI Europe

104. Kobayashi H, Fleming SE (2001) The source of dietary fiber influences – short chain fatty acid production and concentrations in large bowel. Boca Raton: CRC Press

105. Rafter JJ, Child P, Anderson AM, Alder R, Eng V, Bruce WR (1987) Cellular toxicity of fecal water depends on diet. Am J Clin Nutr 45:559–563

106. Lapre JA, DeVries HT, Van der Meer R (1993) Cytotoxicity of fecal water is dependent on the type of dietary fat and is reduced by supplemental calcium phosphate in rats. J Nutr 123: 578–585

107. Van der Meer R, Termont DS, DeVries HT (1991) Differential effects of calcium ions and calcium phosphate on cytotoxicity of bile acids. Am J Physiol 260:142–147

108. Sesink AL, Termont DS, Kleibeuker JH, Van Der MR (2000) Red meat and colon cancer: dietary haem, but not fat, has cytotoxic and hyperproliferative effects on rat colonic epithelium. Carcinogenesis 21:1909–1915

109. Sesink AL, Termont DS, Kleibeuker JH, Van Der MR (1999) Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. Cancer Res 59:5704–5709

110. Glinghammar B, Venturi M, Rowland IR, Rafter J (1997) Shift from a dairy product-rich to a dairy product-free diet: influence on cytotoxicity and genotoxicity of fecal water – potential risk factors for colon cancer. Am J Clin Nutr 66:1277–1282

111. Venturi M, Hambly RJ, Glinghammar B, Rafter JJ, Rowland IR (1997) Genotoxic activity in human faecal water and the role of bile acids: a study using the alkaline comet assay. Carcinogenesis 18:2353–2359

112. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumour development. N Engl J Med 319:525–532

113. Rieger MA, Parlesak A, Pool-Zobel BL, Rechkemmer G, Bode C (1999) A diet high in fat and meat but low in dietary fibre increases the genotoxic potential of 'faecal water'. Carcinogenesis 20: 2311–2316

114. Osswald K, Becker TW, Grimm M, Jahreis G, Pool Zobel BL (2000) Inter- and intra-individual variation of faecal water – genotoxicity in human colon cells. Mutation Research 472: 59–70

115. Ahlquist DA, Gilbert J (1996) Stool markers for colorectal cancer screening. Future considerations. Dig Dis Sci 14:132–44

116. Ahlquist DA, Shuber AP (2002) Stool screening for colorectal cancer: evolution from occult blood to molecular markers. Clin Chim Act 315:157–68

117. Ahlquist DA, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE (2000) Colorectal cancer screening by detection of altered human DNA in stool: Feasibility of a multitarget assay system. Gastroenterol 119:1219–1227

118. Ruoslahti E (2002) Specialisation of tumour vasculature. Nat Rev Cancer 2:83–90

119. Dormond O, Foletti A, Paroz C, Ruegg C (2001) NSAIDs inhibit alpha V beta 3 integrin-mediated and Cdc42/Rac-dependent endothelial-cell spreading, migration and angiogenesis. Nat Med 7:1041–1047

120. Adlercreutz H, Fotsis T, Lampe JW, et al. (1993) Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas-chromatography mass-spectrometry. Scand J Clin Lab Invest 53:5–18

121. Masso-Welch PA, Zangani D, Ip C, Vaughan MM, Shoemaker S, Ramirez RA, Ip MM (2002) Inhibition of angiogenesis by the cancer chemopreventive agent conjugated linoleic acid. Cancer Res 62:4383–4389

122. Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, Schweigerer L (1993) Genistein, a dietary-derived inhibitor of in vitro angiogenesis. Proc Natl Acad Sci USA 90:2690–2694

123. Minagawa N, Nakayama Y, Hirata K, Onitsuka K, Inoue Y, Nagata N, Itoh H (2002) Correlation of plasma level and immunohistochemical expression of vascular endothelial growth factor in patients with advanced colorectal cancer. Anticancer Res 22:2957–2963

124. Tamura M, Ohta Y, Nakamura H, Oda M, Watanabe G (2002) Diagnostic value of plasma vascular endothelial growth factor as a tumour marker in patients with non-small cell lung cancer. Int J Biol Markers 17:275–279

125. Choi JH, Ahn MJ, Jang SJ, Park CK, Park YW, Oh HS, Lee YY, Choi IY, Kim IS (2002) Absence of clinical prognostic value of vascular endothelial growth factor and microvessel density in multiple myeloma. Int J Hematol 76:460–464

126. Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2:161–174

127. McCabe A, Wallace J, Gilmore WS, Strain JJ, McGlynn H (1999) The effects of eicosopentanoic acid on matrix metalloproteinase gene expression. Lipids 34:217–218

128. Shaker MR, Yang G, Timme TL, Park SH, Kadmon D, Ren CZ, Ji XR, Lee HM, Sehgal I, Anzano M, Sporn MB, Thompson TC (2001) Dietary 4-HPR suppresses the development of bone metastasis in vivo in a mouse model of prostate cancer progression. Clin Exper Metastasis 18:429–438

129. Nangia-Makker P, Hogan V, Honjo Y, Baccarini S, Tait L, Bresalier R, Raz A (2002) Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. J Natl Cancer Inst 94:1854–1862

130. McIntosh GH, Royle PJ, Playne MJ (1999) A probiotic strain of L acidophilus reduces DMH-induced large intestinal tumours in male Sprague-Dawley rats. Nutr Cancer 35:153–159

131. Tsunezuka Y, Kinoh H, Takino T, Watanabe Y, Okada Y, Shinagawa A, Sato H, Seiki M (1996) Expression of membrane-type matrix metalloproteinase 1 (MT1-MMP) in tumour cells enhances pulmonary metastasis in an experimental metastasis assay. Cancer Res 56:5678–5683

132. Hua J, Muschel RJ (1996) Inhibition of matrix metalloproteinase 9 expression by a ribozyme blocks metastasis in a rat sarcoma model system. Cancer Res 56:5279–5284

133. Dyce M, Sharif SF, Whalen GF (1992) Search for anti-metastatic therapy: effects of phenytoin on B16 melanoma metastasis. J Surg Oncol 49:107–112

134. Xing RH, Rabbani SA (1999) Regulation of urokinase production by androgens in human prostate cancer cells: effect on tumour growth and metastases in vivo. Endocrinology 140:4056–4064

135. Arlt M, Kopitz C, Pennington C, Watson KL, Krell HW, Bode W, Gansbacher B, Khokha R, Edwards DR, Kruger A (2002) Increase in gelatinase-specificity of matrix metalloproteinase inhibitors correlates with antimetastatic efficacy in a T-cell lymphoma model. Cancer Res 62:5543–5550

136. Leather AJ, Gallegos NC, Kocjan G, Savage F, Smales CS, Hu W, Boulos PB, Northover JM, Phillips RK (1993) Detection and enumeration of circulating tumour cells in colorectal cancer. Br J Surg 80:777–780

137. Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmuller G (1992) Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet 340:685–689

138. Moreno JG, O'Hara SM, Gross S, Doyle G, Fritsche H, Gomella LG, Terstappen LW (2001) Changes in circulating carcinoma cells in patients with metastatic prostate cancer correlate with disease status. Urology 58:386–392

139. Mayhew E, Glaves D (1984) Quantitation of tumorigenic disseminating and arrested cancer cells. Br J Cancer 50:159–166

140. Molnar B, Ladanyi A, Tanko L, Sreter L, Tulassay Z (2001) Circulating tumour cell clusters in the peripheral blood of colorectal cancer patients. Clin Cancer Res 7:4080–4085

141. Fehm T, Sagalowsky A, Clifford E, Beitsch P, Saboorian H, Euhus D, Meng S, Morrison L, Tucker T, Lane N, Ghadimi BM, Heselmeyer-Haddad K, Ried T, Rao C, Uhr J (2002) Cytogenetic evidence that circulating epithelial cells in patients with carcinoma are malignant. Clin Cancer Res 8:2073–2084

142. Wattenberg LW (1992) Inhibition of carcinogenesis by minor dietary constituents. Cancer Res 52 (Suppl): 2085s–2091s

143. Zhang BC, et al. (1997) Oltipraz Chemoprevention Trial in Qidong, Jiangsu Province, People's Republic of China. J Cellular Biochemistry Supplements 28/29:166–173

144. Kensler TW, Helzlsouer KJ (1995) Oltipraz: Clinical opportunities for cancer chemoprevention. J Cell Biochem 22 (Suppl):101–107

145. Wang JS, Shen X, He X, Zhu YR, Zhang BC, Wang JB, Qian GS, Kuang SY, Zarba A, Egner PA, Jacobson LP, Munoz A, Helzlsouer KJ, Groopman JD, Kensler TW (1999) Protective alterations in phase 1 and 2 metabolism of aflatoxin B1 by oltipraz in residents of Qidong, People's Republic of China. J Natl Cancer Inst 91:347–354

146. deBethizy JD, Hayes JR (1994) Metabolism, a determinant of toxicity. In: Hayes AW (ed) Principles and methods of toxicology, 3 edn. Raven Press Ltd., New York, pp 59–100

147. Sivaraman L, Leatham MP, Yee J, Wilkens LR, Lau AF, Marchand LL (1994) CYP1A1 genetic polymorphisms and in situ colorectal cancer. Cancer Res 54:5692–5695

148. Smith G, Stanley LA, Sim E, Strange RC, Wolf CR (1995) Metabolic polymorphisms and cancer susceptibility. In: Ponder H, Bruce AJ (eds) Genetics and cancer, a second look. Cold Spring Harbor Laboratory Press, New York, pp 27–65

149. Bartsch H, Hietanen E (1996) The role of individual susceptibility in cancer burden related to environmental exposure. Environ Health Perspect 104: 569–577

J. Rafter et al.
PASSCLAIM – Diet-related cancer

II/79

150. Sachse C, Smith G, Wilkie MJV, Barrett JH, Waxman R, Sullivan F, Forman D, Bishop DT, Wolfl CR, The Colon Cancer Study Group (2003) A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis 23: 1839–1849

151. Guengerich FP (2000) Metabolism of chemical carcinogens. Carcinogenesis 21:345

152. Kaderlik KR, Minchin RF, Mulder GJ, Ilett KF, Daugaard-Jenson M, Teitel CH, Kadlubar FF (1994) Metabolic activation pathway for the formation of DNA adducts of the carcinogen 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) in rat extrahepatic tissues. Carcinogenesis 15: 1703–1709

153. Murray BP, Edwards RJ, Murray S, Singleton AM, Davies DS, Boobis AR (1993) Human hepatic CYP1A1 and CYP1A2 content, determined with specific anti-peptide antibodies, correlates with the mutagenic activation of PhIP. Carcinogenesis 14:585–592

154. Turesky RJ (2002) Heterocyclic aromatic amine metabolism, DNA adduct formation, mutagenesis, and carcinogenesis. Drug Metabolism Reviews 34:625–650

155. Palli D, Masala G, Vineis P, Garte S, Saieva C, Krogh V, Panico S, Tumino R, Munnia A, Riboli E, Peluso M (2003) Biomarkers of dietary intake of micronutrients modulate DNA adduct levels in healthy adults. Carcinogenesis 24:739

156. World Cancer Research Fund, American Institute for Cancer Research (1997) Food, nutrition and the prevention of cancer: a Global perspective. American Institute for Cancer Research, Washington DC

157. Lampe JW, Peterson S (2002) Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. J Nutr 132:2991–2994

158. Murray S, Lake BG, Gray S, Edwards AJ, Springall C, Bowey Ea, Williamson G, Boobis AR, Gooderham NJ (2001) Effect of cruciferous vegetable consumption on heterocyclic aromatic amine metabolism in man. Carcinogenesis 22:1413–1420

159. van Poppel G, Verhoeven DT, Verhagen H, Goldbohm RA (1999) Brassica vegetables and cancer prevention. Epidemiology and mechanisms. Adv Exp Med Biol 472:159–168

160. Seow A, Yuan JM, Sun CL, Van Den Berg D, Lee HP, Yu MC (2002) Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. Carcinogenesis 23: 2055–2061

161. Johnson IT (2002) Glucosinolates: bioavailability and importance to health. Int J Vitamins and Nutr Res 72:26–31

162. Slattery ML, Kampman E, Samowitz W, Caan B, Potter JD (2000) Interplay between dietary inducers of GST and the GSTM-1 genotype in colon cancer. Int J Cancer 87:728–733

163. Lampe JW, Chen C, Li S, Prunty J, Grate MT, Meehan DE, Barale KV, Dightman Da, Feng ZPJD (2000) Modulation of human glutathione S-transferases by botanically defined vegetable diets. CancerEpidemiol.Biomarkers Prev 9: 787–793

164. Simmons PT, Portier CJ (2002) Toxicogenomics: the new frontier in risk analysis. Carcinogenesis 23:903

165. Roses AD (2000) Pharmacogenetics and the practice of medicine. Nature 405:857–865

166. Hayes JD, Pulford DJ (1995) The glutathione S-transferase supergene family. regulation of GST* and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Critical Reviews in Biochemistry and Molecular Biology 30:445–460

167. Armstrong RN (1997) Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem Res Toxicol 10:2–18

168. Cerutti PA (1985) Prooxidant states and tumour promotion. Science 227: 375–381

169. Hayes JD, Strange RC (1995) Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. Free Rad Res 22:193–207

170. Berhane K, Widersten M, Engstrom A, Kozarich JW, Mannervik B (1994) Detoxication of base propenals and other a,b-unsaturated aldehyde products of radical reactions and lipid peroxidation by humane glutathione transferases. Proc Natl Acad Sci USA 91:1480–1484

171. Waleh NS, Calaoagan J, Murphy BJ, Knapp AM, Sutherland RM, Laderoute KR (1998) The redox-sensitive human antioxidant responsive element induces gene expresseion under low oxygen conditions. Carcinogenesis 19: 1333–1337

172. Rushmore TH, Morton Mr, Pickett CB (1991) The antioxidant responsive element. J Biol Chem 18:11632–11639

173. Chen C, Yu R, Owuor ED, Kong AN (2000) Activation of antioxidant-response element (ARE), mitogen activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. Arch Pharm Res 23: 605–612

174. Pinkus R, Weinert LM, Daniel V (1996) Role of oxidants and antioxidants in the induction of AP-1, NF-kB, and glutathione S-transferase gene expression. J Biol Chem 271:13422–13429

175. Desmots F, Rauch C, Henry C, Guillouzo A, Morel F (1998) Genomic organization, 5'-flanking region and chromosomal localization of the human glutatione transferase A4 gene. Biochem J 336:437–442

176. Whalen R, Boyer T (1998) Human glutathione S-transferases. Seminars in Liver Disease 18:345–358

177. Johnson IT, Williamson G, Musk SRR (1994) Anticarcinogenic factors in plant foods: A new class of nutrients? Nutr Res Rev 7:175–204

178. Ebert MN, Beyer-Sehlmeyer G, Liegibel UM, Kautenburger T, Becker TW, Pool-Zobel BL (2001) Butyrate-induced activation of glutathione S-transferases protects human colon cells from genetic damage by 4-Hydroxynonedienal. Nutr Cancer 41: 156–164

179. Knoll N (2002) Modulation der GST Aktivität in HT29Zellen und Konsequenzen für die Genotoxizität von 4-Hydroxynonena. 2002. Friedrich-Schiller University Jena

180. Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N, Ookuma R, Bell DA (1996) Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. Carcinogenesis 17: 1855–1859

181. de Jong MM, Nolte IM, te Meerman GJ, van der Graaf WTA, de Vries EGE, Sijmons RH, Hofstra RMW, Kleibeuker JH (2002) Low-penetrance genes and their involvement in colorectal cancer susceptibility. Cancer Epidemiol Biomarkers Prev 11:1332–1352

182. Brockton NT (2002) UGT1A1 polymorphisms and colorectal cancer susceptibility. Gut 50:749

183. Ichiba M, Wang Y, Oishi H, Iyadomi M, Shono N, Tomokuni K (1996) Smoking-related DNA adducts and genetic polymorphism for metabolic enzymes in human lymphocytes. Biomarkers 1:211–214

184. Marchand LL, Hankin JH, Wilkens LR, Pierce LM, Franke A, Kolonel LN, Seifried A, Custer LJ, Chang W, Lum-Jones A, Donlon T (2002) Combined effects of well-done red meat, smoking, and rapid N-acetyltransferase 2 and CYP1A2 phenotypes in increasing colorectal cancer risk. Canc Epid Biom Prev 10:1259–1266

185. Hu X, Herzog C, Zimniak P, Singh SV (1999) Differential protection against benzo [a]pyrene-7.8-dihydro-diol-9.10-epoxide-induced DNA damage in HepG2 Cells stably transfected with allelic variants of p class human glutathone S-transferases. Cancer Res 59:2358–2362

186. Chou MW, Mikhailova MV, Nichols J, Poirier LA, Warbritton A, Beland FA (2000) Interactive effects of methyl-deficiency and dietary restriction on liver cell proliferation and telomerase activity in Fischer 344 rats pretreated with aflatoxin B(1). Cancer Lett 152: 53–61

187. Primiano T, Sutter TR, Kensler TW (1997) Antioxidant-inducible genes. Adv Pharmacol 38:293–328

188. Stein J, Schröder O, Bonk M, Oremek G, Lorenz M, Caspary WF (1996) Induction of glutathione-S-transferase-pi by short-chain fatty acids in the intestinal cell line Caco-2. Europ J Clin Invest 26:84–87

189. Treptow-van Lishaut S, Rechkemmer G, Rowland IR, Dolara P, Pool-Zobel BL (1999) The carbohydrate crystalean and colonic microflora modulate expression of glutathione S-transferase subunits in colon of rats. Eur J Nutr 38:76–83

190. van Lieshout EMM, Bedaf MMG, Pieter M, Ekkel C, Nijhoff WA, Peters WHM (1998) Effects of dietary anticarcinogens on rat gastrointestinal glutathione S-transferase theta 1–1 levels. Carcinogenesis 19:2055–2057

191. Bogaards JJP, Verhagen H, Willems MI, van Poppel G, van Bladeren PJ (1994) Consumption of Brussels sprouts results in elevated a class glutathione S-transferase levels in human blood plasma. Carcinogenesis 15:1073–1075

192. Kensler TW, Groopman JD, Wogan GN (1996) Use of carcinogen-DNA and carcinogen-protein adduct biomarkers for cohort selection and as modifiable end points in chemoprevention trials. In: Steward BW, McGregor DB, Kleihues P (eds) Principles of Chemoprevention. International Agency for Research on Cancer, Lyon, pp 237–248

193. Szarka CE, Pfeiffer GR, Hum ST, Everly LC, Balshem AM, Moore DF, Litwin S, Goosenberg EB, Frucht H, Engstrom PF, Clapper ML (1995) Glutathione S-transferase activity and glutathione S-transferase m expression in subjects with risk for colorectal cancer. Cancer Res 55:2789–2793

194. Ebert MN, Klinder A, Schäferhenrich A, Peters WHM, Sendt W, Scheele J, Pool-Zobel BL (2003) Expression of glutathione S-transferase (GST) in human colon cells and inducibility of GSTM2 by butyrate. Carcinogenesis 24:1637–1644

195. Morel F, Schulz WA, Sies H (1994) Gene structure and regulation of expression of human glutathione S-transferases alpha. Biol Chem 375:641–649

196. Thier R, Taylor JB, Pemble EE, Griffeth HW, Persmark M, Ketterer B, Guengerich FP (1993) Expression of mammalian glutathione S-transferase 5–5 in Salmonella typhimurium TA 1535 leads to base-pair mutations upon exposure to dihalomethanes. Proc Natl Acad Sci USA 90:8576–8580

197. Kim DJ, Han BS, Ahn B, Hasegawa R, Shirai T, Ito N, Tsuda H (1997) Enhancement by indole-3-carbinol of liver and thyroid gland neoplastic development in a rat medium-term multiorgan carcinogenesis model. Carcinogenesis 18:377–381

198. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice RR, Waters MD, Aitio A (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutation Research 463:111–172

199. Verhagen H, Aruoma OI, van Delft JHM, Dragsted LO, Ferguson LR, Knasmüller S, Pool-Zobel BL, Poulsen HE, Williamson G, Yannai S (2003) The 10 basic requirements for a scientific paper reporting antioxidant, antimutagenic or anticarcinogenic potential of test substances in in vitro experiments and animal studies in vivo. Food Chem Toxicol 41:603–610

200. Ames BN, Wakimoto P (2002) Are vitamin and mineral deficiencies a major cancer risk? Nature Reviews Cancer 9:694–704

201. Pool-Zobel BL, Bub A, Müller H, Wollowski I, Rechkemmer G (1997) Consumption of vegetables reduces genetic damage in humans: first results of an intervention trial with carotenoid-rich foods. Carcinogenesis 18: 1847–1850

202. Bub A, Watzl B, Blockhaus M, Briviba K, Liegibel UM, Müller H, Pool-Zobel BL, Rechkemmer G (2003) Fruit juice consumption modulates antioxidative status, immune status and DNA damage. J Nutr Biochem 14:98

203. Fenech M, Stockley C, Aitken C (1997) Moderate wine consumption protects against hydrogen peroxide-induced DNA damage. Mutagenesis 12: 289–296

204. Pool-Zobel BL, Münzner R, Holzapfel WH (1993) Antigenotoxic properties of lactic acid bacteria in the Salmonella typhimurium mutagenicity assay. Nutr Canc 20:261–270

205. Pool-Zobel BL, Neudecker C, Domizlaff I, Ji S, Schillinger U, Rumney CJ, Moretti M, Villarini M, Scassellati-Sforzolini G, Rowland IR (1996) Lactobacillus- and Bifidobacterium-mediated antigenotoxicity in colon cells of rats: Prevention of carcinogen-induced damage in vivo and elucidation of involved mechanisms. Nutr Canc 26:365–380

206. Pool-Zobel BL, Lotzmann N, Knoll M, Kuchenmeister F, Lambertz R, Leucht U, Schröder HG, Schmezer P (1994) Detection of DNA damage in gastric, colonic and nasal mucosa cells derived from the rat and from human biopsy specimens. Environ Mol Mutagen 24: 23–45

207. Pool-Zobel BL, Abrahamse SL, Collins AR, Kark W, Gugler R, Oberreuther D, Siegel EG, Treptow-van Lishaut S, Rechkemmer G (1999) Analysis of DNA strand breaks, oxidized bases and glutathione S-transferase P1 in human colon cells. Canc Epid Biom Prev 8:609–614

208. Schäferhenrich A, Sendt W, Scheele J, Kuechler A, Liehr T, Claussen U, Rapp A, Greulich KO, Pool-Zobel BL (2003) Endogenously formed cancer risk factors induce damage-of p53 in human colon cells obtained from surgical samples. Fd Chem Toxicol 41:655–664

209. Singh NP, Tice RR, Stephens RE, Schneider EL (1991) A microgel electrophoresis technique for the direct quantitation of DNA damage and repair in individual fibroblasts cultured on microscope slides. Mutation Res 252:289–296

210. Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO (1983) Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutation Research 113:357–391

211. Pool BL, Brendler SY, Liegibel UM, Tompa A, Schmezer P (1990) Employment of adult mammalian primary cells in toxicology: In vivo and in vitro genotoxic effects of environmentally significant N-nitrosodialkylamines in cells of the liver, lung and kidney. Environm Mol Mutagen 15:24–35

212. Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V, Tice RR (2003) Recommendations for conducting the in vivo alkaline Comet assay. Mutagenesis 18:45–51

213. Kassie F, Parzefall W, Knasmuller S (2000) Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. Mutat Res 463: 13–31

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214. Veglia F, Matullo G, Vineis P (2003) Bulky DNA adducts and risk of cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 12:157–160

215. Fang JL, Vaca CE (1997) Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers. Carcinogenesis 18:632

216. Schabath MB, Spitz MR, Grossman HB, Zhang K, Dinney CP, Zheng PJ, Wu X (2003) Genetic instability in bladder cancer assessed by the Comet assay. JNCI Cancer Spectrum 95:540

217. van Poppel G, Poulson HE, Loft S, Verhagen H (1995) No influence of beta carotene on oxidative DNA damage in male smokers. J Natl Cancer Inst 87:310–311

218. Duthie SJ, Ma A, Ross MA, Collins AR (1996) Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer Res 56: 1291–1295

219. Mitchell JH, Collins AR (1999) Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men – a pilot study. Eur J Nutr 38:143–148

220. Gedik CM, Boyle SP, Wood SG, Vaughan NJ, Collins AR (2002) Oxidative stress in humans: validation of biomarkers of DNA damage. Carcinogenesis 23:1441–1446

221. Giovannelli L, Saieva C, Masala G, Testa G, Salvini S, Pitozzi V, Riboli E, Dolara P, Palli D (2002) Nutritional and lifestyle determinants of DNA oxidative damage: a study in a Mediterranean population. Carcinogenesis 23: 1483–1489

222. Vulimiri SV, Smith CV, Randerath E, Randerath K (1994) 32P-Postlabeling of bile components: bulky adduct-like behavior in polyethyleneimine-cellulose thin layer chromatography. Carcinogenesis 15:2061–2064

223. Randerath K, Zhou GD, Monk SA, Randerath E (1997) Enhanced levels in neonatal rat liver of 7.8-dihydro-8-oxo-2'deoxyguanosine (8-hydroxy-deoxyguanosine), a major mutagenic oxidative DNA lesion. Carcinogenesis 18:1419–1421

224. Friedberg EC (2001) How nucleotide excision repair protects against cancer. Nature Reviews Cancer 1:22–33

225. Hoeijmakers JHJ (2001) Genome maintenance mechanisms for preventing cancer. Nature 411:366–374

226. Margison GP, Povey AC, Kaina B, Santibanez Koref MF (2003) Variability and regulation of O6-alkylguanine-DNA alkyltransferase. Carcinogenesis 24:625

227. Hesketh R (1997) The Oncogene and Tumour Suppressor Gene Facts Book, 2 edn

228. Fodde R, Smits R, Clevers H (2001) APC signal transduction and genetic instability in colorectal cancer. Nature Reviews Cancer 1:55–67

229. Smerhovsky Z, Landa K, Rössner P, Juzova D, Brabec M, Zudova Z, Hola N, Zarska H, Nevsimalova E (2002) Increased risk of cancer in radon-exposed miners with elevated frequency of chromosomal aberrations. Mutation Res 514:176

230. Bonassi S, Hagmar L, Stromberg U, Montagud AH, Tinnerberg H, Forni A, Heikkila P, Wanders S, Wilhardt P, Hansteen IL, Knudsen LE, Norppa H (2000) Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res 60:619–625

231. Ramsey MJ, Moore DH, Briner JF, Lee DA, Olsen L, Senft JR, Tucker JD (1995) The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. Mutation Res 338:95–106

232. Fenech MF, Dreosti I, Aitken C (1997) Vitamin-E supplements and their effect on vitamin-E status in blood and genetic damage rate in peripheral blood lymphocytes. Carcinogenesis 18:359–364

233. Fenech MF, Dreosti I, Rinaldi JR (1997) Folate, vitamin B12, homocysteine status and chromosome damage rate in lymphocytes of older men. Carcinogenesis 18:1329–1336

234. Zijno A, Andreoli C, Leopardi P, Marcon F, Rossi S, Caiola S, Verdina A, Galati R, Cafolla A, Crebelli R (2003) Folate status, metabolic genotype, and biomarkers of genotoxicity in healthy subjects. Carcinogenesis 24:1097–1103

235. Fenech M, Aitken C, Rinaldi J (1998) Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. Carcinogenesis 19: 1163–1171

236. Fenech M (2001) The role of folic acid and Vitamin B12 in genomic stability of human cells. Mutation Res 475: 57–67

237. Levine AJ, Salvan A, Talaska G, Boeniger MF, Suruda A, Schulte PA (1997) The utility of epithelial-cell micronuclei in the assessment of intermittent exposures. Biomarkers 2: 135–138

238. Desai SS, Ghaisas SD, Jakhi SD, Bhide SV (1996) Cytogenetic damage in exfoliated oral mucosal and circulating lymphocytes of patients suffering from precancerous oral lesions. Cancer Lett 109:9–14

239. Schatzkin A, Gail M (2002) The promise and peril of surrogate endpoints in cancer research. Nature Reviews Cancer 2:1–9

240. Yamasaki H, Omori Y, Zaidan-Dagli ML, Mironov N, Mesnil M, Krutovskikh V (1999) Genetic and epigenetic changes of intercellular communication genes during multistage carcinogenesis. Cancer Detect Prev 23: 273–279

241. Chaumontet C, Bex V, Gaillard-Sanchez I, Seillan-Heberden C, Suschetet M, Martel P (1994) Apigenin and tangeretin enhance gap junctional intercellular communication in rat liver epithelial cells. Carcinogenesis 15:2325–2330

242. Bex V, Mercier T, Chaumontet C, Gaillard-Sanchez I, Flechon B, Mazet F, Traub O, Martel P (1995) Retinoic acid enhances connexin43 expression at the post- transcriptional level in rat liver epithelial cells. Cell Biochem Funct 13:69–77

243. Sies H, Stahl W (1997) Carotenoids and intercellular communication via gap junctions. Int J Vitam Nutr Res 67:364–367

244. Trosko JE, Chang CC (2001) Mechanism of up-regulated gap junctional intercellular communication during chemoprevention and chemotherapy of cancer. Mutat Res 480–481:219–229

245. Auvinen M, Paasinen A, Andersson LC, Holtta E (1992) Ornithine decarboxylase activity is critical for cell transformation. Nature 360:355–358

246. Thomas T, Thomas TJ (2001) Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci 58: 244–58

247. Tabib A, Bachrach U (1999) Role of polyamines in mediating malignant transformation and oncogene expression. Int J Biochem Cell Biol 31: 1289–1295

248. Lavelle F, Riou JF, Laoui A, Mailliet P (2000) Telomerase: a therapeutic target for the third millennium? Crit Rev Oncol Hematol 34:111–126

249. Ouellette MM, Lee K (2001) Telomerase: diagnostics, cancer therapeutics and tissue engineering. Drug Discov Today 6:1231–1237

250. Schmidt PM, Lehmann C, Matthes E, Bier FF (2002) Detection of activity of telomerase in tumour cells using fiber optical biosensors. Biosens Bioelectron 17:1081–1087

251. Steele VE, Hawk ET, Viner JL, Lubet RA (2003) Mechanisms and applications of non-steroidal anti-inflammatory drugs in the chemoprevention of cancer. Mutat Res 523–524:137–44

252. Ding XZ, Hennig R, Adrian TE (2003) Lipoxygenase and cyclooxygenase metabolism: new insights in treatment and chemoprevention of pancreatic cancer. Mol Cancer 2:10

253. Li JK, Lin-Shia SY (2001) Mechanisms of cancer chemoprevention by curcumin. Proc Natl Sci Counc Repub China B 25:59–66

254. de la Puerta R, Ruiz Gutierrez V, Hoult JR (1999) Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. Biochem Pharmacol 57:445–449

255. Issa JP (1999) Aging, DNA methylation and cancer. Crit Rev Oncol Hematol 32:31–43

256. Verma M, Srivastava S (2002) Epigenetics in cancer: implications for early detection and prevention. Lancet Oncol 3:755–763

257. Kim YI, Baik HW, Fawaz K, Knox T, Lee YM, Norton R, Libby E, Mason JB (2001) Effects of folate supplementation on two provisional molecular markers of colon cancer: a prospective, randomized trial. Am J Gastroenterol 96:184–195

258. Stover PJ GC (2002) Molecular and genetic considerations for long-term nutrition interventions. Asia Pacific J Clin Nutr 11:S129–S136

259. Cairns BR (2001) Emerging roles for chromatin remodeling in cancer biology. Trends Cell Biol 11:S15–S21

260. Hinnebusch BF, Meng S, Wu JT, Archer SY, Hodin RA (2002) The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. J Nutr 132:1012–1017

261. Joyce D, Albanese C, Steer J, Fu M, Bouzahzah B, Pestell RG (2001) NF-kappaB and cell-cycle regulation: the cyclin connection. Cytokine Growth Factor Rev 12:73–90

262. Gilmore T, Gapuzan ME, Kalaitzidis D, Starczynowski D (2002) Rel/NF-kappa B/I kappa B signal transduction in the generation and treatment of human cancer. Cancer Lett 181:1–9

263. Yu R, Hebbar V, Kim DW, Mandlekar S, Pezzuto JM, Kong AN (2001) Resveratrol inhibits phorbol ester and UV-induced activator protein 1 activation by interfering with mitogen-activated protein kinase pathways. Mol Pharmacol 60:217–224

264. DiSepio D, Sutter M, Johnson AT, Chandraratna RA, Nagpal S (1999) Identification of the AP1-antagonism domain of retinoic acid receptors. Mol Cell Biol Res Commun 1:7–13

265. Hill BT, Perrin D, Kruczynski A (2000) Inhibition of RAS-targeted prenylation: protein farnesyl transferase inhibitors revisited. Crit Rev Oncol Hematol 33:7–23

266. Haluska P, Dy GK, Adjei AA (2002) Farnesyl transferase inhibitors as anticancer agents. Eur J Cancer 38:1685–1700

267. Kong AN, Yu R, Hebbar V, Chen C, Owuor E, Hu R, Ee R, Mandlekar S (2001) Signal transduction events elicited by cancer prevention compounds. Mutat Res 480–481:231–241

268. Philipp-Staheli J, Payne SR, Kemp CJ (2001) p27(Kip1): regulation and function of a haploinsufficient tumour suppressor and its misregulation in cancer. Exp Cell Res 264:148–168

269. Gupta S, Afaq F, Mukhtar H (2002) Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. Oncogene 21:3727–3738

270. Bloom J, Pagano M (2003) Deregulated degradation of the cdk inhibitor p27 and malignant transformation. Semin Cancer Biol 13:41–47

271. Hambly RJ, Rumney CJ, Cunninghame M, Fletcher JME, Rijken PJ, Rowland IR (1997) Influence of diets containing high and low risk factors for colon cancer on early stages of carcinogenesis in human flora-associated (HFA) rats. Carcinogenesis 18:1535–1539

272. Goldin BR (1986) In situ bacterial metabolism and colon mutagens. Ann Rev Microbiol 40:367–393

273. Mallett AK, Rowland IR (1988) Factors affecting the gut microflora. In: Role of the Gut Flora in Toxicity and Cancer. Academic Press

274. Carman RJ, Van Tassel RL, Kingston DGI (1988) Conversion of IQ, a dietary pyrolysis carcinogen to a direct mutagen by intestine bacteria of humans. Mutation Res 206:335–342

275. Clinton SK, Bostwick DG, Olson LM, Mangian HJ, Visek WJ (1988) Effects of ammonium acetate and sodium cholate on N-methyl-N'-nitro-N-nitrosoguanidine-induced colon carcinogenesis of rats. Cancer Res 48:3035–3039

276. Pickering JS, Lupton JR, Chapkin RS (1995) Dietary fat, fiber and carcinogen alter fecal diacylglycerol composition and mass. Cancer Res 55:2293–2298

277. Reddy BS, Simi B, Engle A (1994) Biochemical epidemiology of colon cancer: effect of various types of dietary fiber on colonic diaclglycerols in women. Gastroenterology 106:883–889

278. Hinzman MJ, Novotny C, Ullah A, Shamsuddin AM (1987) Fecal mutagen fecapentaene-12 damages mammalian colon epithelial DNA. Carcinogenesis 8:1475–1479

279. Shamsuddin AM, Ullah A, Baten A, Hale E (1991) Stability of fecapentaene-12 ans its carcinogenicity in F-344 rats. Carcinogenesis 12:601–607

280. Schiffman MH, Van Tassell RL, Robinson A, Smith L, Daniel J, Hoover RN, Weil R, Rosenthal J, Nair PP, Schwartz S (1989) Case-control study of colorectal cancer and fecapentaene excretion. Cancer Res 49:1322–1326

281. Macfarlane GT, Cummings JH (1991) The colonic flora, fermentation and the large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter RG (eds) The Large Intestine: Physiology, Pathophysiology and Disease. Raven Press, New York, pp 51–91

282. Crofts F, Taioli E, Trachman J, Cosma GN, Currie D, Tonniolo P, Garte SJ (1994) Functional significance of different human CYP1A1 genotypes. Carcinogenesis 15:2961–2963

283. Dai R, Jacobson KA, Robinson RC, Friedman FK (1997) Differential effects of flavonoids on testosterone metabolizing cytochrome P450s. Life Sciences 6:75–80

284. Kawata S, Tamura S, Matsuda Y, Ito N, Matsuzawa Y (1992) Effect of dietary fiber on cytochrome P450IAI induction in rat colonic mucosa. Carcinogenesis 13:2121–2125

285. Langouet S, Coles B, Morel F, Becquemont L, Beaune P, Guengerich FP, Ketterer B, Guillouzo A (1995) Inhibition of CYP1A2 and CYP3A4 by oltipraz results in reduction of aflatoxin B1 metabolism in human hepatocytes in primary culture. Cancer Res 55:5574–5579

286. LeCluyse E, Madan A, Hamilton G, Carroll K, DeHaan RPA (2000) Expression and regulation of cytochrome P450 enzymes in primary cultures of human hepatocytes. J Biochem Mol Toxicol 14:177–188

287. Roland N, Migpm-Baudon L, Flinois JP, Beaune PH (1994) Hepatic and Intestinal cytochrome P450, Glutathone s-transferase and UDP Glucuronosyl transferase are affected by six types of dietary fiber in rats inoculated with human whole fecal flora. J Nutr 124:1581–1587

288. Rosenberg DW (1991) Tissue specific induction of the carcinogen inducible cytochrome P450 isoform, P450IA1 in colonic epithelium. Arch Biochemistry Biophysics 284:223–226

289. Sinha R, Rothman N, Brown ED, Mark SD, Hoover RN, Caporaso NE, Levander OA, Knize MG, Lang NP, Kadlubar FF (1994) Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycyclic aromatic hydrocarbons induces cytochrome P4501A2 activity in humans. Cancer Res 54:6154–6159

J. Rafter et al.
PASSCLAIM – Diet-related cancer

II/83

290. Stillwell WG, Kidd LCR, Wishnok JS, Tannenbaum SR, Sinha R (1997) Urinary excretion of unmetabolized and phase II conjugates of 2-amino-1-methyl-6-phenylimidazo [4, 5-b]pyridine and 2-amino-3.8-dimethylimidazo [4, 5-f]quinoxaline in humans: relationship to cytochrome p4501A2 and N-acetyltransferase activity. Cancer Res 57:3457–3464

291. Vang O, Jensen H, Autrup H (1991) Induction of cytochrome p-450IAq, IA2, IIB1, IIB2 and IIE1 by broccoli in rat liver and colon. Chem-Biol Interac 78: 85–96

292. Wolf CR, Mahmood A, Henderson CJ, McLeod R, Manson MM, Neal gE, Hayes JD (1996) Modulation of the cytochrome p450 system as a mechanism of chemoprotection. In: Steward BW, McGregor DB, Kleihues P (eds) Principles of Chemoprevention. International Agency for Research on Cancer, Lyon, pp 165–174

293. Cashman JR (2001) Flavin monooxygenases. In: Ioannides C (ed) John Wiley and sons, Chicester, pp 67–93

294. Cashman JR (2002) Human and plant flavin-containing monooxygenase N-oxygenation of amines: detoxication vs. bioactivation. Drug Metabolism Rev 34:513–521

295. Krueger SK, Williams DE, Yueh M-F, Martin SR, Hines RN, Raucy JL, Dolphin CT, Shepard EA, Phillips IR (2002) Genetic polymorphisms of flavin-containing monooxygenase (FMO). Drug Metabolism Reviews 34:532

296. Ziegler DM (2002) An overview of the mechanism, substrate specificities and the structure of FMOs. Drug Metabolism Reviews 34:503–511

297. Benson AM, Hunkeler MJ, Talalay P (1980) Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. Proc Natl Acad Sci USA 77:5216–5220

298. Montano MM, Katzenellenbogen BS (1997) A quinone reductase gene: a unique estrogen receptor-regulated gene that is activated by antiestrogens. Proc Natl Acad Sci USA 94:2581–2586

299. Smith MT (1999) Benzene, NQO1, and genetic susceptibility to cancer. Proceedings of the National Academy of Sciences USA 96:7624–7626

300. Tawfig N, Wanigatunga S, Heaney RK, Musk SRR, Williamson G, Fenwick GR (1994) Induction of the anti-carcinogenic enzyme quinone reductase by food extracts using murine hepatoma cells. Eur J Cancer Prevention 3: 285–292

301. Tawfig N, Heaney RK, Plumb JA, Fenwick GR, Musk SRR, Williamson G (1995) Dietary glucosinolates as blocking agents against carcinogenesis: Glucosinolate breakdown products assessed by induction of quinone reductase activity in murine hepa1c1c7 cells. Carcinogenesis 16: 1191–1194

302. Uda Y, Price KR, Williamson G, Rhodes MJC (1997) Induction of the anticarcinogenic marker enzyme, quinone reductase, in murine hepatoma cells in vitro by flavonoids. Cancer Lett 120: 213–216

303. Wang W, Liu LQ, Higuchi CM, Chen H (1998) Induction of NADPH: Quinone reductase by dietary phytoestrogens in colonic Colo205 cells. Biochem Pharmacol 56:189–195

304. Yannai S, Day AJ, Williamson G, Rhodes MJC (1998) Characterization of flavonoids as monofunctional or bifunctional inducers of quinone reductase in murine hepatoma cell lines. Fd Chem Toxicol 36:623–630

305. Clapper ML, Szarka CE (1998) Glutathione S-transferases – biomarkers of cancer risk and chemopreventive response. ChemBiol Interac 111–112: 377–388

306. Hayes JD, McLeod R, Ellis EM, Ulford DJ, Ireland LS, McLellan LI, Udah DJ, Anson MM, Eal GE (1996) Regulation of glutathione S-transferases and aldehyde reductase by chemoprotectors: studies of mechanisms responsible for inducible resistance to aflatoxin B1. In: Steward BW, McGregor DB, Kleihues P (eds) Principles of Chemoprevention. International Agency for Research on Cancer, Lyon, pp 175–188

307. Kirlin WG, Cai J, DeLong MJ, Patten EJ, Jones DP (1999) Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. J Nutr 129:1827–1835

308. Nijhoff WA, Mulder TPJ, Verhagen H, van Poppel G, Peters WHM (1995) Effects of consumption of Brussels sprouts on plasma and urinary glutathione S-transferase class-a and -p in humans. Carcinogenesis 16: 955–957

309. Embola CW, Weisburger JH, Weisburger MC (2001) Urinary excretion of N-OH-2-amino-3-methylimidazo [4, 5-f]quinoline-N-glucuronide in F344 rats is enhanced by green tea. Carcinogenesis 22:1095–1098

310. Embola CW, Sohn OS, Fiala ES, Weisburger JH (2002) Induction of UDP-glucuronosyltransferase 1 (UDP-GT1) gene complex by green tea in male F344 rats. Fd Chem Toxicol 40:841–844

311. Fisher MB, Paine MF, Strelevitz TJ, Wrighton SA (2001) The role of hepatic and extrahepatic UDP-glucuronosyltransferases in human drug metabolism. Drug Metabolism Reviews 33:273–297

312. Malfatti MA, Felton JS (2001) N-glucuronidation of 2-amino-1-methyl-6-phenylimidazo [4, 5-b]pyridine (PhIP) and N-hydroxy-PhIP by specific human UDP -glucuronosyltransferases. Carcinogenesis 22:1087–1093

313. Yang CS, Chen L, Lee MJ, Landau JM (1996) Effects of tea on carcinogenesis in animal models and humans. In: American Institute for Cancer Research (ed) Dietary Phytochemicals in Cancer Prevention and Treatment, 401 edn. Plenum Press, New York and London, pp 51–62

314. Zhang Y, Hendrich S, Murphy PA (2003) Glucuronides are the main isoflavone metabolites in women. J Nutr 133:399–404

315. Levy GN, Weber WW (2001) Arylamine acetyltransferases. In: Ioannides C (ed) Enzyme systems that metabolise drugs and other xenobiotics. John Wiley and Sons, Chicester, pp 441–457

316. Grant DM (1993) Molecular genetics of the N-acetyltransferases. Pharmacogenetics 3:45–50

317. Martin FL, Venitt S, Carmichael PL, Crofton-Sleigh C, Stone EM, Cole KJ, Gusterson BA, Grover PL, Phillips DH (1997) DNA damage in breast epithelial cells: detection by the single-cell gel (comet) assay and induction by human mammary lipid extracts. Carcinogenesis 18:2299–2305

318. Jacobson JS, Begg MD, Wang LW, Wang Q, Agarwal M, Norkus E, Singh VN, Young TL, Yang D, Santella RM (2000) Effects of a 6-month vitamin intervention on DNA damage in heavy smokers. Cancer Epidemiol Biomarkers Prev 9:1303–1311

319. Perera FP, Mooney LA, Stampfer M, Phillips DH, Bell DA, Rundle A, Cho S, Tsai WY, Ma J, Blackwood A, Tang D (2002) Associations between carcinogen-DNA damage, glutathione S-transferase genotypes, and risk of lung cancer in the prospective Physicians' Health Cohort Study. Carcinogenesis 23:1641–1646

320. Marnett LJ (2000) Oxyradicals and DNA damage. Carcinogenesis 21:361

321. Mayne ST (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 133:933S–9940

322. Verhagen H, Poulson HE, Loft S, van Poppel G, Willems MI, van Bladeren PJ (1995) Reduction of oxidative DNA-damage in humans by Brussels sprouts. Carcinogenesis 16:969–970

323. Hu WW, Feng Z, Eveleigh J, Iyer G, Pan J, Amn S, Chung F, Tang MS (2002) The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma. Carcinogenesis 23:1781–1789

324. Nair J, Vaca CE, Velic I, Mutanen M, Valsta LM, Bartsch H (1997) High dietary w-6 polyunsaturated fatty acids drastically increase the formation of etheno-DNA base adducts in white blood cells of female subjects. Canc Epid Biom Prev 6:597–601

325. Zhang Y, Chen SY, Hsu T, Santella RM (2002) Immunohistochemical detection of malondialdehyde-DNA adducts in human oral mucosa cells. Carcinogenesis 23:207–211

326. Loeb KR, Loeb LA (2000) Significance of multiple mutations in cancer. Carcinogenesis 21:379–385

327. Bol SAM, Horlbeck J, Markovic J, de Boer JG, Turesky RJ, Constable A (2000) Mutational analysis of the liver, colon and kidney of Big Blue(R) rats treated with 2-amino-3-methylimidazo [4, 5-f]quinoline. Carcinogenesis 21:1

328. Kulling SE, Rosenberg B, Jacobs E, Metzler M (1999) The phytoestrogens coumoestrol and genistein induce structural chromosomal aberrations in cultured human peripheral blood lymphocytes. Arch Toxicol 73:50–54

329. Renner HW, Münzner R (1991) The possible role of probiotics as dietary antimutagens. Mutation Res 262:239–245

330. Goldman R, Shields PG (2003) Food Mutagens. J Nutr 133:965S–9973

331. Tucker JD, Carrano AV, Allen NA, Christensen ML, Knize MG, Strout CL, Felton JS (1989) In vivo cytogenetic effects of cooked food mutagens. Mutation Research 224:105–113

332. Fenech M, Rinaldi J (1995) A comparison of lymphocyte micronuclei and plasma micronutrients in vegetarians and non-vegetarians. Carcinogenesis 16:223–230

333. Fenech MF, et al. (2003) Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. Results of an international slide-scoring exercise by the HUMN project. Mutation Res 534:45–64

334. Moore LE, Smith AH, Hopenhayn-Rich C, Biggs ML, Kalman DA, Smith MT (1997) Micronuclei in exfoliated bladder cells among individuals chronically exposed to arsenic in drinking water. Canc Epid Biom Prev 6:31–36

335. Pastor S, Gutierrez S, Creus A, Xamena N, Piperakis S, Marcos R (2001) Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells. Mutagenesis 16:539–545

336. Piyathilake CJ, Macaluso M, Hine RJ, Vinter DW, Richards EW, Krumdieck CL (1995) Cigarette smoking, intracellular vitamin deficiency, and occurrence of micronuclei in epithelial cells of the buccal mucosa. Cancer Epidemiol Biomarkers Prev 4:751–758

337. Titenko-Holland N, Jacob RA, Shang N, Balaraman A, Smith MT (1998) Micronuclei in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. Mutation Res 417:101–114

338. Elahi A, Zheng Z, Park J, Eyring K, McCaffrey T, Lazarus P (2002) The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. Carcinogenesis 23:1229–1234

339. Hazra TK, Izumi T, Kow YW, Mitra S (2003) The discovery of a new family of mammalian enzymes for repair of oxidatively damaged DNA, and its physiological implications. Carcinogenesis 24:155–157

340. Martin FL, Cole KJ, Orme MH, Grover PL, Phillips DH, Venitt S (1999) The DNA repair inhibitors hydroxyurea and cytosine arabinoside enhance the sensitivity of the alkaline single-cell gel/electrophoresis 'comet' assay in metabolically-competent MCL-5 cells. Mutation Research 445:21–43

341. de Boer J, Hoeijmakers JHJ (2000) Nucleotide excision repair and human syndromes. Carcinogenesis 21:453

342. Berwick M, Vineis P (2000) Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. JNCI Cancer Spectrum 92:874

343. Aaltonen LA, Peltomäki P, Lee FS, Sistonen P, Pylkkänen L, Meo JP, Järvinen H, Powell SM, Stanley JJ, Petersen GM, Kinzler W, Vogel B, de la Chapelle A (1993) Clues to the pathogenesis of familial colorectal cancer. Science 260:812–815

344. Thibodeau SN, Bren G, Schaid D (1993) Microsatellite instability in cancer of the proximal colon. Science 260:816–819

345. Howlett J, Shortt C (2004) PASS-CLAIM-Report of the Second Plenary Meeting: review of a wider set of interim criteria for the scientific substantiation of health claims. Eur J Nutr 43(Suppl 2):174–183