Review

Berry phytochemicals, genomic stability and cancer: Evidence for chemoprotection at several stages in the carcinogenic process

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Consumption of a diet high in plant-based foods is associated with a decreased risk of epithelial cell cancers at several sites. Cytoprotectants in fruits and vegetables include vitamins, minerals and numerous micronutrients. While there is little evidence uniquely linking berry consumption with lower cancer risk, berries contain high levels of compounds believed to reduce malignant transformation, including the polyphenol flavonoids and anthocyanins. There is strong and convincing evidence that berry extracts and berry phytochemicals modulate biomarkers of DNA damage and indicators of malignant transformation *in vitro* and *in vivo*. Data from numerous cell culture and animal models indicate that berry components such as the anthocyanins are potent anticarcinogenic agents and are protective against genomic instability at several sites in the carcinogenic pathway. Anticarcinogenic mechanisms include modulation of carcinogen activation and detoxification, decreased DNA binding of the carcinogen, inhibition of oxidative DNA damage, alteration in cell signalling and malignant transformation and inhibition of cell invasiveness and metastasis. Exactly which berry constituents are cytoprotective remains uncertain and in the majority of *in vitro* and *in vivo* studies the concentration of extract or phytochemical employed is non-nutritional. Evidence for an anticarcinogenic effect in human studies is weak.

Keywords: Berries / Cancer / Cytoprotective / DNA stability / Phytochemical

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1 Introduction

It is widely accepted that consumption of a diet high in plant-based foods is associated with a decreased risk of cancer [1]. Potential cytoprotectants in fruits and vegetables include vitamins, minerals and numerous phytochemicals. While there is little unequivocal evidence linking berry consumption with lower cancer risk, berries contain high levels of compounds believed to reduce malignant transformation, including the B vitamins, such as folic acid, essential minerals such as calcium and selenium and complex dietary fibre. The genoprotective properties of these dietary components have been reviewed in detail elsewhere and will not be discussed here. However, berries such as cranberries, blueberries, strawberries and raspberries also contain significant amounts of non-nutritive phytochemicals including the polyphenols and are proposed to reduce cancer risk [2]. Intake of one group of flavonoids, the anthocyanins, has been estimated to exceed 200 mg/day [3]. Anthocyanins are particularly prevalent in berries, reaching concentrations in excess of 10 g/kg in some cultivars. Moreover, anthocyanins can modify cancer biomarkers *in vitro*, decreasing DNA damage in normal human cells [4] and also inhibiting cancer cell growth in culture [5]. It remains to be established whether berries themselves possess anticarcinogenic properties.

A detailed review of the micronutrients found in edible berries is provided elsewhere in this journal together with the current evidence relating berry intake to human cancer risk. This article will focus on the *in vitro* and *in vivo* mechanistic evidence for the genoprotective or anticarcinogenic potential of edible berries that are widely available and commonly consumed in the human diet.

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Abbreviations: ACF, aberrant crypt foci; **AZM,** azoxymethane; **B(*a*)P,** benzo[*a*]pyrene; **DMBA,** 7,12-dimethylbenz[*a*]anthracene; **MMPs,** metalloproteinases; **NFκB,** nuclear factor kappaB; **NMBA,** *N*-nitrosomethylbenzylamine; **O^{8}oxo-dG,** 8-oxo-deoxyguanosine; **TPA,** 12-*O*-tetradecanoylphorbol-13-acetate

2 Experimental evidence for a genoprotective effect of berries, berry juice and whole berry extracts

2.1 In vitro

Cancer is a highly complex multistage process beginning with initiation of a cancer cell via overt DNA damage and accumulation of mutations, promotion of cell proliferation and tumour expansion and finally progression to a malignant phenotype with subsequent invasion and metastasis to other sites in the body. Berry extracts and individual berry phytochemicals may act to alter genomic stability at a number of points along this sequence of malignant transformation, modulating initiation, promotion and progression of cancer. Several short-term *in vitro* and *in vivo* systems have been employed to determine the antimutagenic and anticarcinogenic potential of these fruits and their principal components.

Mutagenesis is an essential and early event in the carcinogenic process. Accumulation of mutations in genes regulating, for example, cell division, apoptosis and DNA repair result in the creation of a cell capable of uncontrolled growth and invasiveness. Phytochemicals in berries can block mutagenesis by chemical carcinogens and endogenous mutagens. Juice and homogenate prepared from several cultivars of blackberry, suppress 2-amino anthracene (2-AA)-induced mutagenesis in bacteria by 90% in the Ames test [6]. Similarly, juice from strawberry, blueberry and raspberry fruits strongly inhibit mutagenesis caused by the alkylating agent, methylmethanesulfonate (MMS) and the procarcinogen benzo[*a*]pyrene (B(*a*)P) [7]. In this study, strawberry juice suppressed MMS mutagenesis by 37% and B(*a*)P carcinogenesis by 76%. The other berry species were less effective antimutagens [7]. Efficacy of inhibition appears highly dependent on the berry species and the variety [6]. In a recent study, eight blackberry cultivars were tested for their ability to inhibit mutations induced by UV-C irradiation in the Ames test. All varieties were cultivated and harvested under identical conditions, yet two were found to inhibit mutagenesis strongly, two had no effect on the levels of mutations and the remaining four extracts had intermediate effects [8]. The Ames test is a good *in vitro* indicator of mutagenic potential *in vivo*, with a 90% correlation between a positive response in the assay and carcinogenicity in animal models [6].

Mutagenesis, uncontrolled cell proliferation and resistance to programmed cell death or apoptosis are characteristic features of transformed cells. Berry extracts can modify these processes *in vitro*. Blackberry juice and blackberry extract protect cultured human vascular endothelial cells against oxidative peroxynitrite-induced DNA strand breakage [9] and inhibit HT29 colon cancer cell proliferation [10]. Cranberry extract and cranberry presscake (the material remaining after the juice is squeezed from the berries), strongly inhibit breast, prostate, skin, brain and liver cancer cell growth by arresting proliferation in G1 of the cell cycle and by initiating apoptosis [11–13]. Inhibition of breast cancer cell growth and apoptosis are dose dependent at concentrations ranging from 5 to 50 mg/mL [13]. Bilberry extracts induce programmed cell death in human leukaemia cells [14]. Proliferation of the breast cancer line MCF-7 and the colon cancer cell line HT29 is reduced up to 74% following exposure to extracts of several fruits and berries including blueberries, blackcurrant, black chokeberries and raspberries at concentrations ranging from 0.025 to 0.5% [15]. However, few studies have investigated the ability of berry extracts to differentially effect DNA damage or inhibition of growth in cancerous versus normal cells derived from the same tissue, although bilberry and chokeberry extract (10–75 μg/mL) inhibits HT29 colon cancer cell growth more strongly than cells derived from normal human colonic epithelium [16].

Fractions of freeze-dried strawberries and black raspberries, together with ellagic acid were analysed for their ability to prevent malignant transformation following exposure to B(*a*)P in the Syrian Hamster embryo (SHE) cell transformation model [17]. This *in vitro* transformation assay mimics the stages that occur during *in vivo* carcinogenesis and is used extensively to determine both the carcinogenic and chemopreventive potential of chemicals. Ellagic acid, one of the most abundant phytochemicals found in these berries, inhibits malignant transformation *in vitro* and *in vivo*. Both ellagic acid (0.3–4.5 μg/mL) and methanol extracts from both berry types (2–100 μg/mL), dose-dependently decreased malignant transformation, achieving greater than 80% inhibition at the highest concentration. Methanol-derived berry extracts do not contain detectable quantities of ellagic acid suggesting that other berry phytochemicals must be genoprotective *in vitro*. Moreover, ellagic acid and the berry extracts appear to act at different points in the carcinogenic pathway [17]. This is elegantly demonstrated in a recent study where blackberry extract inhibited proliferation of A549 human lung cancer cells and decreased neoplastic transformation in normal mouse epidermal JB6 cells exposed to the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Inhibition was associated with down-regulation of several genes involved in tumour initiation and promotion [18]. A more detailed description of how berry components can influence intracellular signalling and cancer progression will be described later in this review.

2.2 *In vivo*

Berry extracts protect against carcinogenesis in animal models. *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal tumour incidence, multiplicity and grade of disease were significantly reduced in rats fed 5 and 15% freeze-dried black raspberries and strawberries [19, 20].

Chemoprevention was effective at both initiation and promotion stages of cancer development. However, no protective effect was observed when extract from blueberries, one of the most widely consumed berries in the American diet, was fed to the animals prior to and during exposure to the same chemical carcinogen. DNA damage (alkylated DNA adducts) measured in the tumours, tumour incidence, multiplicity and size were all unaffected by pre- and cotreatment with blueberry extract, suggesting that blueberries, despite having a higher antioxidant activity than strawberries and several other berry species, lack one or more cytoprotective phytochemicals present in other berry types that inhibit chemically-induced cancer in this model [21]. One way in which blueberries differ from strawberries and black raspberries is that they do not contain equivalent quantities of ellagic acid, which is able to reduce alkylation damage in esophageal DNA from NMBA-treated rats [22].

Lyophilised black raspberries can also inhibit carcinogenesis in a rodent model of oral cavity cancer, in a rat model of chemically induced colon cancer and in a strain of mice genetically susceptible to colon adenoma formation. Black raspberry extract is chemopreventive against tumorigenesis in the cheek pouches of male Syrian Golden Hamsters exposed to 7,12-dimethylbenz[*a*]anthracene (DMBA). Treatment with black raspberry extract (5% of diet) 2 wk prior to and 10 wk after treatment with DMBA reduced DNA adduct formation and tumour number [23]. Freeze-dried black raspberry extract inhibited azoxymethane (AZM)-induced aberrant crypt foci (ACF), a tentative biomarker of dysplasia and malignant transformation, by 21–36%, adenocarcinoma formation by 80% at the top concentration and colon tumour multiplicity by 42–71% when fed to rats at dietary concentrations of 2.5–10% post-initiation [24] indicating that berry extracts are effective at inhibiting both dysplasia and malignant progression. Oxidative DNA damage, such as that seen when 8-oxo-deoxyguanosine (O^8oxo-dG) is formed in DNA, is highly mutagenic and has been implicated in cancer development [25]. O^8oxo-dG levels were decreased more than 80% in the urine from animals treated with the highest dose of extract [24]. Similarly, anthocyanin extracts from chokeberry, bilberry and grape (fed at 35 mg/day) inhibited ACF in rats treated with AZM [26]. In the same model, extracts from bilberry and chokeberry dose-dependently reduced ACF formation, colonic epithelial cell proliferation rate and faecal bile acid concentration [27]. Bilberry anthocyanins, commercially available as "Mirtoselect", dose-dependently reduced adenoma formation in *Apc*^{min} mice fed the anthocyanin-rich mixture at 0.03, 0.1 or 0.3% in the diet for 12 wk. Moreover, anthocyanins were detectable in the intestinal mucosa of these animals [28].

2.3 Human intervention studies

Many phytochemicals with antioxidant properties are bioavailable from berries. Plasma total antioxidant capacity is elevated for up to 4 h after consumption of strawberry fruit (240 g) and blueberry extract (100 g) in elderly women and middle-aged men [29, 30]. The potent cytoprotective flavonol quercetin is also bioavailable from berries. Compared with control subjects, plasma quercetin was elevated 32–51% in healthy Finnish men consuming 100 g of blackcurrants, lingonberries and bilberries daily for 8 wk [31]. However, the relative bioavailability of anthocyanins compared with other dietary berry polyphenols appears to be poor. Anthocyanins have been detected at very low concentrations in blood and urine from subjects fed elderberry juice but only when consumed at pharmacological doses (500–1500 mg/day) [32–34]. The bioavailability of berry components, including antioxidant flavonols and anthocyanins will be covered elsewhere in this edition.

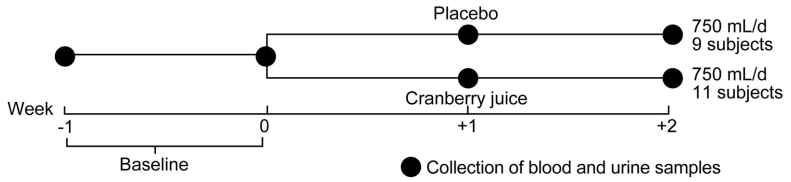
While there is good evidence that berries or berry phytochemicals can influence genomic stability *in vitro* and in rodent models, their ability to modify markers of DNA damage and carcinogenesis in humans is highly contentious.

Strawberry consumption (300 g) reduces endogenous formation of the carcinogen NDMA by 70% in healthy young volunteers fed nitrate (400 mg/day) together with an amine-rich diet [35]. Consumption of Aronia, blueberry and boysenberry juice for 5 wk significantly decreased oxidative DNA damage in blood cells isolated from human volunteers [36]. Consumption of wolfberries, a medicinal berry consumed in China that contains significant levels of ellagic acid in addition to being high in carotenoids and vitamin C, reduced DNA strand breakage in buccal cell scrapings from a small group of subjects compared with controls [37]. However, as with the rodent experiments, it is impossible in this type of study using whole food, to ascertain which are the most bioeffective phytochemicals. In direct contrast, supplementation of a group of twenty healthy subjects with blackcurrant juice (containing 600 mg/L anthocyanin and 210 mg/L vitamin C) for 3 wk caused a small but significant increase in oxidative DNA purine base damage [38]. No change in genomic stability was seen in subjects consuming a drink containing equivalent concentrations of anthocyanin but without vitamin C, suggesting that the detrimental effect seen in this study may have been due to a pro-oxidant effect of ascorbic acid [38]. However, anthocyanins were not protective in this study. Similarly, no significant decrease in oxidative DNA damage was found in female subjects consuming a polyphenol-rich blueberry and apple juice for 4 wk, despite a significant increase in plasma antioxidant capacity [39]. Cranberry juice, consumed at nutritionally relevant levels, has little effect on DNA stability in healthy volunteers [40]. Healthy female subjects (aged between 18 and 40 years) were randomly allocated to receive either 750 mL/day of cranberry juice ($n = 11$) or a placebo drink ($n = 9$) for 2 wk. The cranberry juice employed in this study contained high levels of vitamin C, total phenols, catechins and anthocya-

Anthocyanin human intervention trial: cranberry juice

Phytochemical content and antioxidant potential of cranberry juice and placebo

	Cranberry Juice	Placebo
total phenols (mg/IGAE)	1136 ± 3.50	8.9 ± 0.14
total anthocyanins (mg/L)	2.8 ± 0.19	not detectable
catechins (mg/L)	29.1 ± 0.40	not detectable
∆FRAP (µmM FeII)	14.0 ± 0.01	0.06 ± 0.01
ESR (radicals reduced/10^{18}/ml)	9.2 ± 0.07	0.02 ± 0.01



Anthocyanins in cranberry juice:

A. Cyanidin-3-galactoside (26.1%)
B. Cyanidin-3-glucoside (1.4%)
C. Cyanidin-3-arabinoside (27.1)
D. Peonidin-3-galactoside (29.2%)
E. Peonidin-3-glucoside (4.1%)
F. Peonidin-3-arabinoside (17.5%)

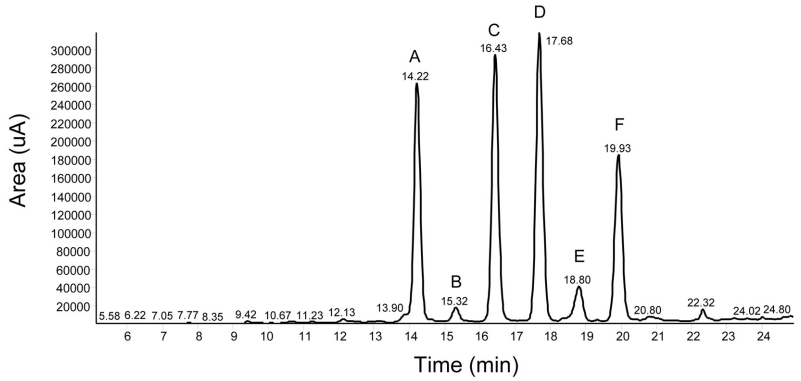


Figure 1. Panel showing the phytochemical content and antioxidant potential of cranberry juice and placebo drink, the volunteer sampling protocol and the anthocyanin profile of cranberry juice identified by HPLC-MS-MS with the contribution (as percentage) of individual anthocyanins [40].

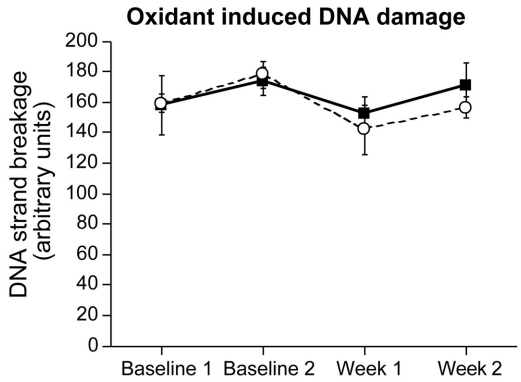
nins and displayed significant antioxidant activity, measured as ferric reducing antioxidant potential (FRAP) and by electron spin resonance (ESR) in the laboratory (Fig. 1). Endogenous or background DNA damage was measured in urine as O^8oxo-dG and as DNA strand breaks and oxidised pyrimidines in lymphocytes isolated from the volunteers. In addition, the ability of cranberry juice to protect against induced oxidative DNA damage was measured in lymphocytes exposed to hydrogen peroxide. Despite a calculated daily intake of 850 mg of total phenols, 2.2 mg of anthocyanins and 22 mg of catechins, anthocyanins and catechins were undetectable in the blood or urine of volunteers consuming cranberry juice daily for 2 wk. Unsurprisingly, there was no change in the antioxidant capacity of the plasma and consumption of cranberry juice had no benefit on DNA stability. Cellular DNA damage remained unchanged in both groups and there was no difference in the excretion rate of O^8oxo-dG. Similarly, DNA strand breakage induced in response to oxidative stress (H_2O_2 treatment) was similar in lymphocytes isolated from both treatment groups indicating that the antioxidant capacity of the juice conferred no protection against oxidative attack *in vivo* (Fig. 2). Lack of efficacy in this study undoubtedly reflects low bioavailability of these compounds at nutritionally relevant levels.

Despite the lack of evidence for a clear protective effect of berry extract or juice on genomic stability, clinical studies investigating the efficacy of berries and berry components to inhibit carcinogenesis in people with existing cancers or patients at risk of cancer recurrence are underway. Interim findings from a 6-month pilot study of ten patients with Barrett's esophagus, a condition associated with a 30–40-fold increased risk for esophageal adenocarcinoma, show that gram quantities of lyophilised black raspberries did not significantly reduce the urinary excretion of 8-oxodG in the group as a whole [41]. Changes in this bio-

Anthocyanin human intervention trial: cranberry juice

The effect of cranberry juice on blood biomarkers in human volunteers

	Cranberry Juice	Placebo
total anthocyanins (plasma/urine)	not detectable	not detectable
∆FRAP (µmM FeII)	24.8 ± 1.5	25.4 ± 1.2
ESR (radicals reduced/10^{18}/ml)	6.73 ± 0.23	7.10 ± 0.17
8-oxodG (ng/mg creatinine)	9.47 ± 0.65	10.18 ± 1.53
Oxidised pyrimidines (AU)	54.2 ± 5.8	49.0 ± 5.9
DNA strand breaks (AU)	37.4 ± 4.8	33.8 ± 4.0



Supplementation had no effect on antioxidant capacity or genomic stability (oxidised bases or endogenous and induced DNA strand breakage).

Figure 2. Panel showing the effect of cranberry juice on plasma anthocyanin levels, antioxidant potential (measured as FRAP and ESR) and background and induced DNA damage in human volunteers [40].

marker in response to supplementation were small and highly variable between subjects and it is plainly too soon to draw conclusions from this study.

3 Evidence for a genoprotective ability of specific berry constituents

Without a doubt, berries and berry extracts do positively affect biomarkers of genomic stability and indicators of carcinogenesis *in vitro* and *in vivo*. However, one major disadvantage of studying whole food or food extracts is that it is impossible to establish with any degree of certainty which dietary components (*e.g.* folic acid, flavonols, vitamin C, anthocyanins, stilbenes, *etc.*) are the effective cytoprotective agents. Nevertheless, there is considerable evidence that individual berry components have chemopreventive or anticancer properties *in vitro* and *in vivo*, with ability to function at several stages along the cancer progression pathway.

Numerous experimental studies indicate that dietary flavonoids, such as quercetin, myricetin and kaempferol protect against DNA instability. A comprehensive review of the evidence linking flavonoid exposure with genomic stability *in vitro*, *in vivo* and in human intervention trials has been provided previously [2]. Fewer studies have investigated how anthocyanins specifically modulate DNA stability.

Anthocyanins, including cyanidin and several glycosides are antimutagens in both the Ames and sister chromatid exchange (SCE) genotoxicity tests [42] and effectively inhibit oxidative DNA damage in rat smooth muscle cells [43]. Ellagic acid (found in many berry species including cranberry and black raspberry) inhibits carcinogen activation and binding to DNA and mutagenesis in the Ames test. It also protects supercoiled plasmid DNA against oxidative damage and modulates cell cycle control and programmed cell death in cancer cells [44]. Anthocyanidins, including delphinidin, malvidin, peonidin and petunidin inhibit proliferation of cancer cells derived from various tissues including colon, breast, blood and lung at high micromolar concentrations (reviewed in 45). Similarly, cyanidin and a mixture of several of its glycosides dose-dependently inhibit HCT116 and HT29 colon cancer cell growth, with an IC50 of 63 and 780μM, respectively [9]. In general, potency reflects the chemical structure of the polyphenol with the anthocyanin glycoside less effective than the parent aglycone [45]. Cyanidin and its glucoside cyanidin-3-glycoside, reduce oxidant-induced DNA strand breakage in normal human lymphocytes ex vivo and are as potent chemoprotectants as the flavonols quercetin and myricetin. Moreover, the anthocyanins were more effective than several other antioxidants including kaempferol, silymarin, vitamin C, vitamin E and its water-soluble analogue Trolox (Fig. 3). Cyanidin 3-rutinoside and cyanidin 3-glucoside

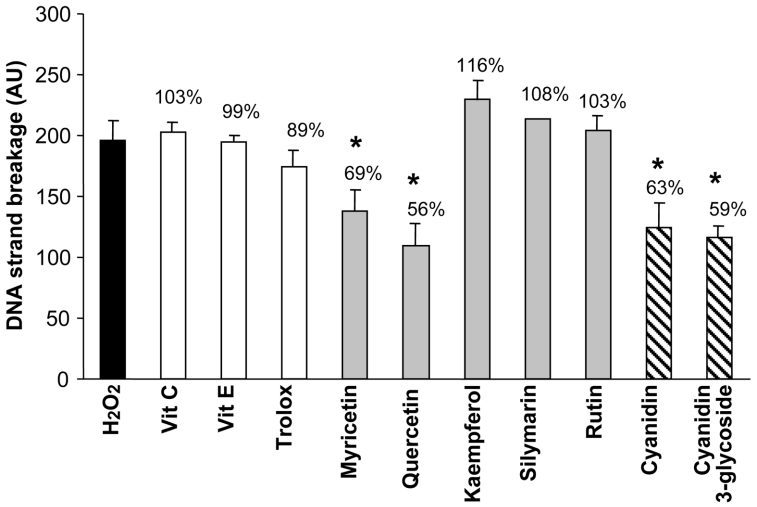


Figure 3. The effect of flavonols, anthocyanins and antioxidant vitamins on induced oxidative DNA damage in human lymphocytes. Lymphocytes were pretreated for 4 h with phytochemical (50 μM), washed with PBS and exposed to the model oxidant hydrogen peroxide (H_2O_2, 200 μM) for 5 min on ice. DNA strand breaks were measured by single cell gel electrophoresis. Phytochemical-mediated cytoprotection is shown as percentage DNA strand breakage compared with H_2O_2-treated cells alone. Results are mean ± SEM for $n > 4$, $^*p < 0.05$.

suppress cancer cell metastasis by inhibiting the motility, adhesiveness and invasiveness of the metastatic human lung cancer cell line A549 [46]. Cultured lung cells pretreated with anthocyanin metabolites (0–100 μM) for 24 h have a reduced capacity to migrate through a matrigel layer. Both cyanidin 3-glucoside and cyanidin 2-rutinoside inhibited cell invasion by approximately 40% at the highest concentration tested [46]. Cyanidin 3-glucoside was also a potent inhibitor of cell adhesion [46].

Anthocyanins are bioactive in different *in vivo* model systems. Hepatic O^8oxo-dG was significantly decreased in vitamin E-deficient rats fed a complex anthocyanin extract containing glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin (1 g/kg diet) for 2 wk [47]. However, in a study using only cyanidin-3-glycoside at a more nutritionally relevant concentration, no effect was seen on endogenous DNA strand breakage and oxidative base damage in isolated lymphocytes and O^8oxo-dG levels in liver and colon from vitamin E-deficient rats. Moreover, lymphocytes isolated from vitamin E-deficient rats fed cyanidin-3-glycoside were as susceptible to induced oxidative stress as rats fed control diet [4]. Several studies report that anthocyanins inhibit tumour formation *in vivo*. Mammary tumour formation, multiplicity and tumour volume is decreased in DMBA-treated female rats fed 15 different anthocyanins from grape juice [48], while anthocyanins, given either as a mixture (estimated as 6.4 mg/day), a single pure cyanidin solution (estimated as 1.6 mg/day) or as freeze-dried tart cherries (600 mg/day) inhibit dysplasia in cancer-susceptible mice (*Apc*^{min}). Mice consuming anthocyanins, cyanidin or tart cherries had significantly fewer and smaller caecal adenomas than mice consuming the control diet. The authors calculate that the comparative human doses required to modulate tumour formation would be approximately 2.4 or 0.6 g of anthocyanins or cyanidin, respectively, which is obviously supraphysiological and unachievable by normal dietary intervention [9]. Disturbingly, tart cherry consumption was associated with a significantly increased tumour volume in the small intestine. Conversely, cyanidin 3-glucoside decreased the number of malignant and benign skin tumours in mice treated with DMBA as an initiator and TPA as promotor [49].

These data suggest that certain polyphenols can modulate oxidative DNA damage *in vivo*, but others cannot. Moreover, in those studies where complex anthocyanin extracts or single anthocyanin aglycones or glycosides have been shown to be affective against oxidative damage, the phytochemicals have generally been fed at supra-nutritional or pharmacological doses. In those few studies where the bioactivity of anthocyanins has been investigated at nutritionally-relevant concentrations, evidence of a protective affect is weak.

4 Berry consumption and potential anticancer mechanisms

High fruit and vegetable consumption is associated with a decreased risk of certain epithelial cancers. When added to bacteria or cultured mammalian cells, berry extracts or specific phytochemicals found in high quantities in berries, inhibit mutagenesis, DNA damage, cell proliferation and malignant transformation and metastasis. Similarly, feeding berry extract inhibits carcinogenesis at various sites, including the colon, esophagus, lung and breast in animals. While it is apparent that berry phytochemicals exert their anticarcinogenic effects at several points along the cancer progression pathway, exactly how these phytoprotectants act is unclear.

4.1 Antioxidant effects

Accumulation of oxidative DNA damage over the human lifespan may be a significant factor in cancer development [25]. Reactive oxygen species generated endogenously through aerobic metabolism are potent genotoxins, causing

mutations, DNA strand breakage and oxidative DNA base damage *in vitro* and *in vivo*. The anticarcinogenic value of a diet high in fruits and vegetables may be due to the relatively high abundance of antioxidants in these foods, which act to inhibit oxidative DNA damage through a variety of mechanisms including free radical scavenging and metal chelation (reviewed in [2]). Berry extracts and individual berry components, including the anthocyanins, are strong antioxidants *in vitro* and exhibit a broad spectrum of antioxidant activity in chemical and cellular systems [50]. Flavonoids are strong metal chelators and are able to suppress peroxyl and hydroxyl radical-induced supercoiled DNA strand scission and generation of reactive oxygen species from activated human granulocytes [2, 42]. Quercetin, the anthocyanin cyanidin and its glycoside cyanidin 3-glycoside are powerful inhibitors of oxidant-induced DNA damage in normal human colon mucosal cells *in vitro* [4], while 3-glucopyranosides of delphinidin, cyanidin, petunidin, peonidin and malvidin ameliorate the effects of vitamin E depletion on oxidative DNA damage in rat liver [47]. As described elsewhere in this review, black raspberry extract significantly inhibits O^8oxo-dG formation and tumorigenesis in animals treated with the carcinogen AZM [24]. The ability of polyphenols, including anthocyanins, to modulate free radical generated DNA damage and malignant transformation is reviewed elsewhere [2, 50].

4.2 Modulation of phase 1 and 2 drug metabolising enzyme activities and inhibition of DNA damage

Berry extracts inhibit mutagenesis by metabolically activated carcinogens in the Ames test, but are not as effective against carcinogens acting directly on DNA [6]. Several freeze-dried berry extracts inhibit NMBA-induced esophageal cancer *in vivo*, with strawberry, black raspberry and blackberry preparations decreasing esophageal papilloma numbers by 24–56% relative to control animals. Inhibition of tumorigenesis was associated with a concomitant decrease in the level of the DNA adduct, O-6-methylguanine, suggesting that the berry extract was acting to inhibit formation of the mutagenic agent from the procarcinogen [51]. Moreover, tumour burden was reduced up to 64% when berry extract was fed after cancer initiation by NMBA indicating that berry extracts function as potent chemoprotectants at a number of sites along the cancer cascade. Berry phytochemicals may exert their anticarcinogenic effect by modulating the enzyme systems that metabolise carcinogens or procarcinogens to genotoxins. Activation of the procarcinogen may be inhibited or it may be converted to a less reactive compound before it is able to bind to DNA and initiate mutagenesis and carcinogenesis. The cytochrome P450 superfamily of enzymes metabolise a large number of procarcinogens to reactive intermediates that covalently bind to DNA and induce malignant transformation. P450 activity can be induced or inhibited by flavonoids. Ellagic acid inhibits mutagenesis and carcinogenesis by acting on both P450 xenobiotic metabolism and several phase 2 detoxifying enzymes. Rats fed ellagic acid have significantly fewer carcinogen-induced hepatic and esophageal tumours and this reduction in tumour burden is associated with changes in the activity of several enzymes involved in carcinogen activation and detoxification. Liver P450 reductase activity was decreased significantly (25%) in rats fed ellagic acid for 23 days, while glutathione transferase (GST), NADPH quinone reductase and UDP glucuronyltransferase (UDPGT) activities increased. *In vitro* studies indicate that specific isozymes of rat liver P450s are inhibited by ellagic acid, with those isozymes inhibited the most, generally the most important in the bioactivation of procarcinogens in rats and humans [52]. In addition to altering activation or binding of the chemical carcinogen with DNA, black raspberry extract also alters expression of genes associated with inflammation and carcinogenesis. COX-2 gene expression and subsequently prostaglandin production and nitric oxide synthase activity are inhibited in premalignant rat esophageal cells following feeding with berry extract [53].

4.3 Inhibition of cell proliferation, malignant transformation and invasiveness

Some anthocyanins suppress cancer cell growth *in vitro* by modifying cell signalling pathways. Benzo[*a*]pyrene diolepoxide (BPDE) treatment of mouse epidermal JB6 CI 41 cells induces activated protein 1 (AP-1) and nuclear factor kappaB (NFκB). Changes in expression of these proteins, which function normally to regulate cell proliferation and cell cycle control, are believed to be involved in human cancer development. Pretreatment of JB6 CI 41 mouse cells with methanol-extracted black raspberry fractions (1–100 μg/mL) dose-dependently inhibits carcinogen-induced AP-1 or NFκB protein expression in a luciferase reporter assay without affecting carcinogen/DNA binding [54]. Inhibition of the AP-1 pathway or NFκB activation was mediated by a decrease in the phosphorylation of members of the MAPK protein family and inhibition of IκBα phosphorylation and degradation, respectively [54]. Berry extracts were inhibitory only when given prior to or during carcinogen exposure. Similarly, methanol-extracted blackberry extract dramatically inhibited BPDE-induced activation of AP-1 and NFκB and subsequent expression of vascular endothelial growth factor and COX-2, proteins believed to have a key role in tumour promotion and progression. Further studies indicated that the blackberry extract was altering the cell signalling pathways responsible for activating AP-1 and NFκB by inhibiting MAPKs activity and IκBα phosphorylation [55]. Blackberry and strawberry extracts and cyanidin 3-glucoside produced similar effects in the AP-1 and MAPK pathways in JB6 mouse cell

treated with UVB or TPA [18, 49, 56]. Whether down-regulation of this gene cascade alters tumorigenesis *in vivo* remains to be established.

The process of metastasis, the spread of malignant cancer cells from their primary location to secondary sites in the body, is dependent on specific changes within the tumour. Cancer cells must attain increased motility, surface adhesion properties and increased extracellular protease activity to facilitate cell movement and invasiveness. Degradation and rupture of the extracellular matrix by proteases such as metalloproteinases (MMPs), serine proteinases and cathepsins, allow cancer cell migration and metastasis. Two glucosides of the anthocyanidin cyanidin (cyanidin 3-rutinoside and cyanidin 3-glucoside) reduce the invasive ability of the highly metastatic human lung cancer cell line A549 by differentially altering transcription of several proteases involved in carcinogenesis [46]. Expression of matrix metalloproteinase-2 (MMP-2) and urokinase-plasminogen activator (u-PA) was inhibited, while conversely, expression of tissue inhibitor of matrix metalloprotein-2 (TIMP-2) and plasminogen activator inhibitor (PAI), endogenous inhibitors of MMP-2 and u-PA, respectively, was increased [46]. Extracts from blueberry down-regulate MMP activity and increase TIMP-1 and TIMP-2 activity in human prostate cancer cells *in vitro* [34].

4.4 Inhibition of angiogenesis

Angiogenesis is the process by which new blood vessels are formed from pre-existing vessels. Angiogenesis, a normal physiological process in growth and development and in wound healing, is also a fundamental step in the transition of tumours from a benign to a malignant or invasive state. Tumours induce angiogenesis by secreting growth factors such as VEGF to induce capillary growth into the tumour allowing tumour expansion and spread. Cancer cells can dissociate from the main tumour and be carried in the circulation to a site distant from the primary tumour where they implant and begin formation of a secondary tumour. Several berry derivatives have potent anti-angiogenesis properties *in vitro*. In a series of elegant experiments, berry extracts (wild blueberry, bilberry, cranberry, elderberry, raspberry seed and strawberry) were tested for their ability to inhibit angiogenesis via altered VEGR expression and invasiveness. Human HaCaT keratinocytes, which are normally quiescent, retain the ability to initiate angiogenesis in response to certain stimuli. Each of the berry extracts inhibited hydrogen peroxide and TNF-α induced VEGF expression in these cells. Inducible VEGF expression was related to the flavonoid component of the berry extracts, independently of their antioxidant potential [57]. Moreover, berry extracts were able to inhibit angiogenesis in human dermal microvascular endothelial cells. Similarly, crude whole black raspberry extract (at a concentration of 0.1% w/v) was antiangiogenic in a human placental tissue-based fibrin clot angiogenesis assay [58]. Fractionation of crude extract revealed a highly potent anti-angiogenic component that accounted for only 1% of the fresh weight of the whole berries. This highly active fraction completely inhibited angiogenic initiation and vessel sprouting. Several active compounds, including gallic acid, were subsequently identified, although none was as effective individually as the whole fraction, indicating that these compounds act synergistically [58].

Berry extracts can also inhibit angiogenesis in animals. Hemangiomas are abnormally dense collections of dilated capillaries that can occur in the skin or internal organs and have been used to model angiogenesis *in vivo*. Mouse endothelioma cells (EOMA) injected into compatible host animals proliferate to form blood vessel conduits that fuse with the systemic circulation, drawing blood into the hemangioma. EOMA cells pretreated in culture either with wild blueberry or a proprietary berry mix (OptiBerry) were unable to form hemangioma tumours as effectively as placebo-treated cells after injection into host mice. In the wild blueberry treatment group, less that 50% of the animals tested positive for the presence of a hemangioma and in those animals that tested positively, tumour mass was below 50% of that observed in the untreated group. Moreover, macrophage infiltration was significantly lower in these animals [59]. Macrophages, recruited to sites of inflammation or infection, produce growth factors and cytokines that regulate angiogenesis. Berry extracts may work to inhibit angiogenesis by inhibiting inducible expression of NFκB and basal expression of monocyte chemotactic protein 1 (MCP-1) [59].

5 Concluding remarks

Clearly, berry extracts modulate biomarkers of DNA damage and indicators of malignant transformation *in vitro* and *in vivo*. Data from numerous cell culture and animal models suggest that berry extracts or berry-derived phytochemicals such as the anthocyanins are potential cancer chemotherapeutic agents and can be protective against genomic instability at several sites in the carcinogenic pathway. However, exactly which berry constituents provoke this cytoprotective response remains uncertain and in the vast majority of studies the concentration of extract or phytochemical employed is substantially greater than could ever be achieved nutritionally. Moreover, evidence for an anticarcinogenic ability of berries in controlled human intervention studies is weak. While the lack of effect of berry extracts on DNA stability in human studies may reflect the relatively short duration of these interventions, alternatively, these compounds at concentrations appropriate to the human diet may not be effective genoprotectants *in vivo*.

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6 References

[1] World Cancer Research Fund and American Institute for Cancer Research, *In Food, Nutrition and Prevention of Cancer; a global perspective*, World Cancer Research Fund and American Institute for Cancer Research 1997.

[2] Duthie, G. G., Duthie, S. J., Kyle, J. A. M., Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants, *Nutr. Res. Rev.* 2000, *13*, 79 – 106.

[3] Clifford, M. N., Anthocyanins: Nature, occurrence and dietary burden, *J. Sci. Food. Agric*. 2000, *80*, 1063 – 1072.

[4] Duthie, S. J., Gardner, P. T., Morrice, P. C., Wood, S. G. *et al.*, DNA stability and lipid peroxidation in vitamin E deficient rats in vivo and colon cells in vitro: Modulation by the dietary anthocyanin, cyanidin 3-glycoside, *Eur. J. Nutr.* 2005, *44*, 195 – 203.

[5] Kamei, H., Koide, T., Kojimam, T., Hasegawa, M. *et al*., Influence of OH group and sugar bonded to flavonoids on flavonoid-mediated suppression of tumour growth in vitro, *Cancer Biother. Radiopharm.* 1996, *11*, 193 – 196.

[6] Tate, P., Kuzmar, A., Smith, S. W., Wedge, D. E. *et al*., Blackberry extracts inhibit UV-induced mutagenesis in *Salmonella typhimurium* TA100, Comparative effects of eight varieties of blackberry on mutagenesis, *Nutr. Res*. 2003, *23*, 971 – 979.

[7] Hope Smith, S., Tate, P. L., Huang, G., Mage, J. B. *et al*., Antimutagenic activity of berry extracts, *J. Med. Food* 2004, *7*, 450 – 455.

[8] Tate, P., Stanner, A., Shields, K., Smith, S., Larcom, L., Blackberry extracts inhibit UV-induced mutagenesis in *Salmonella typhimurium* TA100, *Nutr. Res.* 2006, *26*, 100 – 104.

[9] Serraino, V., Dugom, L., Dugom, P., Mondellom, L. *et al*., Protective effects of cyanidin 3-O-glucoside from blackberry extract against peroxynitrite-induced endothelial dysfunction and vascular failure, *Life Sci*. 2003, *73*, 1097 – 1114.

[10] Kang, S. Y., Seeram, N. P., Nair, M. G., Bourquin, L. D., Tart cherry anthocyanins inhibit tumour development in Apcmin mice and reduce proliferation of human colon cancer cells, *Cancer Lett*. 2003, *194*, 13 – 19.

[11] Sun, J., Chu, Y. F., Wu, X. Z., Liu, R. H., Antioxidant and antiproliferative activities of common fruits, *J. Agric. Food Chem*. 2002, *50*, 7449 – 7454.

[12] Ferguson, P. J., Kurowska, E., Freeman, D. J., Chambers, A. F. *et al*., A flavonoid fraction from cranberry extract inhibity proliferation of human tumor cell lines, *J. Nutr.* 2004, *134*, 1529 – 1535.

[13] Sun, J., Liu, R. H., Cranberry phytochemical extracts induce cell cycle arrest and apoptosis in human MCF-7 breast cancer cells, *Cancer Lett.* 2006, *241*, 124 – 134.

[14] Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K. *et al*., Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins, *J. Agric. Food Chem*. 2003, *51*, 68 – 75.

[15] Olsson, M. E., Gustavsson, K. E., Andersson, S., Nilsson, A. *et al*., Inhibition of cenacer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels, *J. Agric. Food Chem*. 2004, *52*, 7264 – 7271.

[16] Zhao, C., Giusti, M. M., Malik, M., Moyer, M. P. *et al*., Effects of commercial anthocyanin-rich extracts on colonic and nontumorigenic colonic cell growth, *J. Agric. Food Chem*. 2004, *52*, 6122 – 6128.

[17] Xue, H. W., Azizm, R. M., Sunm, N. J., Cassadym, J. M. *et al*., Inhibition of cellular transformation by berry extracts, *Carcinogenesis* 2001, *22*, 351 – 356.

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[18] Feng, R., Bowman, L. L., Lu, Y. J., Leonard, S. S. *et al*., Blackberry extracts inhibit activating protein 1 activation and cell transformation by perturbing the mitogenic signalling pathway, *Nutr. Cancer* 2004, *50*, 80 – 89.

[19] Carlton, P. S., Kresty, L. A., Siglin, J. C., Morse, M. A. *et al*., Inhibition of N-nitrosomethylbenzylamine-induced tumorigenesis in the rat esophagus by dietary freeze-dried strawberries, *Carcinogenesis* 2001, *22*, 441 – 446.

[20] Kresty, L. A., Morse, M. A., Adams, C. A., Stoner, G. D., Inhibitory effect of lyophilised black raspberries on esophageal tumorigenesis and O6-methylguanine levels in the F344 rat, *Proc. Am. Assoc. Cancer Res*. 1998, *39*, 120.

[21] Aziz, R. M., Nines, R., Rodrigo, K., Harris, K. *et al*., The effect of freeze-dried blueberries on N-nitrosomethylbenzylamine tumorigenesis in the rat esophagus, *Pharm. Biol.* 2002, *40*, 43 – 49.

[22] Mandal, S., Stoner, G. D., Inhibition of *N*-nitrosobenzylmethylamine-induced esophageal tumorigenesis in rats by ellagic acid, *Carcinogenesis* 1990, *11*, 55 – 61.

[23] Casto, B. C., Kresty, L. A., Kraly, C. L., Pearl, D. K. *et al*., Chemoprevention of oral cancer by raspberries, *Anticancer Res*. 2002, *22*, 4005 – 4015.

[24] Harris, G. K., Gupta, A., Nines, R. G., Kresty, L. A. *et al*., Effects of lyophilised black raspberries on azoxymethane-induced colon cancer and 8-hydrozy-2′-deoxyguanosine levels in the Fischer 344 rat, *Nutr. Cancer* 2001, *40*, 125 – 133.

[25] Halliwell, B., Effect of diet on cancer development: is oxidative DNA damage a biomarker? *Free Rad. Biol. Med*. 2002, *32*, 968 – 974.

[26] Magnusson, B. A., Lala, G., Kwon, Y. J., Anthocyanin-rich extracts inhibit growth of human colon cancer cells and azoxymethane-induced colon aberrant crypts in rats: Implications for colon cancer Chemoprevention, *Cancer Epidemiol. Biomark. Prev.* 2003, *12*, 1323s – 1324s.

[27] Lala, G., Malik, M., Zhao, C. W., He, J. *et al*., Anthocyanin rich extracts inhibit multiple biomarkers of colon cancer in rats, *Nutr. Cancer* 2006, *54*, 84 – 93.

[28] Cooke, D., Schwartz, M., Boocock, D., Winterhalter, P. *et al*., Effect of cyanidin 3-glucoside and an anthocyanin mixture from bilberry on adenoma development in the Apc min mouse model of intestinal carcinogenesis – relationship with tissue anthocyanin levels, *Int. J. Cancer* 2006, *119*, 2213 – 2220.

[29] Cao, G., Russell, R. M., Lischner, N., Prior, R. L., Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women, *J. Nutr.* 1998, *128*, 2383 – 2390.

[30] Kay, C. D., Holub, B. J., The effect of wild blueberry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects, *Br. J. Nutr.* 2002, *88*, 389 – 397.

[31] Erlund, I., Marniemi, J., Hakala, P., Alfthan, G. *et al*., Consumption of black currants, lingonberries and bilberries increases serum quercetin concentrations, *Eur. J. Clin. Nutr.* 2003, *57*, 37 – 42.

[32] Murkovic, M., Mulleder, U., Adam, U., Pfannhauser, W., Detection of anthocyanins from elderberry juice in human urine, *J. Sci. Food Agric*. 2001, *81*, 934 – 937.

[33] Wu, X., Cao, G., Prior, R. L., Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry, *J. Nutr*. 2002, *132*, 1865 – 1871.

[34] Manach, C., Scalbert, A., Morand, C., Remesy, C. *et al*., Polyphenols: Food sources and bioavailability, *Am. J. Clin. Nutr.* 2004, *79*, 727 – 747.

[35] Chung, M. J., Lee, S. H., Sung, N. J., Inhibitory effect of whole strawberries, garlic juice or kale juice on endogenous formation of N-nitrosomethylamine in humans, *Cancer Lett.* 2002, *182*, 1 – 10.

[36] Bub, A., Watzl, B., Blockhaus, M., Fruit juice consumption modulates antioxidant status, immune status and DNA damage, *J. Nutr. Biochem*. 2003, *14*, 90 – 98.

[37] Szeto, Y. T., Benzie, I. F. F., Collins, A. R., Choi, S. W. *et al*., A buccal cell model comet assay: Development and evaluation for human biomonitoring and nutritional studies, *Mutat. Res*. 2005, *578*, 371 – 38.

[38] Moller, P., Loft, S., Alfthan, G., Freese, R., Oxidative DNA damage in circulating mononuclear blood cells after ingestion of blackcurrant juice or anthocyanin-rich drink, *Mutat. Res*. 2004, *551*, 119 – 126.

[39] Wilms, L. C., Hollman, P. C. H., Boots, A. W., Kleinjans, J. C. S., Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes, *Mutat. Res*. 2005, *582*, 155 – 162.

[40] Duthie, S. J., Jenkinson, A. McE., Crozier, A., Mullen, W. *et al*., The effect of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy volunteers, *Eur. J. Nutr.* 2006, *45*, 113 – 122.

[41] Kresty, L. A., Frankel, W. L., Hammond, C. D., Baird, M. E. *et al*., Transitioning from preclinical to clinical chemopreventive assessment of lyophilised black raspberries: Interim results show berries modulate markers of oxidative stress in Barrett's esophagus patients, *Nutr. Cancer* 2006, *54*, 148 – 156.

[42] Gasiorowski, K., Szyba, K., Brokos, B., Kolaczynska, B. *et al*., Antimutagenic activity of anthocyanins isolated from *Aronia melanocarpa* fruits, *Cancer Lett*. 1997, *119*, 37 – 46.

[43] Lazze, M. C., Pizzalo, R., Savio, M., Stivala, L. A. *et al*., Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells, *Mutat. Res. Genet. Toxicol. Environ. Mutagen*. 2003, *535*, 103 – 115.

[44] Vattem, D. A., Jang, H. D., Levin, R., Shetty, K. J., Synergism of cranberry phenolics with ellagic acid and rosmarinic acid for antimutagenic and DNA protection functions, *Food Biochem.* 2006, *30*, 98 – 116.

[45] Cooke, D., Steward, W. P., Gescher, A. J., Marczylo, T., Anthocyanisn from fruits and vegetables: Dose bright colour signal cancer chemopreventive activity? *Eur. J. Cancer* 2005, *41*, 1931 – 1940.

[46] Chen, P. N., Chu, S. C., Chiou, H. L., Kuo, W. H. *et al*., Mulberry anthocyanins, cyanidin 3-rutioside and cyanidin 3-glucoside exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line, *Cancer Lett.* 2006, *235*, 248 – 259.

[47] Ramirez-Tortosa, C., Anderson, O., Gardner, P. T., Morrice, P. C. *et al*., Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E depleted rats, *Free Rad. Biol. Med*. 2001, *31*, 1033 – 1037.

[48] Singletary, K. W., Stansbury, M. J., Giusti, M., Inhibition of rat mammary tumorigenesis by Concord grape juice constituents, *J. Agric. Food. Chem*. 2003, *51*, 7280 – 7286.

[49] Ding, M., Feng, R. T., Wang, S. Y., Bowman, L. *et al*., Cyanidin 3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity, *J. Biol. Chem*. 2006, *281*, 17359 – 17368.

[50] Beattie, J., Crozier, A., Duthie, G. G., Potential health benefits of berries, *Curr. Nutr. Food Sci*. 2005, *1*, 71 – 86.

[51] Kresty, L. A., Morse, M. A., Adams, C. A., Stoner, G. D., Chemoprevention of NMBA-induced rat esophageal tumorigenesis by lyophilised black raspberries, *Cancer Res*. 1997, *40*, 392.

[52] Ahn, D., Putt, D., Kresty, L., Stoner, G. D. *et al*., The effects of dietary ellagic acid on rat hepatic and esophageal mucosal cytochromes P450 and phase II enzymes, *Carcinogenesis* 1996, *17*, 821 – 828.

[53] Stoner, G. D., Chen, T., Kresty, L. A., Aziz, R. M. *et al*., Protection against esophageal cancer in rodents with lyophilised berries: Potential mechanisms, *Nutr. Cancer* 2006, *54*, 33 – 46.

[54] Huang, C. S., Huang, Y., Li, J. X., Hu, W. W. *et al*., Inhibition of benzo(a)pyrene diol-epoxide-induced transactivation of activated protein 1 and nuclear factor kB by black raspberry extracts, *Cancer Res*. 2002, *62*, 6857 – 6863.

[55] Lu, H. T., Li, J. X., Zhang, D. Y., Stoner, G. D. *et al*., Molecular mechanisms involved in Chemoprevention of black raspberry extracts: from transcription factors to their target genes, *Nutr. Cancer* 2006, *54*, 69 – 78.

[56] Wang, S. Y., Feng, R. T., Lu, Y. J., Bowman, L. *et al*., Inhibitory effect of activator protein 1, nuclear factor-kappaB, and cell transformation by extracts of strawberries (*Fragaria x ananassa* Duch), *J. Agric. Food Chem*. 2005, *53*, 4187 – 4193.

[57] Roy, S., Khanna, S., Alessio, H. M., Vider, J. *et al*., Anti-angiogenic property of edible berries, *Free Rad. Res*. 2002, *36*, 1023 – 1031.

[58] Liu, Z. J., Schwimer, J., Liu, D., Greenway, F. L. *et al*., Black raspberry extract and fractions contain angiogenesis inhibitors, *J. Agric. Food Chem.* 2005, *53*, 3909 – 3915.

[59] Atalaya, M., Gordillo, G., Roy, S., Rovin, B. *et al*., Anti-angiogenic property of edible berry in a model of hemangioma, *FEBS Lett.* 2003, *544*, 252 – 257.

