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Berry Fruits for Cancer Prevention: Current Status and Future Prospects

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Overwhelming evidence suggests that edible small and soft-fleshed berry fruits may have beneficial effects against several types of human cancers. The anticancer potential of berries has been related, at least in part, to a multitude of bioactive phytochemicals that these colorful fruits contain, including polyphenols (flavonoids, proanthocyanidins, ellagitannins, gallotannins, phenolic acids), stilbenoids, lignans, and triterpenoids. Studies show that the anticancer effects of berry bioactives are partially mediated through their abilities to counteract, reduce, and also repair damage resulting from oxidative stress and inflammation. In addition, berry bioactives also regulate carcinogen and xenobiotic metabolizing enzymes, various transcription and growth factors, inflammatory cytokines, and subcellular signaling pathways of cancer cell proliferation, apoptosis, and tumor angiogenesis. Berry phytochemicals may also potentially sensitize tumor cells to chemotherapeutic agents by inhibiting pathways that lead to treatment resistance, and berry fruit consumption may provide protection from therapy-associated toxicities. Although a wide variety of berry fruits are consumed worldwide, this paper focuses on those commonly consumed in North America, namely, blackberries, black raspberries, blueberries, cranberries, red raspberries, and strawberries. In addition, a large body of studies on singly purified berry bioactives is available, but this paper focuses on studies of "whole berries" per se, that is, as berry extracts and purified fractions, juices, and freeze-dried powders. Potential mechanisms of anticancer action and bioavailability of berry phenolics, as well as gaps in knowledge and recommendations for future berry research, are also briefly discussed.

KEYWORDS: Berries; phenolics; cancer; in vitro; in vivo

INTRODUCTION

Among small soft-fleshed colorful fruits, berries make up the largest proportion that is consumed in our diet. Berry fruits are popularly consumed not only in fresh and frozen forms but also as processed and derived products including canned fruits, yogurts, beverages, and jams and jellies. In addition, there has been a growing trend in the intake of berry extracts as ingredients in functional foods and dietary supplements, which may or may not be combined with other colorful fruits, vegetables, and herbal extracts.

Berry fruits commonly consumed in North America include blackberries (*Rubus* spp.), black raspberries (*Rubus occidentalis*), blueberries (*Vaccinium corymbosum*), cranberries (*Vaccinium macrocarpon*), red raspberries (*Rubus idaeus*) and strawberries (*Fragaria* × *ananassa*). Other "niche-cultivated" berries and forest/wild berries, for example, bilberries, black currant, lingonberry, and cloudberry, are also popularly consumed in other regions of the world. In addition, there is a growing trend in the consumption of exotic "berry-type" fruits and their products, including the pomegranate (*Punica granatum*), goji berries (*Lycium barbarum*; also known as the wolfberry), fruits of *Garcinia mangostana*, the Brazilian açaí berry (*Euterpe oleraceae*), and the Chilean maqui berry (*Aristotelia chilensis*). Although the types of berry fruits consumed worldwide are many, this paper focuses on the aforementioned berries that are commonly consumed in North America.

A large and growing body of studies has convincingly established the anticancer potential of singly purified constituents found in berry fruits (*1, 2*). These phytochemicals include phenolics such as anthocyanins (pigments that impart the attractive colors to berry fruits and colorful vegetables), quercetin (a ubiquitous flavonol also found in onions, apple skins, etc.), proanthocyanidins (flavanol polymers common to green tea, grape skin and seeds, blueberries, cranberries, dark chocolate, etc.), hydrolyzable tannins (particularly ellagitannins, found in strawberries, black raspberries, red raspberries, blackberries, muscadine grapes, some nuts, oak-aged beverages, etc.), and other flavonoid-related molecules. However, this paper focuses on studies of "whole-berries", as their freeze-dried powders, berry extracts and/or their purified fractions, beverages, and single and combined berry formulations. Although the majority of published studies are with in vitro cell culture models, there

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is a growing body of published studies in animals and, recently, a few human studies have appeared in the peer-reviewed literature.

Reviews on the anticancer potential of berry fruits are available (*1*–*8*), but many of these focused on specific berry fruits, for example, cranberries and blueberries (*4, 5*), freeze-dried black raspberries (*6, 7*), and a berry formulation (*8*). Because of the rapidly growing body of studies in this field, this paper provides an update since the published literature reviews (see refs *1* and *3*). Potential mechanisms of anticancer action of berry bioactives, bioavailability, and metabolism of berry phenolics, as well as gaps in knowledge and recommendations for future berry research in the area of cancer prevention and therapy, are also briefly discussed herein.

BERRY PHYTOCHEMICALS

Some of the known chemopreventive agents present in berries include vitamins A, C, and E and folic acid; calcium and selenium; β-carotene, α-carotene, and lutein; phytosterols such as β-sitosterol and stigmasterol; triterpene esters; and phenolic molecules such as anthocyanins, flavonols, flavanols, proanthocyanidins, ellagitannins, and phenolic acids. Berries contain high levels of a diverse range of phytochemicals, most of which are phenolic molecules.

The chemistry of berry phenolics directly influences their bioavailability, metabolism, and biological effects in vivo (*9, 10*). The structural diversity of berry phenolics is observed in several ways including (a) their degree of oxidation and substitution patterns of hydroxylation, (b) their abilities to exist as stereoisomers, (c) glycosylation by sugar moieties and other substituents, and (d) conjugation to form polymeric molecules, such as tannins and other derived molecules (*11*). The major structural classes of berry phenolics are flavonoids (anthocyanins, flavonols and flavanols), condensed tannins (proanthocyanidins), hydrolyzable tannins (ellagitannins and gallotannins), stilbenoids, phenolic acids (hydroxybenzoic and hydroxycinnamic acids), and lignans (*1*).

IN VITRO ANTICANCER STUDIES

That berry phenolics exhibit potent antioxidative properties is widely accepted, but their biological properties extend beyond antioxidation (reviewed in ref *2*). In fact, berry phenolics also exhibit anti-inflammatory properties, are able to induce carcinogen detoxification (phase-II) enzymes, and modulate subcellular signaling pathways of cancer cell proliferation, apoptosis, and tumor angiogenesis (reviewed in ref *1*). These as well as other potential mechanisms of action of berry bioactives on carcinogenesis are briefly discussed below.

Recent studies have shown that berry extracts and their singly purified phenolic constituents inhibit cell proliferation, modulate cell cycle arrest, and induce apoptosis (programmed cell death) in cancer cells with little or no cytotoxic effects in normal cells. For example, our laboratory recently showed that blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit the growth of human oral, breast, colon, and prostate cancer cell lines in a dose-dependent manner (*12*). Furthermore, the berry extracts stimulated apoptosis of a human colon cancer cell line, HT-29, which expresses the cyclooxygenase-2 enzyme (COX-2). It is noteworthy that several cherry and berry extracts have been shown to inhibit COX-2 enzyme activity in vitro (*13*).

Strawberry, raspberry, both highbush and lowbush blueberries, blackberry, and cranberry juices were evaluated for antiproliferative and anti-inflammatory activities and also abilities to induce apoptosis and cell cycle arrest against a panel of human stomach, prostate, intestine, and breast cancer cell lines (*14*). Strongest inhibition of cell growth was observed for the raspberry, lowbush blueberry and cranberry juices. The authors reported that the inhibition of proliferation by the berry juices was independent of caspase-dependent apoptosis but appeared to involve cell-cycle arrest, as evidenced by down-regulation of the expression of cyclin kinases, cdk 4, cdk 6, cyclin D1 and cyclin D3. Some of the berry juices also significantly inhibited the tumor necrosis factor-induced activation of the COX-2 enzyme expression and activation of the transcription factor, nuclear factor kappa B (NFκB). The authors concluded that different berry fruits might act through different mechanisms in their cancer preventive ability (*14*).

A red raspberry extract, obtained after a digestion procedure that mimicked the physiochemical conditions of the upper gastrointestinal tract, was shown to decrease the population of human HT29 colon cancer cells in the G1 phase of the cell cycle (*15*). In addition, the raspberry extract imparted significant protective effects against DNA damage induced by hydrogen peroxide in the colon cancer cells. Finally, the authors reported that the raspberry extract significantly inhibited the invasion of HT115 colon cancer cells in a matrigel invasion assay (*15*).

A study recently investigated whether the regulations of apoptosis and the phase-II enzymes glutathione-*S*-transferase (GST) and quinone reductase (QR) are potential mechanisms through which blueberry may prevent cancer (*16*). The authors showed that anthocyanin-enriched fractions purified from the blueberries induced apoptosis of human HT29 colon cancer cells using both DNA fragmentation and caspase-3 activity methods. However, at the test concentrations, the blueberry anthocyanins decreased rather than induced QR and GST activities (*16*).

The differential effects of proanthocyanidin-enriched fractions obtained from blueberries on androgen sensitive (LNCaP) and androgen insensitive (DU145) human prostate cancer cell lines have been reported (*17*). The authors found that the blueberry proanthocyanidins had a greater effect on the androgen-dependent growth of prostate cancer cells (*17*).

Several berry extracts, including strawberry and raspberry, were recently evaluated for their effects on cell viability and expression of markers of cell proliferation and apoptosis in human HT29 colon cancer cells (*18*). The authors concluded that the berry extracts inhibited cancer cell proliferation mainly via the p21WAF1 (a member of the cyclin kinase inhibitors) pathway. The pro-apoptosis marker, Bax, was found to be increased in cells treated with the berry extracts in the apoptosis experiments. In addition, the authors reported that in addition to anthocyanins, other phenolic compounds, such as ellagitannins, and nonphenolic compounds, might also contribute greatly toward the antiproliferative activity of berries (*18*).

Isolated cell lines from human oral squamous cell carcinoma tumors were recently used to investigate the effects of a freeze-dried black raspberry ethanol extract on cellular growth (*19*). The authors showed that the black raspberry extract suppressed cell proliferation without perturbing viability, inhibited translation of the complete angiogenic cytokine vascular endothelial growth factor, suppressed nitric oxide synthase activity, and induced both apoptosis and terminal differentiation (*19*).

Three cultivars of blueberries ('Briteblue', 'Tifblue', and 'Powderblue') were extracted and assessed for antiproliferative and pro-apoptotic effects on liver HepG2 cancer cells (*20*). The greatest inhibitory effects were observed for the blueberry anthocyanin fractions (ranging from 70 to 150 μg/mL concentrations) with 50% inhibition of cancer cell population growth.

Induction of apoptosis was assessed by DNA fragmentation, and the blueberry anthocyanin fraction showed a 2−4-fold increase in apoptosis compared to control (*20*).

Cranberry extracts were shown to significantly inhibit the growth of human breast cancer MCF7 cells, which was attributed to the ability of the extracts to initiate apoptosis and induce G1 phase arrest in the cell cycle (*21*). The same group of workers isolated and identified 20 pure compounds from cranberries, including ursolic acid, quercetin, and 3,5,7,3′,4′-pentahydroxyflavonol-3-*O*-β-D-glucopyranoside and showed that these compounds have potent antiproliferative activities against liver HepG2 and breast MCF7 cancer cell growth (*22*).

The antiproliferative effects of organically versus conventionally cultivated strawberries on human colon and breast tumor cells have been reported (*23*). The organically grown strawberries showed higher antiproliferative activity than the conventionally grown fruits, which was accounted for by the higher content of secondary metabolites with anticarcinogenic properties in those fruits (*23*).

Berry-derived products such as their seed flours have also been evaluated for anticancer properties. Black raspberry, red raspberry, blueberry, and cranberry seed flours were shown to inhibit the proliferation of human HT29 colon cancer cell line (*24*). The authors suggested that the berry fruit seed flours might have potential for the development of value-added products for cancer prevention and optimal health. In a separate study, the same group of researchers also showed that strawberries treated with essential oils, such as thymol, menthol, or eugenol, exhibited stronger inhibition of human HT29 colon cancer cell growth than those from untreated fruit (*25*).

Apart from the potential mechanisms of anticancer action described above, berry fruits have also been shown to inhibit the activities of enzymes, which play a significant role in cancer metastasis, such as matrix metalloproteinases (MMPs) (*1*). A recent study investigated the ability of flavonoid-enriched fractions from lowbush blueberry to down-regulate MMP activity in DU145 human prostate cancer cells (*26*). Differential down-regulation of MMPs was observed in cells exposed to both anthocyanin-and proanthocyanidin-enriched blueberry fractions. The possible involvement of protein kinase-C and mitogen-activated protein kinase pathways in the flavonoid-mediated decreases in MMP activity was observed. The authors concluded that the down-regulation of MMP activities by the blueberry flavonoids might occur through multiple mechanisms (*26*).

The potential of negating drug resistance in cancer cells has important clinical implications, and berry fruits have immense potential in this area. Recently, cranberry proanthocyanidin fractions have been reported to show cytotoxicity toward platinum-resistant human ovarian cancer cell lines, neuroblastoma, and prostate cancer cell lines (*27*). The cranberry fractions sensitized human ovarian SKOV3 cancer cells to the platinum drug, paraplatin, and the authors concluded that this suggested a significant synergistic effect between cranberry proanthocyanidins and the chemotherapy drug (*27*).

Berry fruits also show potential in the inhibition of absorption of environmental carcinogens. Mahadevan et al. evaluated the potential of red raspberry extracts to inhibit the absorption of environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs) using a Calu-3 cell monolayer model (*28*). The authors reported that the phytochemicals present in red raspberries inhibited PAH absorption across the Calu-3 cell monolayers and are likely to influence the exposure of lung epithelial cells to PAH-induced DNA damage (*28*).

IN VIVO ANTICANCER STUDIES

Animal Studies. The possible effects of berries (blueberries, blackberries, and cranberry juice) and other high-antioxidant fruits (pomegranate, watermelon, mangoes, and plum) on azoxymethane (AOM)-induced aberrant crypt foci (ACF) in Fisher 344 male rats were recently investigated (*29*). After 17 weeks, the rats fed fruits and fruit juices showed significant ($p < 0.05$) reductions in the formation of AOM-induced ACF compared to untreated animals. In addition, GST activity in the liver of the rats fed fruits and fruit juices was significantly ($p < 0.05$) higher compared to control. The authors concluded that among the fruits and fruit juices tested, blueberry and pomegranate juices contributed to significant ($p < 0.05$) reductions in the formation of AOM-induced ACF (*29*).

A rodent model of human esophageal squamous cell carcinoma was used to evaluate the chemopreventive effects of freeze-dried black raspberry powder (BRB) for this disease and to determine potential mechanisms of action (*30*). The authors showed that dietary BRB inhibited *N*-nitrosomethylbenzylamine (NMBA)-induced tumor development in the rat esophagus by inhibiting the formation of DNA adducts and reducing the proliferation rate of preneoplastic cells. On a molecular level, the BRB down-regulated the expression of c-Jun, COX-2, and inducible nitric oxide synthase (iNOS). The authors also analyzed the effect of BRB on angiogenesis, a process critical to tumor growth and metastasis that involves the formation of new blood vessels. Vascular endothelial growth factor (VEGF) is an important angiogenic activator, and BRB significantly suppressed VEGF expression from a (2.38 ± 0.34)-fold increase in animals treated with NMBA alone to a (1.08 ± 0.22)-fold increase in animals treated with NMBA plus BRB ($p < 0.005$). In addition, the microvessel density of the esophagus was decreased from 53.7 ± 5.6 vessels/cm in animals treated with NMBA alone to 22.6 ± 2.6 vessels/cm in animals treated with NMBA plus BRB ($p < 0.0001$). This study also showed that the down-regulation of VEGF was correlated with suppression of COX-2 and iNOS. The authors concluded that because high vascularity is a risk factor for metastasis and tumor recurrence, BRB might have cancer therapeutic effects in human esophageal cancer (*30*).

It is noteworthy that in an earlier study, the same group of workers had treated F344 rats with NMBA, three times per week for 5 weeks (*31*). Beginning 1 week later, the animals were fed a diet containing 5% BRB for the duration of the bioassay (25 weeks) and were sacrificed at weeks 9, 15, and 25. The expression and enzymic activities of COX-2 and iNOS, as well as the expression of c-Jun in the esophagi, were evaluated to investigate the potential mechanism(s) by which BRBs modulate tumorigenesis. At week 25, BRB inhibited tumor multiplicity, from 3.78 ± 0.41 tumors per rat in NMBA-treated animals to 2.23 ± 0.21 tumors per rat in animals treated with NMBA plus BRB ($p < 0.005$). BRB reduced mRNA and protein expression levels of COX-2, iNOS, and c-Jun as well as the level of prostaglandin E_2 in preneoplastic lesions of the esophagus at week 25. The BRB inhibited mRNA expression of iNOS and c-Jun, but not COX-2, in papillomatous lesions of the esophagus. Prostaglandin E_2 and total nitrite levels were also decreased by BRB in papillomas. The authors concluded that this suggested a novel tumor suppressive role of BRB through the inhibition of COX-2, iNOS, and c-Jun (*31*).

The mechanistic basis of the cancer anti-initiating effects of BRB by studying NMBA metabolism in esophageal explant cultures, and in liver microsomes taken from rats fed a control diet versus a control diet containing BRB (at 5 or 10%,

concentrations), were investigated (*32*). At both test concentrations, dietary BRB inhibited NMBA metabolism in explants (26 and 20%) and in microsomes (22 and 28%). The authors identified individual components of BRBs, ellagic acid, and the anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, as active constituents. NMBA metabolism in explants was inhibited maximally by cyanidin-3-rutinoside (47%) followed by ellagic acid (33%), cyanidin-3-glucoside (23%), and then the BRB extract (11%). Similarly, in liver microsomes, the inhibition was maximal with cyanidin-3-rutinoside (47%), followed by ellagic acid (33%) and cyanidin-3-glucoside (32%). Finally, dietary BRB was shown to induce glutathione-*S*-transferase activity in the liver (*32*).

Human Studies. Increased fruit and vegetable consumption has been associated with the decreased risk of a number of cancers of epithelial origin, including esophageal cancer. As discussed above, dietary administration of lyophilized BRB has been shown to significantly inhibit chemically induced oral, esophageal, and colon carcinogenesis in animal models.

A 6 month chemopreventive pilot study conducted by administering 32 or 45 g (female and male, respectively) of BRB to patients with Barrett's esophagus (BE), a premalignant esophageal condition in which the normal stratified squamous epithelium changes to a metaplastic columnar-lined epithelium, has been reported (*33*). BE's importance lies in the fact that it confers a 30−40-fold increased risk for the development of esophageal adenocarcinoma, a rapidly increasing and extremely deadly malignancy. At the time of the publication, interim findings from 10 patients with BE supported the finding that daily consumption of BRB promoted reductions in the urinary excretion of two markers of oxidative stress, 8-epi-prostaglandin $F_{2\alpha}$ and, to a lesser more variable extent, 8-hydroxy-2′-deoxyguanosine (*33*).

It is noteworthy that this group of researchers has also investigated the formulation and characterization of a novel gel formulation for local delivery of the BRB chemopreventive compounds to human oral mucosal tissues (*34*). Anthocyanins contained in mucoadhesive berry gel formulations were readily absorbed into human oral mucosa tissue as evidenced by detectable blood levels within 5 min after gel application. There was a trend for greater penetration of berry anthocyanins into tissue explants for berry gels with a final pH of 6.5 versus 3.5. The results from this study showed that the berry anthocyanin stability was dependent upon gel pH and storage temperature and also demonstrated that the gel composition was well suited for absorption and penetration into the target oral mucosal tissue site (*34*).

A recent study evaluated the effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers (*35*). Twenty healthy female volunteers aged 18–40 years consumed 750 mL/day of either cranberry juice or a placebo drink (with artificial flavor and color but containing no phenolics or vitamin C) for 2 weeks, and fasted blood and urine samples were obtained over 4 weeks. Vitamin C, total phenolic, anthocyanin, and catechin concentrations and antioxidant potential (by the FRAP assay) were significantly higher in the volunteers who drank the cranberry juice compared with those levels in the volunteers who drank the placebo. Cyanidin and peonidin glycosides comprised the major anthocyanin metabolites [peonidin galactoside (29.2%) > cyanidin arabinoside (26.1%) > cyanidin galactoside (21.7%) > peonidin arabinoside (17.5%) > peonidin glucoside (4.1%) > cyanidin glucoside (1.4%)]. Plasma vitamin C increased significantly ($p < 0.01$) in volunteers consuming cranberry juice. No anthocyanins (plasma) or catechins (plasma or urine) were detectable, and plasma total phenolics, triglycerides, and both high-density and low-density lipoproteins levels were unchanged. The antioxidant potentials of the plasma, glutathione peroxidase, catalase, and superoxide dismutase activities and malondialdehyde were similar for both groups of subjects. Supplementation with cranberry juice did not affect 8-oxo-deoxyguanosine in urine or endogenous or H_2O_2-induced DNA damage in lymphocytes. The authors concluded that cranberry juice consumption did not alter blood or cellular antioxidant status or several biomarkers of lipid status. Similarly, the cranberry juice had no effect on basal or induced oxidative DNA damage (*35*).

DIETARY INTAKE OF BERRY PHYTOCHEMICALS

Unfortunately, data on the dietary intake of berry bioactives in humans are limited. This is partially due to the difficulties in estimation of berry phytochemicals (namely, phenolics including proanthocyanidins, and hydrolyzable tannins) in foods due to their wide structural diversity and ill-defined structures and the unavailability of commercial standards. As a result, only partial data for certain berry phenolics, such as flavonols, have been published on the basis of direct food analysis or bibliographic compilations (*10*). In the United States, federal agencies such as the U.S. Department of Agriculture (USDA) have established databases in which the flavonoid contents of selected foods, compiled from various bibliographic sources, are available.

Studies have shown a high variability in phenolic intake based on variations in individual food preferences. A high daily intake of fruits and vegetables is estimated to provide up to 1 g of phenolics (*36*). However, among berry phenolics, research has targeted individual data for specific classes of compounds. For example, consumption of flavonols has been estimated at 20–25 mg/day in different regions of the world such as the United States, Denmark, and The Netherlands (*37–39*). However, in Italy, flavonol consumption can reach an average amount of 35 mg/day (*40*). In countries such as Finland, where high amounts of berry fruits are consumed, anthocyanin intake may exceed 200 mg/day (*41*).

The dietary burden of specific classes of berry bioactives such as anthocyanins (*42*), ellagitannins (*43*), proanthocyanidins (*44*), sterols (*45*), hydroxybenzoic acid derivatives (*46*), chlorogenic acid and other cinnamates (*47*), lignans and stilbenes (*48*), and flavonols, flavones, and flavanols (*49*) has been reviewed.

BIOAVAILABILITY OF BERRY BIOACTIVES

The bioavailability and metabolism of phenolics, the predominant phytochemicals present in berry fruits, have been reviewed (*1, 9, 10, 50–53*) and therefore will not be discussed in detail in this paper. It is noteworthy that the bioavailability of phenolics, the predominant phytochemicals present in berry fruits, is widely accepted by the scientific community to be poor on the basis of their relatively "low" levels detected in circulation. However, these compounds are extensively metabolized in the body's tissues and by the colonic microflora. When the gut microflora is involved, the efficiency of absorption is often reduced because the flora also degrades the aglycons that it releases and produces various simple phenolic and aromatic acids in the process. During the course of absorption, phenolics are conjugated (usually methylated, sulfated, and glucuronidated) in the small intestine and later in the liver, a metabolic detoxification process that facilitates biliary and urinary elimination. Many of these metabolites are still largely unknown and not accounted for, and knowledge on their tissue

disposition is scarce. In addition, there are also large variations in polyphenol bioavailability observed among individuals due to nutrigenetic and nutrigenomic effects. Therefore, it is critical that these aforementioned factors be taken into consideration for a complete evaluation of the impact of the bioavailability and metabolism of berry phenolics on cancer prevention.

SUMMARY AND FUTURE PERSPECTIVES

An overwhelming and rapidly growing body of studies suggests that berry fruits may have immense potential for cancer prevention and therapy, but there are still important gaps in our knowledge [see the introduction to this group of papers (*54*)]. Although our understanding of some of the potential mechanisms of the action of berry phytochemicals in cancer prevention has increased over the past decade, research efforts should continue to focus on the elucidation of mechanisms of action at the cellular and molecular levels. In addition, as research into the potential health benefits of berries continues in a postgenomic era, it will bring ever-increasing demands to observe and characterize variations within biological systems. Research focus on nutrigenomics (effects of nutrients on the genome, proteome, and metabolome) and nutrigenetics (effects of genetic variation on the interaction between diet and disease) will be essential. Because extrapolations cannot be made between in vitro and in vivo systems, further studies should be designed to investigate the cancer-preventive potential of berry fruits in animal models and human subjects. In addition, although our knowledge on the bioavailability, metabolism, and tissue disposition of berry bioactives, gained from cell culture and animal studies, has increased over the past decade, future studies in humans are also needed. Whether the chemopreventive potential of berry bioactives is increased by complex interactions of multiple substances within the natural food matrix of berry fruits, and/or in combination with phytochemicals from other foods, should be investigated. In addition, studies probing potential "herb−drug" interactions of berries and pharmaceutical drugs should be investigated in carefully planned and controlled human clinical studies. Finally, interdisciplinary research is highly recommended so that basic and preclinical studies can lead to translational research (from laboratory to bedside).

In conclusion, it is strongly recommended that this area of research for berry fruits continue to be explored, as this will lay the foundation for the development of diet-based strategies for the prevention and therapy of several types of human cancers.

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